SETAC EUROPE
Milan 2011

SETAC Europe 21st Annual Meeting

Ecosystem Protection in a Sustainable World:

A Challenge for Science and Regulation

15–19 May 2011

abstract book

SETAC

ABSTRACT BOOK
SETAC EUROPE 21ST ANNUAL MEETING
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This book composes the abstracts of the presentations for the platform and poster sessions of the 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Milano Convention Centre in Milan, Italy from 15-19 May 2011.
The abstracts are reproduced as accepted by the Scientific Committee of the 21st Annual Meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

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Milan 2011

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on growing membership, meetings attendance, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors / Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 30 years.

Like many other professional societies, SETAC publishes esteemed scientific journals and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur.

SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989; SETAC Asia / Pacific in 1997 and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these "geographic units" into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, Central and Eastern Europe, Africa and soon-to-be organized Japan), through regional chapters, and through national language branches (Germany). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 2.915 pages in 2001. Since January 2005, SETAC publishes a second scientific journal: *Integrated Environmental Assessment and Management*. IEAM is devoted to bridge the gap between scientific research and the use of science in decision making, regulation and environmental management. SETAC publishes the global newsletter SETAC Globe, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

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Keynote speaker abstracts

Ecotoxicology examined: current issues and trends

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In the second half of 20th century ecotoxicology developed to a large extent through "jumping" from one urgent issue to another. We had thus the bioconcentration and biomagnification boom in 1970s through 1980s, standardization of ecotoxicological tests took our minds mostly in 1990s, and we entered 21st century diving enthusiastically into the 'omics'. While this picture is probably typical for any young science, the grown-up fields let the scientists harvest many crops. I believe that ecotoxicology is just entering its maturity. Hence, when asked by the conference organizers to give a plenary lecture on "New trends in ecotoxicology", I realized that it is not as easy to identify them today as it used to be in the past. The 'omics' is still with us but can hardly be called now the "new trend". At the same time, we have come back to problems noticed already years ago, like toxicity of complex mixtures and influence of natural factors on toxic effects of pollutants. We are also looking beyond simple standard tests and urge the decision makers to consider more elaborate methods of ecological risk assessment. Not being quite sure whether this is just my personal viewpoint – possibly a reflection of loosing the track? – I made a short poll among colleague ecotoxicologists. The result was the same: none (sic!) of the responders was eager to name just one novel trend, and the responses were widely spread across different topics. Almost equal number of researchers found the most important issues in toxicokinetics and toxicodynamics, landscape and ecosystem ecotoxicology, interactions between chemicals and natural factors, population-level modeling, or chemical mixtures. Further topics included nanoparticles – the only problem that can be indeed called reasonably novel, and methods for extrapolating results of laboratory tests to real-field scenarios. Is that a bad sign that top scientists are not able to identify novel trends in the subject of their interest? I do not think so: as mentioned above, I believe that this is the sign of maturation (of ecotoxicology, not the scientists...). Despite the difficulty with naming novel issues, there are, however, some very interesting trends and processes undergoing in ecotoxicology nowadays, certainly deserving a closer look and a discussion. Among them are both purely scientific ones and societal, and both groups will be addressed in my lecture.

Ecosystem services, environmental protection and SETAC: preventing and adapting to the "perfect storm"

Lorraine Maltby
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Ecosystems provide us with the essentials for life – food to eat, water to drink, fibre for clothes and shelter, fuel to keep us warm. However, they do more than that; they play a key role in climate regulation through carbon sequestration, flood prevention through water retention and runoff regulation, and water purification through filtration processes and microbial activity. They are places where we go to relax, to participate in recreational activities or simply to be inspired by the wonders of the natural world. Moreover, ecosystems perform essential processes such as nutrient and water cycling and the production of biomass. These benefits to people are termed ecosystem services and they are provided by all ecosystems.

The growing human population is putting increased pressure on ecosystems and their ability to provide the services we require. It is estimated that by 2030, world food demands will increase by 50%, energy demands will rise by 50%, water demands will increase by over 30% and 60% of the world's population will live in urban areas. Overlay climate change and you have the ingredients for what was described by John Beddington, the UK government's chief scientist, as the "perfect storm".

Managing landscapes for the provision of the ecosystem services we require (e.g. food, water, energy, minerals), whist at the same time protecting the biodiversity on which many of these services depend, is a major challenge, but one to which the SETAC community can make a valuable contribution. This keynote presentation will consider this challenge and the opportunities it provides for environmental scientists.

Exposure science - the link between hazard and risk

Marika Berglund
Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

We are facing the challenge of assessing the exposure to a huge number of chemicals and other stressors. Exposure science is the platform for generating better exposure information which is highly needed for reliable risk assessment and protection, prevention and sustainability of the environment and human health. The lack of relevant exposure information is often a problem in regulatory decision-making and risk reduction. Development and harmonization of methods for characterizing, estimating, modelling, measuring, and quantifying exposure will result in more efficient risk reduction in the future. Toxicologists, epidemiologists, environmental and human health scientists, risk assessors and risk managers are all using a number of different data sources and methods to estimate and analyse exposure information. It is time to start working together, and to learn from each other. Together we can identify data gaps and shape the future of exposure science for more reliable decision-making. Exposure science is the crucial link between hazard information and risk. It has a great potential to meet the need for suitable methods to obtain the exposure information required by new chemical legislations and regulatory frameworks.

Toward sustainable solutions

Robert Costanza
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A high and sustainable quality of life is a central goal for humanity. Our current socio-ecological regime and its set of interconnected worldviews, institutions, and technologies all support the vision of unlimited growth of material production and consumption as a proxy for quality of life. However, abundant evidence shows that, beyond a certain threshold, further material growth no longer significantly contributes to improvement in quality of life. Not only does further material growth not meet humanity's central goal, there is mounting evidence that it creates significant roadblocks to sustainability through increasing resource constraints (i.e., peak oil, water limitations) and sink constraints (i.e., climate disruption). Overcoming these roadblocks and creating a sustainable and desirable future will require an integrated, systems level redesign of our socio-ecological regime focused explicitly and directly on the goal of sustainable quality of life rather than the proxy of unlimited material growth. This transition, like all cultural transitions, will occur through an evolutionary process, but one that we, to a certain extent, can control and direct through the process of shared envisioning. Visions and models of integrated sets of worldviews, institutions, and technologies are needed to stimulate and seed this evolutionary redesign. The process of creating a shared vision of the future is also a key element of real democracy.

Special session abstracts

SS01 - New QSAR models for regulatory purposes

SS01-1
Consensus Modeling of Environmental Endpoints
T Martin, DM Young
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A consensus modeling approach was devised to develop models for different ecotoxicological endpoints of large, structurally diverse data sets. In the consensus approach, the endpoint is predicted by taking the average of the predicted values from several different QSAR (quantitative structure-activity relationship) approaches. Several QSAR approaches were used in the consensus model including the hierarchical clustering, multilinear regression, group contribution, and nearest neighbor methods. Models were developed for the following endpoints: acute aquatic toxicity, acute oral rat toxicity, fish bioaccumulation factor, and Ames mutagenicity. In general the consensus models achieved higher prediction accuracy and prediction coverage (the fraction of chemicals predicted) than any of the individual models by themselves.

SS01-2
The development of new tools towards a better exploitation of non testing methods for regulatory purposes
A Roncaglioni, A Manganaro, R Gonella Diaza, A Lombardo, M Petoumenou, E Benfenati
Istituto di Ricerche Farmacologiche Mario Negri, MILAN, Italy
The use of non testing methods, including QSAR and read-across approaches, has the potential ability to obtain fast, costless and reliable data, hence their usage has been promoted in the regulations.
Some difficulties are anyhow experienced in the use of these methods particularly by non experts to properly address the reliability of the estimations so obtained.
For instance more details about QSAR models may be provided, including information about the meaning of the outputs obtained by the models, the applicability domains of models, and the source data that the models are based on.
Here we present methods to fill these information gaps, offering tools to examine the results, their meaning, and the possible uses of the models.
Compared with many existing QSAR models, we have put greater emphasis on ensuring that the models generate transparent, understandable, reproducible and verifiable results. To achieve this, a series of tools has been optimised, which can relate the results obtained for the target chemical to the results obtained for similar (structurally related) compounds.
In addition to obtaining the result of the evaluation, the models we studied provide the user with access to a series of features that are important for regulatory purposes:
* an identification of the possible regulatory uses of the result produced;
* an evaluation of the applicability domain, with an explanation;
* an understanding of the rules and reasoning behind the model.
Moreover these tools also provide a reproducible procedure for read-across.
Acknowledgments: Support by EU projects ANTARES (LIFE programme, project ref. LIFE08 ENV/IT/000435) and ORCHESTRA (FP7-COOPERATION programme, contract nr. 226521) is acknowledged.

SS01-3
Integrating statistical and structural results in predictive modelling for a multi-view evaluation
C Gini, T Ferrari, D Cattaneo
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The application of new rules and regulations calls for a more efficient in-silico screening. In particular, prioritization, classification & labelling, and prediction open new challenges. The modelling systems will need to be accurate and understandable. Either QSAR or SAR are often used alone to model the activity of substances, on the basis of the kind of the output (dose vs presence) property of interest. Many QSAR models are simple or complex relations built over molecular descriptors. The choice of the descriptors to use is relevant since it affects the statistical accuracy of the model; moreover a lot of effort is put in justifying the choice in terms of interpretability of the model (or in terms of mechanicistic steps). Many SAR models are manually built from a set of observations and experiments and consist in a set of rules, usually stated as structural alerts that have been associated with the property. It is not easy to go from a qualitative SAR result to a quantitative QSAR result. On the other hand, the structural nature of chemicals can be explicitly considered by a graph-mining approach, which mines large datasets for frequent substructures, or fragments, that are statistically associated with the property.
We present a new approach that integrates the two SAR-QSAR methods. It can be used to build statistical models using categorical or continuous values, can derive structural alerts, can produce hypotheses of safe substructures, and can accompany the statistical result with a rule.

SS01-4
Towards the application of Nano-QSAR models for regulatory purposes
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The REACH legislation in Europe governs inventory of chemicals that are introduced at the market in large quantities. Every new chemical should be registered first, and the registration must be precluded by a comprehensive risk assessment. The REACH system promotes the use of alternative ways of toxicity testing, including Quantitative Structure-Activity Relationship methods. When properly validated, QSAR models can replace costly and ethically questionable experiments with use of laboratory animals.
Regarding the extensive growth of nanotechnology observed during last few years nanomaterials are becoming a distinct group of widely used chemicals. Since nanoparticles differ from bulk substances by physicochemical properties and toxicity, the registration requirements for them should be reviewed and adapted considering their specificity.
This presentation reviews current status of nanomaterials under the REACH regulation and discusses advances and challenges of QSAR development for nanomaterials (Nano-QSAR). The most challenging problems are: (i) scarce and/or inconsistent experimental data available and lack of conceptual frameworks for grouping nanoparticles according to mode of physicochemical properties and toxic action; (ii) lack of appropriate descriptors able to express specificity of "nano"structure; (iii) very limited knowledge on the interactions between nanoparticles and biological systems (DNA, proteins, membranes etc.); and (iv) lack of rational modeling procedures to screen large numbers of structurally diversified nanoparticles. Majority of the existing Nano-QSARs refer to physical-chemical enpoints. However, our last contribution demonstrated the first Nano-QSAR model for predicting toxicity (cytotoxicity to bacteria E. coli) of nanosized metal oxides. This would be the first step towards creating a comprehensive tool for computational risk assessment of nanomaterials to be used for regulatory purposes.

SS01-5
Regulators' role and mission: Feedback from various stakeholder contexts
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[4]PublicSpace Ltd., ULVERSTON, United Kingdom
The ORCHESTRA project (2009-12, EC #226521) aims to promote wider understanding, awareness and appropriate use of in silico methods. The project web portal will become a central resource for sharing of knowledge and experience and will also provide downloadable software.
In September 2010, we consulted regulators, scientists and industry stakeholders on their use of and perceptions of in silico methods (see our poster presentation for details; view questionnaire and fill out online at www.in-silico-methods.eu)
While the self-selected sample was very restricted, among the points of interest are the positions taken by regulators, compared to or contextualized by those of other stakeholders. For instance, regulators appear to have tested in silico methods in a distinctly broader array of areas (with human toxicology leading the field - in particular contrast to industry experience).
Regulators experiment with in silico methods for the full variety of functions these may serve, ranging from a formal decision focus (supporting information, or weight-of-evidence input), to less formal convenience features (fast evaluation of chemical properties). Use of these decision aids is expected to grow, most strikingly for the prioritisation of compounds for further analysis, and as input to the "key study".
Outstanding is the gap between perceptions by scientists and industry users, and by regulators, regarding benefits of in silico methods. Regulators surveyed give little emphasis to in silico's ability to reduce laboratory use of vertebrates (in stark contrast also to lay demands as judged by an informal tracking of press articles about REACH.) One regulator commented: "Reduction of vertebrate testing is a major goal, however, it is secondary to improved risk management". Do regulators thus weigh the benefits strictly in regard to their weighted priorities (goals)? In this way, regulators' primary evaluation of in silico would be (appropriately) in terms of whether it is "fit for purpose". Is that purpose, however, centred for regulators on absolute human and environmental protection, or on optimisation of risk management?
Do other stakeholder categories take for granted that regulators' role is the absolute protection of humans (leaving thus a margin for demands of animal protection as well)? Is optimisation understood? Insight is offered from the area of nuclear power and radioactive waste - where major studies have focussed on societal demands on regulation.

SS02 - International reference life cycle data system (ILCD) handbook and data network

SS02-1
LCA and the ILCD in the context of Sustainability Assessment
C Ciupagea
European Commission, ISPRA, Italy
The new Unit 'Sustainability Assessment' of the Institute for Environment and Sustainability of the European Commission's Joint Research Centre (JRC) is intended to provide scientific and technical support to EU services that are engaged in enhancing sustainable development and resource efficiency.
It will develop and apply new approaches and solutions to integrated socio-economic and environmental assessments and impact analyses across policies at local, regional and global scales in a customer-driven manner.
The deliverables will equally help industry and other actors of society to actively work on the improvement of their products and daily operations as well as advice procurers and citizen towards more sustainable consumption.
A core component and expertise of the new Unit is related to the life-cycle thinking concept and, in particular, to the (environmental) Life Cycle Assessment (LCA). This Special Symposium focuses on the development of the International Reference Life Cycle Data System (ILCD) Handbook and Data Network. The ILCD has been developed in context of the European Platform on LCA to support life cycle based policies and business instruments related to sustainable production and consumption as well as resource-efficiency with guidance and with consistent and quality-assured data. Very important next to the tool LCA itself are the concepts and principles that make up the strengths of LCA, which plays a double role both as one specific method and component in the sustainability toolbox and inspiring a wider sustainability assessment with the named concepts and principles it draws on. The presentation will briefly place LCA into the wider integrated sustainability assessment framework envisaged for the current and future work of this new Unit

SS02-2
The role of the ILCD Handbook as methodological basis for robust life cycle based environmental policies
M Galatola
European Commission, DG Environment, BRUSSELS, Belgium

SS02-3
Overview of the International Reference Life Cycle Data System (ILCD)
MA Wolf, R Pant, K Chomkhamsri, DW Pennington, S Sala, M Brandao
European Commission, Joint Research Centre, ISPRA, VA, Italy
The International Reference Life Cycle Data System (ILCD) has been developed as ISO-conform but further specified guidance on Life Cycle Assessment (LCA) and to facilitate access to quality-assured Life Cycle Inventory (LCI) data. This is in response to the Integrated Product Policy (IPP) Communication and to support the implementation of sustainable production and consumption as well as resource efficiency policies in the EU and internationally. The ILCD consists primarily of a Handbook and a Data Network: The ILCD Handbook is a series of technical

guidance documents that provides the basis for developing consistent and quality-assured life cycle data and assessments. The upcoming decentralised ILCD Data Network provides access to ILCD Handbook-consistent and quality-assured Life Cycle Inventory data sets. It is open to all providers globally, such as businesses, national LCA projects, researchers, consultants. The data owners maintain their own data and give access via their own servers, based on their own license conditions. To ease the build-up phase, initially also data that meets only the less demanding 'ILCD Data Network - entry-level requirements' can be published via the network. These data are to be ISO-conform and be properly documented and independently reviewed as well as use the common ILCD elementary flows; the next step is to move to fully consistent data. As a foreseen contribution to the ILCD Data Network the European Reference Life Cycle Database (ELCD) is available, covering core LCI data relevant to the European market.
The ILCD has been developed in coordination by the European Commission's Joint Research Centre (JRC), Institute for Environment and Sustainability (IES) together with Directorate-General Environment and through a series of invited and public consultations with global outreach. This has aimed at best-attainable consensus, reflecting best available practice in industry and government. The invited consultations have involved UNEP, the 27 EU Member States, the national LCA projects of Brazil, China, Japan, Malaysia, Thailand and Japan, consultation with the U.S. EPA, a range of industry associations, as well as representatives of leading life cycle database, software and impact assessment method developers.

SS02-4
Steel industry requirements on the methodological basis for life cycle assessment - the role of the ILCD Handbook and Data Network
C Broadbent
Worldsteel Association, BRUSSELS, Belgium

SS02-5
Plastics industry needs for a common method basis in life cycle assessment for Eco-profiles and Environmental footprinting - the role of the ILCD Handbook and Data Network.
A Schanssema
PlasticsEurope, BRUSSELS, Belgium
In 1990 the predecessor of PlasticsEurope, the Association of Plastics Manufacturers Europe (APME) decided to develop detailed LCI datasets for processes operated by its member companies with the firm intention of making this information available for public use. It currently has about 70 datasets for intermediates, polymers and some specific polymer products freely available.
The datasets are European industry averages and cover the major proportion of the production in Europe, in some cases the sample size is even up to 100%. These cradle-to-gate LCI datasets - which are called eco-profiles - are compiled by independent consultants, and updated when needed. In 2007 the program was extended with a Product Category Rule for Environmental Product Declarations, in total 10 EPD's were developed. In the meantime PlasticsEurope became the data provider for 25 chemical intermediates and polymers to the European LCA Platform operated by the JRC. The aim of the Platform is to provide a pragmatic standardized toolbox for life cycle supported policy making towards sustainability.
The eco-profiles provide data on fuel and raw material consumption, emissions into air and water and waste generation, the EPD's convert these data into impacts in categories such as abiotic depletion of fuel and elements, global warming, acidification, eutrification, ozone depletion and ozone creation potentials.
In 2008 PlasticEurope decided to organize a public consultation on its methodology document. With inputs from academia, LCA practitioners and the JRC a revised document was prepared which actually was a merger of the original methodology document and the PCR. One of the key objectives of updating this methodology was to achieve the next level of quality by making it more transparent and prescriptive, and to make it "consultant proof"in order to allow different qualified external consultants to compile the LCI datasets. One of the points of critique on the eco-profiles is the lack of an external review. Triggered by the ILCD developments of which the PlasticsEurope dataset will become part, an external review will be included and reported.
After having updated 2 eco-profiles using the improved methodology an accelerated program will be developed to update as much as needed the eco-profiles. Details on the program and how the updating is organized will be explained at the symposium.

SS02-6
Environmentally friendly vehicle design throughout the life cycle - expectations on the ILCD Handbook and Data Network
S Krinke
Volkswagen AG, WOLFSBURG, Germany
Life cycle assessment (LCA) is a powerful tool which supports life cycle management. It can be used as an environmental management instrument within the product development.
For successful life cycle management the formal incorporation of life cycle thinking into the company policy is a necessary pre-requisite. Additional success factors which have to be met are the transformation of LCA results into measurable targets for engineers. Based on given environmental targets, such as a certain target value for greenhouse gas emissions, LCA can be used to calculate a specific technical target such as the weight of a component, the fuel consumption of a vehicle or the minimum amount of recycled content in a product. The transformation of pure LCA results into measurable technical targets, which can be understood by engineers, will clearly show the added value which LCA can give in terms of life cycle management.
Even for very complex products with a huge variety of different materials and a complex value chain life cycle assessment can be performed with a reasonable time demand, with good quality and integrated efficiently into business processes.
Essential for Volkswagen is that LCA can be fully integrated into internal processes, that the duration of analysis and resource demand for using the tool is reasonable, and of course that it leads to reliable and stable recommendations. Acceptance by customers, government and other stakeholders is equally important. In this context, Volkswagen welcomes the development of the International Reference Life Cycle Data System (ILCD) Handbook, while we also see the need to further operationalize its use in product and sector specific manuals, to be more time and cost efficient in daily routine work. The related ILCD Data Network is understood to help in future to increase the availability of consistent and quality general background data, where such cannot be obtained through the supply-chain, which is the preferred mechanism for Volkswagen.

SS03 - Marine antifouling - perspectives and recent developments

SS03A-1
Regulatory aspects on antifouling products in the EU.
KA Haglund
Swedish Chemicals Agency, SUNDBYBERG, Sweden
Antifouling products are regulated within the EU through the Biocidal Products Directive 98/8/EC. An overview will be given on how this regulation works and what types of products are regulated or may fall outside the scope of the Directive. The current state of the art regarding the review of new and existing antifouling active substances will be given. The Directive is now more than 10 years old and will be replaced by a new Biocidal Products Regulation. Updated information on the advancements of this process and possible implications on how the regulation of antifouling products may be affected will be given. Some examples of alternative solutions for keeping fouling away will also be presented.

SS03A-2
Recent developments in antifouling paints, the industry perspective

SS03A-3
The next generation antifouling-Selektope®
M Lindblad
I-Tech AB, GOTHENBURG, Sweden
There is a vast range of attempts in finding new antifouling substances with lesser environmental impact. At the same time, the industry demands for sustained antifouling performance are ever increasing in order to minimize fuel consumption and consequently, minimize green house gas emissions. Balancing emissions to the sea and emissions to the air is a delicate optimisation process which involves different aspects where one is marine ecotoxicology. Human risk assessment, regulatory compliance and safe manufacturing are equally important issues. Among those different factors, efficacy are the overall most important factor.
Efficacy can be met by different means, either by killing the organisms or using specific biological cues. Lethal doses are in general high but an alternative is to take advantage of the biology itself which demands a deeper knowledge. This can be used to develop targeted technologies to decrease harmful emissions to the sea without compromising with efficacy.
Selektope® is a new antifouling substance taking advantage of the barnacle biology. Selektope activates specific receptors and interferes with larval behavior at the surface. The larva's attempt to attaching itself to the surface becomes interrupted by Selektope. The effect on the larval behavior is reversible and allow for larval settlement on untreated surfaces.
The specific mode of action allows for low concentration of Selektope. To avoid barnacles, a 0.1% in marine paint is sufficient and should be compared to 3-5% of other organic biocides and 30% or more of cuprous oxide. Further, the specificity is also an advantage in the overall risk assessment regarding regulatory compliance. The different risk assessments will be driven by reversible receptor specific end points. General toxicity appears in concentrations far above the efficacy concentration and is therefore of less concern. Further, it has the advantage of being degradable and demands less resources in a life cycle perspective.
Selektope is now under BPD evaluation. The dossier contains environmental risk assessment, human risk assessment, chemical risk assessment and efficacy evaluation. The risk assessments are based on more than 60 performed studies, both university performed and regulatory compliant studies.
To secure efficacy, paint companies are performing static and dynamic long term efficacy studies. Presently, a number of ocean going vessels have test patches on their hulls containing Selektope.

SS03A-4
Recent developments on the Chinese market

SS03B-1
Ecotoxicology of antifouling biocides
H Blanck
University of Gothenburg, GÖTEBORG, Sweden
Fouling of ship hulls has been a problem to seafarers since Noah´s Ark. The development of TBT-copolymers in the 1960´s stabilized the antifouling market with highly efficacious coatings for both pleasure craft and merchant fleet. The awareness of TBT-related ecological effects particularly on gastropods in the early 80´s caused a deep reconsideration of antifouling strategies. Biocide-free fouling release coatings were developed, while copper came to dominate the biocide-containing paints. A variety of so-called booster biocides, with different efficacy profiles and mechanisms of action, were introduced to be able to formulate completely fouling-protected coatings. In the 2000´s a high number of organic booster biocides were used in Europe, many of them detectable in coastal surveys. Several of them were later questioned from environmental reasons. The balance between efficacy and risk thus remains a key issue.
It is a dilemma that fouling organisms are antifouling targets when occupying a ship hull, but non-target organisms in their natural habitat. Both efficacy and risk is always strongly related to the toxicity of the biocide. In highly efficacious coatings there is a complete toxic effect to all fouling organisms at or near the ship hull surface. To avoid ecological consequences, this toxicity must be lost before it reaches the biological constituents of the aquatic environments. Readily degradable antifouling compounds and a well-balanced, controlled release without overdosing are therefore crucial. In any biocidal paint it is essential that the high toxicity to fouling organisms on the ship hull is not allowed to be expressed also in the ecosystem. A highly relevant biological aspect - development of tolerance - has recently been put into the European Biocide Regulation as an item to consider in approval of biocidal products. Acquired tolerance of the target organisms will require changed paint formulations that increase release and thus loading to the ecosystems. In this way the area and intensity of detrimental effects is increased. In areas polluted with antifouling biocides, tolerant strains or communities are regularly observed, thus indicating an anthropogenic selection pressure on biota.

SS03B-2
Environmental occurrence and fate of antifouling biocides
V Thomas
NIVA, OSLO, Norway
Antifouling biocides are the active ingredients in antifouling paints that prevent the adhesion and growth of organisms to a painted surface. A wide range of chemicals are used as antifouling biocides which have very different physico-chemical properties and therefore differing environmental fates, behaviour and effects. For biocides that have been widely used over a number of decades, for example Irgarol 1051 and diuron, there are a large amount of public domain environmental data, including for their respective metabolites that allow for their environmental safety and potential risk to the environment to be assessed. For other biocides such as dichlofluanid,

DCOIT and zinc/copper pyrithione there is a good understanding of their fate and effects, however few monitoring studies have been performed and not so much is known about the fate and effects of their metabolites. Finally there are new or candidate biocides such as triphenylborane pyridine (TPBP) and medetomidine for which there is very little public domain information. This presentation will provide an overview of the public domain fate and occurrence data available for biocides highlighting some of properties that influence the risks associated with the use of certain biocides.

SS03B-3

Employing classical mixture toxicity concepts for the optimization of biocide combinations for antifouling paints

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The classical mixture toxicity concepts of Concentration Addition (CA) and Independent Action (IA) have been successfully used in the past for modeling the joint action of pesticides, pharmaceuticals and other biologically active compounds. Here we report on their application for the environmental optimization of biocide combinations for antifouling purposes. Both concepts make use of efficacy data of individual compounds and allow the prediction of the total efficacy of all possible mixtures that can be composed from a given set of compounds. This enables a systematic and unbiased in silico exploration of the complete space of possible combinations.
By applying a risk weighting function that accounts for the environmental risks of each individual compound in each mixture it is then possible to rank the mixtures according to their predicted total environmental risk. This provides a detailed map for selecting the most promising combinations for further studies, e.g. for field tests.
The developed algorithms were applied to a set of 7 common and novel antifouling biocides. We experimentally recorded their individual toxicity and efficacy for representatives of the most common fouling organisms (Barnacles, Mussels, Sea Squirt, Bryozoans, Sea Lettuce and Biofilms (slime)) and then modeled the joint efficacy of all possible combinations of the 7 biocides. All combinations with a sufficient efficacy (at least 99% effect on the settling of each test organism) were then ranked according to their modeled environmental risk. We will present the results of this optimization procedure and will relate the efficacy of the modeled mixtures to their environmental risks. In particular we will highlight whether and to what extent combination products can be expected to have lower environmental impacts than products with only one or a limited number of active ingredients, while still providing the same efficacy. The study is part of the Swedish Marine Paint program which is sponsored by Mistra, the Foundation for Strategic Environmental Research.

SS03B-4

Towards novel marine paints with controlled release of biocides - concepts and applications

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The growth of microorganisms, on painted surfaces and other coatings in general and on ship hulls in particular, has increased during the last decades as many anti-growth agents have gradually been prohibited due to toxicity. The current standard way of using biocides in protective coatings against micro-organic surface growth is insufficient as the biocide leaks out from the coating too fast. As most biocides are small molecules, the size results in a fast diffusional leakage and the coating protection is rapidly lost. A new generation of antifouling coatings is therefore needed as most antifouling principles suffer from poor efficacy or pose environmental problems. We investigate a method for prolongation of the coating protection by reducing the release rate of biocides in a controlled manner. This can be achieved by the use of encapsulated biocides in the paint. The biocides are residing in micrometer-sized reservoirs, microspheres, and are subsequently slowly distributed into the coating. The encapsulation procedure results in a high process yield as well as a high loading capacity. So far, uncharged hydrophobic biocides have been successfully encapsulated. Laboratory experiments were designed to provide information of the release rate of various biocides, recorded by various analytical techniques, from painted surfaces in artificial seawater. It was shown that the release is considerably slower from coatings with microspheres compared to an ordinary formulation with freely dispersed biocides. Experimental data were compared to models describing the release by a diffusion mechanism out from spherical particles. The findings have increased our understanding of important parameters in the microsphere design with regard to tuning the release behavior. Further tuning of the release is the focus of two new projects comprising incorporation of nanoclays in the particles and modification of the particle surface using polyelectrolyte layer-by-layer assembly. Considerably comprising field test is due 2010-2011 where we study the effect of fouling on various paint/biocide formulations that have been selected based upon the collective results from the Marine Paint Programme. Microspheres might thus be a beneficial tool for the development of coatings, with a longer protection against biofouling, since they allow for optimization of biocide concentrations. They also increase the lifetime of the protective coating independent on the type of paint system used.

SS04 - Waste treatment in a sustainable world. State-of-the-art and perspectives

SS04-1

Nanomaterials as emerging contaminants at global scale

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Nanotechnology is a major innovative scientific and economic growth area and is fast becoming one of the major areas of chemistry. Its appeal lies in the long-dreamt-of ability to investigate and to manipulate matter at the level of individual atoms and molecules. However, at same time has opened a new window for a wide group of emerging contaminants and a new type of human exposure that needs to be assessed, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics, as well as speculation about various doomsday scenarios. These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted.
In addition, release of manufactured NPs into the aquatic environment is largely an unknown. The surface properties and the very small size of NPs provide surfaces that may bind and transport toxic chemical pollutants, as well as possibly being toxic in their own right by generating reactive radicals.
These questions and the current state of the art on ecotoxicological data will be reviewed together with global applications and industrial development and recent findings for their assessment in the environment. Recent finding in our group for the detection and quantification of fullerenes and dendrimers using analytical methods based on liquid chromatography (LC) coupled to a hybrid triple quadrupole linear ion trap mass spectrometry (QqLIT-MS) for their trace quantification will be exposed. In addition, the results of the investigation of different types of environmental matrices, including air, superficial waters and waste water will be presented. These works constitute the firsts reports on the occurrence of nanoparticles in the environment highlighting the need of nanotechnologies residues assessment for risk evaluation of this materials.

SS04-2

Modelling to assess policy instruments in waste management

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An on-going Swedish research program, Towards Sustainable Waste Management (TOSUWAMA), funded by the Swedish Environmental Protection Agency during 2006-2012, aims at formulating and assessing policy instruments that may contribute to the development of a more sustainable waste management, shifting waste management upwards in the waste hierarchy. The research programme is interdisciplinary and involves the study of many different aspects of waste management: environmental, economic, cultural and psychological. An important part of the assessment is made through soft-linking a set of quantitative models:
- EMEC, a general equilibrium model of the Swedish economy,
- NatWaste, a systems engineering model of the Swedish waste-management system, and
- SWEA, a life cycle assessment model.

This combination of tools is applied to explore how the economy, the waste management sector, and the environment might be affected in the year 2030 by different policy instruments. We use scenario analysis to deal with the large uncertainties inherent in the long-term future: each policy instrument is assessed in five different external scenarios with different assumptions regarding the future development of external aspects of politics, economy, technological development etc. In one scenario, the quantity of Swedish waste continues to rise almost with the economic growth. In other scenarios, the total waste quantity is nearly constant.
An important challenge in this task was to find the appropriate level of detail. The models include municipal as well as most industrial waste flows. We had to make careful decisions on where waste types and economic sectors could and must be aggregated or disaggregated. Another challenge was to link the models in such a way that information generated as output by one tool can easily be used as input by another. This challenge became more severe because the combination of tools has to be flexible enough to be able to assess different types of policy instruments.
We have started assessing economic policy instruments such as a reduction in the VAT of services, tax on waste incineration, tax on raw materials, etc. These all have small effects in the models, compared to the dramatic effects of instruments such as the extended producer responsibility and the landfill bans that are already in place in Sweden.
Further information on our progress and results is posted at the TOSUWAMA website: www.sustainablewaste.info.

SS04-3

A study of WEEE treatment options in Brazil

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Electrical and electronic equipment is one of the most dynamics sectors of the economy, comprising 4.5% of Brazilian gross domestic product. Although business volumes are high, there is no formal specific structure for treatment of waste electrical and electronic equipment (WEEE) in Brazil. A general law on solid waste was approved by Congress and signed by the president on August 2, 2010, but further enabling regulations still need to be issued.
Despite the lack of WEEE treatment, there is the advantage of the inexistence of informal WEEE treatment like in China, India and Africa, due to the higher cost of labor and transaction costs. Nevertheless, other materials are commonly recycled on Brazil, particularly aluminum cans, where the recycling rate was 96% in 2005. This activity developed completely independent of government actions, starting in the early 1990s.
There is a good opportunity to start a formal WEEE treatment structure from the beginning with the support of scavengers and recyclers for improved dismantling to achieve higher recovery of metals and appropriate treatment of hazardous substances.
Nonetheless, the feasibility of such a structure depends on government incentives, through tax advantages and financial support with lower interest rates for investments. Also, the secondary materials market has an important role in the financial equation. This is particularly true in Brazil where primary metals prices are low, since the country is a big global supplier of several metals for many other countries, like China.
Reduction of use is difficult to perceive. Formal recycling will need a strong support from the government along the next years. Reuse and refurbishment of equipments are reputed to be good strategies for WEEE issue considering the decreasing emissions on manufacturing of new equipments, savings of raw primary materials, and social aspects like employment of labor force and digital inclusion of poor communities.
A risk analysis for an after Solid Waste Nationals Politics implementation scenario must evaluate the possibility of contamination of others waste streams since the learning curve of the WEEE system will take some years to reach a good efficiency. The specific regulation of WEEE must establish quantitative and qualitative monitoring of recycling process, control over output secondary material and final disposal of remaining material.

SS04-4

Risk assessment of water effluents in Catalan (NE Spain) waste-water treatment plants based on E-PRTR data

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The Regulation 166/2006 concerning the establishment of a European Pollutant Release and Transfer Register (amending Council Directives 91/689/EEC and 96/61/EC) aims at establishing a Community level register of integrated pollutant release and transfer (known as 'the

European PRTR' or 'E-PRTR'). Its application domain affects certain types of manufacturing and production facilities, among them waste-water treatment plants (WWTPs) with a capacity of more than 100,000 equivalent inhabitants.
Data gathered under the E-PRTR regulation provide a valuable source of information regarding the emission of pollutants to air, water and waste from the assessed installations.
The scenario of the present study covered the 22 WWTP's located in Catalonia (NE Spain) affected by the E-PRTR regulation. 41 micropollutants belonging to different families (heavy metals, anions, volatile organochlorine compounds (VOX), semivolatile organochlorine compounds, volatile aromatic hydrocarbons, policyclic aromatic hydrocarbons, herbicides, endocrine disruptors, phenols and organotins) were determined on water effluent samples (24 h integrated) from the aforementioned plants. Appropriate referenced analytical methods were used to obtain concentration levels of these pollutants during the period 2008 to 2010.
The resulting concentrations were subsequently evaluated using two different risk assessment methods, namely, the COMMPs procedure developed by the Fraunhofer Institut and a method based on fuzzy logic. From the results gathered it has been possible:
(a) To characterize and compare the different sites (WWTPs) according to the associated risk
(b) To prioritize the compounds studied according to their relative contribution to the total risk
(c) To compare the two risk-assessment methods tested

SS04-5
Development and implementation of new techniques for landfill biogas emission assessment
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Assessment of biogas emission from landfills is a relevant issue, both for environmental and economic reasons. In fact methane, a greenhouse gas, is one of the main component of biogas released in the atmosphere from landfills; moreover unexploited methane represents a loss of income for landfill power plants.
In Italy no national/local specific technical rules are in force for landfill gas emission assessment so the procedure recommended by the English Environmental Agency (EA) is commonly applied. This procedure is based on the integration of punctual flux emission measurements from the surface of the landfill.
In this work we propose an alternative method of landfill biogas emission measurement, based on the hypothesis that emitted biogas in stable atmospheric condition (constant wind with velocity lower then a fixed threshold) pertains to an emissive gas plume. The plume may be intercepted by a regular mesh of points located downwind of the landfill, where methane concentration is measured. Afterwards, atmospheric dispersion modelling allow us to estimate the methane emission from the landfill. In order to fulfil the above objective, we handled the following main steps:
a) Identification and set up of methods for measuring biogas concentration;
b) Calculation of total emission by modelling acquired data;
c) Comparison among results obtained applying different measurement methods.
We repeatedly (summer and winter seasons) applied the proposed methods to a set of municipal solid waste (MSW) landfills, either in activity or closed. We estimated methane concentration by means of the Tuneable Diode Laser technique adopted in the USA by the Environmental Protection Agency (EPA), which allowed us to obtain time-series of path-integrated concentration measurements within horizontal and vertical planes located above the landfill surface. We also performed direct sampling of the emissive plume by using a set of bags and remotely controlled air sampler devices, arranged to identify a vertical surface downwind of the landfill. We then measured methane concentration within the bags with a FTIR multigas analyzer.
In order to assess total methane emission, we processed the data obtained by the two above measurement methods by using a numerical code implementing an atmospheric dispersion model. We finally compared these outputs with total emission estimated by applying the EA recommendations as well as with emission estimated by using the BIO5 stechiometric numerical model.

SS05 - LCA network: comparison of experiences

SS05-1
The LCA networks at global level
S Valdivia
UNEP DTIE, PARS, France

SS05-2
IberoAmerican Life Cycle Network: from strategy to action
A Quiros
ECO GLOBAL, SAN JOSE, Costa Rica

SS05-3
The Hungarian LCA network
K Szita Toth
University of Miskolc/Faculty of Economics,, MISKOLC-EGYETEMVÁROS, Hungary

SS05-4
20 years of LCA network activities in Switzerland: The contribution of the LCA forum to the scientific debate
R Frischknecht
ESU-services Ltd., USTER, Switzerland

SS05-5
The Italian LCA network
B Notarnicola[1], FC Cappellaro[2]
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SS05-6
SETAC Networking for Global LCA Science and Practice
BW Vigon
SETAC, PENSACOLA, United States of America
For more than 20 years the Society of Environmental Toxicology and Chemistry (SETAC) has supported the global development and helped advance the scientific underpinnings of LCA. This support occurs through a variety of internal and external networking activities. Among these efforts are internal ones to SETAC where Advisory Groups on LCA at the European and North American levels provide a forum for discussion of critical issues and connection to external activities, such as the UNEP-SETAC Life Cycle Initiative. SETAC provides a scientific component to the Initiative, which it co-founded, and acts as an ISO TC 207 liaison on LCA work items. Recently, the Advisory Group network has been expanded to a global level.
Additionally, on the external side, SETAC has fostered networking globally via strong LCA programs at annual meetings, publication of scientific journal articles and books on LCA, and hosting/organizing of workshops on LCA topics. Four intensive, week-long workshops with global participation, employing a format and process developed by SETAC, have been held to advance the science of LCA. Most recently, one such workshop was held in Japan, under the auspices of the Life Cycle Initiative, to develop guidance principles on LCA datasets and databases. Other networking occurs through international programs, such as the Strategic Approach to International Chemicals Management (SAICM) and the UNEP Mercury Partnership.
This presentation will highlight SETAC's networking efforts on behalf of global LCA science with particular attention paid to the recent Pellston workshop and its forthcoming publication.

SS06 - Emerging exposure science for developing chemical regulatory policy: REACh, biocides, TSCA reform

SS06A-1
A Crosswalk of the Exposure Science Requirements of REACH, TSCA, CEPA and Biocides Legislation
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ICF International, FAIRFAX, United States of America
Thousands of legacy chemicals that have not undergone human and environmental risk assessments exist internationally. Under the United Nations Environment Programme (UNEP) Strategic Approach to International Chemicals Management, legislative bodies worldwide have committed to managing chemical production so that adverse impacts on the environment and human health are minimized. Multiple legislations exist to address domestic or regional chemical management programs, and these statutes also support UNEP objectives. While the objectives of these statutes are comparable, the specific scientific requirements for exposure and risk assessment differ. In order to comply with multiple legislations, scientists and decision makers must understand the differing requirements. There are thousands of pages of guidance to describe the legislative mandates, but navigating the guidance documents is an overwhelming task. The goal of this presentation is to provide an overview of the exposure science requirements of several international legislations, and in so doing to provide context for the regulatory panel discussion and the technical presentations in the Special Session on "Emerging Exposure Science For Developing Chemical Regulatory Policy."This presentation will focus on the scientific requirements of the following legislations: the EU Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) legislation, the US Toxic Substances Control Act (TSCA), the Canadian Environmental Protection Act 1999 (CEPA 1999), and the EU Biocidal Product Directive (BPD), as well as similar biocidal legislation in North America.

SS06A-2
Canada's Experience from Assessment of High Priorities under its Chemicals Management Plan
CA Norman
Health Canada, OTTAWA, Canada
Assessing risk as part of Canada's global commitment to address legacy chemicals by 2020 is implemented through the Chemicals Management Plan. In 2006, Canada categorized approximately 23,000 existing commercial substances through a priority-setting exercise using available data, QSAR modelling, and simple tools that ranked substances based on a number of parameters including their potential for human exposure and persistence and bioaccumulation potential. Since 2007, Health Canada and Environment Canada have addressed the highest priorities from this exercise by jointly conducting screening assessments on approximately 200 substances as part of its 'Challenge' initiative. Experiences from this exercise and opportunities for increased collaboration moving forward will be the focus of the presentation.

SS06A-3
Panel Discussion on Key Exposure Science Needs - A Regulatory Perspective
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Exposure science is a key element in regulatory decision-making. In an interactive panel format, European and North American regulators will share their views on key exposure science needs for human and environmental exposure characterization moving forward within their legislative mandates. Panel members will discuss barriers to addressing these needs, as well as opportunities moving forward. The focus of the discussion will be on exposure science needs related to the following broad themes: access to structured and harmonized supply chain Information; exposure-based priority setting and screening; advancing predictive approaches; data generation and synthesis; informing risk management; and beyond a chemical-by-chemical approach.

SS06B-1
Precision versus practicality; the derivation, construction and experience of the ECETOC TRA human exposure tools
CD Money
ExxonMobil, DIEGEM, Belgium
The ECETOC Targeted Risk Assessment (TRA) model was originally launched in 2003 and a revised version of the TRA was made available in 2009. The TRA facilitates tier-1 risk assessments and allows discriminating between straightforward assessments, which can be successfully resolved by applying the TRA, and those assessments which need a more involved approach. In addition, the TRA produces an output which lends itself to standardized communication. With

these features, the TRA addresses the basic REACH needs for generating safety assessments for all dangerous substances and for communicating the assessment results in the supply chain. It is this improved version that has been endorsed within the REACH Technical Guidance for human health exposure assessment and Exposure Scenario development. The TRA has been applied in the vast majority of the 3400 Phase 1 (2010) REACH substance registrations where exposure assessments were required. As a consequence, ECETOC has been actively soliciting feedback from users of the TRA in order to better understand where improvement opportunities exist (whether for workers, consumers or the environment). No fundamental issues with the core structure of the TRA have been reported. But it is clear that further improvements can be implemented to extend the domain of the tool; the accuracy of exposure predictions; and its overall utility when viewed in the context of its workflows and industry IT platforms. It is also apparent that a significant minority of users have failed to follow some of the key recommendations for its reliable application. This highlights the importance of understanding, defining and refining applicability domains in order that tools such as the TRA can continue to be reliably and usefully applied in the process of chemicals risk assessment.

SS06B-2

Aggregate consumer exposure assessment

N von Goetz
ETH Zurich, ZURICH, Switzerland

Many chemicals that are being used in consumer products are contained in tiny amounts in the respective product. Exposure to the substance from one single product may therefore be insignificant from a risk perspective, while exposure to the same substance from different products can be high enough to pose a risk. Examples are important ingredients of personal care products (PCPs) like stabilizers, preservatives, and UV-filters. These are contained in different PCPs and cosmetics that are used at the same time. Also some high production volume substances like Bisphenol A (BPA) and the flame retardant polybrominated diphenylethers (PBDEs) are found in a large number of products that a consumer comes in close contact with. Examples are epoxy resins in food cans that release BPA into food and PBDE-doped upholstery, electronic equipment, carpets etc. Consequently, in order to obtain an estimate for the overall exposure many single-source exposures have to be added up. The technical term for this overall exposure is the total exposure, which is used when all sources that are currently known are taken into account in the assessment. If only one sector of products is accounted for, the technical term aggregate exposure is used for the sum of single-source exposure.

The main difficulties in assessing these aggregate (or total) exposures are the lack of data on substance sources (e.g. amount of substance included in a product) and the lack of data on consumer use of substances, and here mainly co-use data.

In order to illustrate the challenges of aggregate exposure assessment the key parameters in three exemplary studies on Bisphenol A, Nanoparticles and PBDEs are discussed. As key parameters those parameters were identified that introduce the largest known uncertainty: For Bisphenol A the intake of canned food contributes most to uncertainty, whereas for nanoparticles the lack of co-use data is critical for the assessment. For PBDEs the largest uncertainty is connected to PBDE concentrations in dust and dust intake rates.

Approximations for uncertain or lacking data will be discussed along with the consequences of their use.

SS06B-3

Incorporating Population-Specific Diets and Activity Profiles into Aggregate and Cumulative Exposure Assessments: A Review of Capabilities and Shortcomings

CF Chaisson, CA Franklin
The LifeLine Group, ANNANDALE, United States of America

Starting with a review of the existing and emerging regulatory mandates of Europe and North America, we will present a listing of needed exposure assessment capabilities juxtaposed to capabilities offered by contemporary models and possible sources of information needed for population-specific exposure assessment. Models designed to utilize atypical formats of dietary and activity profile information will be highlighted to illustrate the capacity and relevance to aggregated risk assessment for consumer products using population-specific information. The LifeLine Dietary Record Generator and Activity Profile Generator and will be used to illustrate the structured approach and mathematical processes involved. While these kinds of models provide significant advantages to capture valuable exposure-related information for any population group, regulators and assessors must understand their inherent limitations and know how to ground-truth their predictions. Accurate exposure assessments also require relevance to changes in product use profiles as the seasons change, or in variation across populations because of economics, ethnic traditions, gender or age preferences and because of health related influences on diet and activity and product use. We will demonstrate approaches for amending older existing national survey data using contemporary market information about the products used by the populations in those surveys. Such approaches combine multiple sources of information to extend the shelf life of expensive databases and improve the accuracy and relevance of resulting exposure assessments. Examples will show how information was collected from local and publicly available sources, and incorporated into the exposure assessment. Issues emerge about data quality, qualifications of "experts"at the local level, anticipating product competition and associative use of multiple products.

SS06B-4

Setting the scene: Is mixtures risk assessment necessary and feasible?

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University of London, LONDON, United Kingdom

There is currently considerable interest in considering whether chemicals risk assessment with its traditional focus on chemical-by-chemical evaluations should be expanded to take account of mixture effects. During the last 10 years, research on mixtures has made significant advances, especially in human and mammalian toxicology. This presentation will give a summary of the scientific state of the art of mixture toxicology and will reflect on issues of regulatory relevance for both environmental and human risk assessment. The traditional focus on chemical-by-chemical assessments could be justified, if in typically encountered exposure scenarios only one chemical was toxic, or if the joint action of several chemicals was typically not larger than the most toxic agent in the mixture. However, evidence from experimental studies shows that this is not normally the case. Similarly, there would be no need to take account of mixture effects if there was always sufficient protection when all chemicals are present below their threshold doses. Examples from the literature will be presented which show that combination effects can occur at low doses. This will be followed by a discussion of the general principles behind such phenomena. Experimental mixture studies in ecotoxicology and human toxicology demonstrate that the concepts of dose addition and independent action provide good approximations of observed combination effects. Significant deviations from expected additive effects have only rarely been observed, and when they occurred, the deviations were not large. This argues for using these concepts in mixtures risk assessment. Case studies where mixtures risk assessment has been used in practice will be critically discussed.

The talk will end by highlighting two areas important for better mixtures risk assessment: Firstly, scientifically sound criteria for the grouping of chemicals for cumulative risk assessment are needed. The discussion currently focuses on using toxicological similarity as a grouping criterion, but this may not be decisive in all cases.

Secondly, mixtures risk assessment will have to rely on sound information about cumulative exposures, but our information about combined exposures is fragmentary at best. **Acknowledgement** - The authors thank the Europesn Commission for financial support (study contract 070307/2007/485103/ETU/D.1)

SS06C-1

Challenges ahead in mixtures and cumulative environmental assessments

TP Traas, DTHM Sijm, CWM Bodar
RIVM, BILTHOVEN, The Netherlands

The debate on how to deal with mixtures in risk assessment has been refuelled. This presentation will give an overview of the problems, methods and way forward for environmental risk assessment of mixtures in some current legislative frameworks.

Several studies with mixtures of chemicals with similar and dissimilar modes of action have confirmed that concentration addition (CA) is a conservative estimator of the toxicological effect of mixtures. Due to this - in most cases relatively modest -overprediction, CA be used in risk assessment as the default approach.

Several approaches can be used to express the overall toxicity of the mixture; as toxic units, TEQ values as the sum of risk quotients, or as the fraction of species in the ecosystem that is potentially affected due to the mixture.

Exposure to mixtures of substances presents classical challenges but quite different issues may need to be tackled. Is it an interest in exposure to toxicants not known a priori, or limited to those toxicants known from monitoring or a specific class or category of chemicals? Both monitoring studies and modelling studies have shown that generally, toxicity of mixtures in the field is dominated by a fairly limited number of substances. Even though such findings are encourageing, it is still necessary to characterise these main toxic components by chemical analysis, possibly in combination with smart screening tools for specific modes of action (TIE).

It is difficult to adapt risk assessment procedures in current EU legislation in such a way that one approach fits all problems. As an example, a recent study on mixture toxicity of Dutch surface waters has shown that a few individual substances dominate toxicity at many sites. In such cases, better control and stricter quality objectives can dramatically improve overall water quality. More specifically, risks of mixtures of specific substances (e.g. EDC) can be of interest. In a specific project on estrogenic activity of surface waters in the Netherlands (LOES a few specific hot spots were found but some estrogenic activity could generally be detected at most sampling sites. In this case, targeted measures can be taken at hot spots, reduce the load of e.g. synthetic hormones or adapt water treatment schemes.

Both REACH and the Water Framework Directive offer possibilities to regulate individual chemicals or groups of chemicals that have been identified in mixture toxicity studies.

SS06C-2

Models in environmental chemistry education, science and decision-making

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The last decades have seen a revolution in the development and application of mathematical models as tools for studying environmental and human health problems, and for developing regulatory strategies to address those problems. Models are now indispensible in the daily work of many educators, scientists and decision-makers in the field of environmental chemistry. When faced with a new problem, scientists and policy-makers can often choose from a variety of models with different levels of detail, and differing levels of fidelity to the real system of interest, or they may contemplate developing a new model to address their particular challenge. In this presentation we examine the motivations for developing and applying quantitative models to describe environmental systems, and confront the inherent limitations of such models. Based on this general discussion, we identity the roles that models play in modern education, science and decision-making in environmental chemistry and exposure assessment. Some advantageous uses of models in these applications will be illustrated with case studies from our own experience.

SS06C-3

Regulatory Environmental Exposure Assessments: General Approaches and Use of Market Research Data to Improve Emission Estimates

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This presentation provides an introduction to the methodologies used for environmental exposure estimation as part of the regulatory risk assessment process performed for biocides (under 98/8/EEC) and general chemicals (under REACH). The first-tier emission estimation frameworks used for general chemicals and biocides necessarily include conservative default assumptions. In cases where more specific information is available, it may be justified to refine some of these assumptions. An example is the "10 % rule"which is typically applied to tonnage-based assessments for wide dispersive/consumer uses of substances. By linking population density and country-specific usage statistics it is possible to investigate the validity of this assumption. The results of such an analysis performed for home care products suggest that a refined figure of 4% of EU tonnage used in a hypothetical region (200 [GREEKX] 200 km) region can be justified. A similar analysis performed for consumer uses of insecticide products also indicates that the "10% rule"represents a worst-case for a number of product categories. Thus, it is demonstrated that considerable scope exists for refining emission estimates for some chemical uses.

SS06D-1

Linkages between Human Health and Environmental Assessment

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This presentation will explore similarities in the challenges inherent to human health and

environmental exposure and risk assessments, and how best practices can be applied across the disciplines to increase confidence. All exposure assessments share the same basic goal, to estimate whether a given receptor can be exposed at a high enough dose, for a long enough time, to potentially cause adverse effects. There are, however, major differences that derive in part from differences in risk assessment objectives. Human health assessments aim to protect the individual and usually consider a range of routes and durations of exposure, while environmental assessments generally aim to protect at population, habitat or ecosystem levels of organization and have historically been largely focussed on acute toxicity. The high diversity of ecological assessments, and the wider range of protection goals, means that environmental assessment frameworks are generally less standardised, and in some cases less developed, than those for human health. While both fields are developing approaches to better consider aggregate effects across exposure routes, and cumulative effects across chemical classes, this area is currently more advanced for human risk assessments. Confidence in any assessment depends on the quality of the data available and the scientific strength of the assumptions that underlie the assessment model. Reliance on surrogate data and broad extrapolation of data results in the need to address not only the variability inherent to a situation of interest, but uncertainties in how well the measured or monitored data simulates the "real"situation. Adequate characterisation of variability and uncertainty, and the biases they may introduce, is critical. This presentation will illustrate these challenges, using examples drawn from the assessment of human and environmental exposure to pest control products. The relative utility of simple deterministic and more complex stochastic models will be explored, along with opportunities to better integrate and harmonise research and monitoring resources and model development and validation across the disciplines.

SS06D-2

Thresholds of Toxicological Concern

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Conceived over 40 years ago as a "common sense"approach, the Threshold of Toxicological Concern (TTC) is a useful concept that is becoming of increasing interest as an addition to the arsenal of tools used for characterising the toxicological and environmental risks from low level exposure to chemicals. Initially used by the USA FDA for low levels of indirect additives in foods (packaging migrants), the TTC has subsequently found uses in safety evaluation of flavours, genotoxic contaminants in drug products and contaminants in foods. In these cases the TTC obviates the need for toxicological testing of chemicals where human exposure via the oral route is low. A more recent proposal has been made to extend its use to human exposure via the dermal route (i.e. from low levels of ingredients in cosmetic and personal care products) and from the inhalation route. For these latter two exposure routes, both local effects and systemic effects are taken into consideration. In all cases, threshold levels have been established by using a probabilistic analysis of existing toxicological data, from which it can be concluded that the risk of adverse effects from human exposure to levels of an untested chemical at or below the TTC is minimal. Further proposals have been made to extend the use of the TTC in ensuring environmental protection. These include use for organic chemicals in fresh water systems based on mode of action (Exposure Thresholds of No Concern, or ETNC), and an examination of the feasibility of using a TTC for endocrine active substances in the aquatic environment. Use of the TTC principle may preclude the need for additional toxicity or environmental testing in cases where exposure is sufficiently low. The TTC concept benefits consumers, industry, and regulators, by avoiding unnecessary extensive testing and safety evaluations, thus allowing limited resources of time, animal use, cost, and expertise to be devoted to the testing and evaluation of those substances with greater potential to pose risks to human health or the environment.

SS06D-3

Criteria for Exposure Based Waiving under REACH

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Within the REACH framework, but also within OECD, there is understanding that for reasons of animal welfare, costs and logistics, it is important to limit the number of tests to be conducted. Exposure based waiving (EBW) is a potentially important element in testing strategies. This presentation reflects on criteria for EBW as foreseen in the REACH regulation and proposes a strategy for its implementation. The emphasis will be on the evaluation of exposure assessment models with regard to their suitability for EBW-decisions under REACH. We have developed such decision trees for environmental and human exposure. Human exposure includes occupational exposure, consumer exposure and human exposure via the environment.
The principle behind any EBW is that there are situations when human or environmental exposures are so low or infrequent that there is a very low probability that the acquisition of additional effects information may lead to an improvement in the ability to manage risk. EBW therefore is risk based and needs thorough knowledge on exposure as well as on effects criteria. Both elements are discussed: exposure models are analyzed and the uncertainty in their predictions discussed as well as no-effect criteria. The justification for EBW can be based on qualitative or quantitative argumentations, or on a combination of both. Quantitative justification for EBW needs an assessment proving that exposure is below a 'No-Further-Action Level' (NFAL) such as a PNEC (Predicted No-Effect Concentration), DNEL (Derived No-Effect Level), DMEL (Derived Minimal-Effect Level) or TTC (Threshold of Toxicological Concern). Examples are provided to illustrated the EBW-methods proposed.

SS06E-1

Linking sources with outcomes: The science of stressors and receptors

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Among the issues that create demand for exposure science are (a) the need for identifying and classifying exposures in health surveillance studies, (b) the need in fields such as life-cycle assessment and green chemistry to anticipate exposures from new and existing chemicals in order rank and evaluate products and chemicals, (c) addressing climate change and its impacts on humans and ecosystems, (d) regulating new and existing substances, and (e) the need of industry to provide sustainable products and services. This presentation addresses the factors that create a growing demand for exposure information, the tools we have to address these demands, and the opportunities for expanding these tools and for building new science and new technologies to address the demands for exposure information. To achieve this I will begin with defining and illustrating the core elements of an exposure science "' stressors, receptors, outcomes, and the spatial/temporal contact between stressors and targets the results in outcomes. We will then identify the nature and extent of the demands for exposure science in a number of research efforts. We will consider how activities such as environmental chemistry, biomonitoring, pharmacokinetics, activity tracking, and modeling are addressing these demands. Finally we will consider how these efforts can be expanded and better integrated to improve the relevance and reliability of exposure science.

SS06E-2

From globalization to molecular biology: Extending exposure science across all levels of biological organization for sustainable environmental health policy

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The start of the 21st Century has seen increasing trends toward globalization and continuing advances in molecular biology. These two seemingly disparate drivers provide the imperative for extending consideration of exposure across multiple scales to facilitate systems based analysis for holistic environmental and public health policy design. As understanding of how to assess and mitigate frank impacts from simple exposures to known pollutants improves, scientists are moving toward a broad view of environment, holistic consideration of human-environment interaction, and characterization of biological complexity to understand influence of these on human health. Operationalizing sustainability concepts to address chemicals management will be facilitated by application of approaches such as those used by ecologists to study resilience of linked social-ecological systems. Consideration of the amount of change a cell, organism, community, or social-ecological system can undergo while still retaining important controls on function and structure could provide a holistic approach for assessing cumulative risks, particularly to vulnerable groups. More globally, application of this type of systems-based approach has the potential to improve our ability to predict and minimize unintended consequences of environmental health decisions.

SS06E-3

Exposure Biology and the Exposome: a Health-Based Paradigm for Exposure Science

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Exposure biology is a term that encompasses connections between environmental factors and their interactions with genetic and epigenetic states. Early exposure scientists applied principles of exposure biology in occupational studies to advance our understanding of the toxicodynamics of fibrogenic dusts, respiratory irritants, heavy metals, and some systemic toxicants such as benzene. With the birth of the U.S. EPA, exposure scientists largely abandoned exposure biology in favor of methods to predict air and water concentrations of regulatory pollutants. Such emphasis on air and water pollutants ignores toxic chemicals, arising from the diet and endogenous processes such as inflammation, which are probably much more important contributors to heart disease, diabetes and cancer. Thus, by abandoning exposure biology, exposure science has become only marginally relevant to our understanding of the etiologies of modern chronic diseases. The concept of the exposome, representing all exposures received by a person from both external and internal sources, motivates a holistic view of biologically active chemicals in an individual's internal chemical environment. To characterize the exposome it makes sense to use a top-down approach based upon biomonitoring rather than a bottom-up approach limited to external inputs from air, water and food. Because the exposome varies in space and time, initial investigations could use archived blood and 'omics' techniques to measure important classes of toxic chemicals - including reactive electrophiles, metals, metabolic products, hormone-like substances, and persistent organic compounds - in prospective conort studies. These twin concepts of exposure biology and the exposome offer health scientists an avenue for conducting environment-wide association studies (EWAS) to complement genome-wide association studies (GWAS) that are now commonplace. Indeed, designs which interleaf EWAS with GWAS, can pinpoint key exposures responsible for chronic diseases as well as important gene-environment interactions. Such investigations would also generate vast data resources that can be exploited by systems biologists to enrich our understanding of disease processes and mechanisms. Exposure scientists can play a key role in this exciting research if they adopt a health-based paradigm which embraces exposure biology and the exposome.

SS06F-1

Using pharmacokinetic approaches based on animal and human datasets to track and allocate cumulative exposures and aggregate health impacts

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Multi-chemical exposure is the rule rather than an exception in both the general and the occupational environment. Simultaneous or sequential exposure to multiple chemicals may cause alterations in the pharmacokinetics (PK) and/or pharmacodynamics (PD) of the individual chemicals, resulting in a change in the toxicity predicted based on the summation of the effects of the components. PK interactions occur as a result of one chemical altering the absorption, metabolism, distribution, and/or excretion of other chemical(s). Such interactions often result in a modification of the internal or target dose of one component of a mixture by another component. In such cases, conducting quantitative risk assessment for chemicals present as a mixture is difficult. The uncertainties that arise from changes in the PK of the components can be addressed by developing physiologically based descriptions of the disposition of chemical mixtures that can be used for dose, route, and interspecies extrapolations of the target tissue concentration of the toxic moieties. Further, biologically based models for mechanisms of action and tissue response can be developed to integrate the PD interactions of the chemicals into the predictive modeling framework. The parameters for the PK and PD models can be determined from in vitro experiments using human cells or tissues. Such a quantitative mechanistic approach to the study of chemical interactions is imperative to achieve the ultimate goal of assessing the health risks associated with human exposure to complex chemical mixtures.

SS06F-2

Mobility-based exposure assessment

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People's daily mobility - for activities such as work, shopping, and recreation - brings them in contact with air pollution concentrations that may differ from the concentration at their home location. However, many population exposure assessments are based on census data, which are implicitly home-based.
This presentation compares existing and potential future methods for accounting for mobility in

exposure assessment, and also will discuss when mobility is, or is not, an important component of exposure assessment. These issues are intimately connected with the degree of temporal and spatial variabilty in pollutant concentrations (and people's locations).

SS06F-3

Using exposure models to identify data gaps and develop knowledge infrastructure

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In recent decades regulations and methods have been developed to evaluate tens of thousands of chemicals; however, there are few measured data available for the legislated assessments. In this context, conceptual models are needed for screening and priority setting and mathematical models (mass balance and QSARs) are required to generate data. This presentation illustrates how mass balance exposure models can be used to screen and prioritize chemicals for more comprehensive assessments. The integrated, "holistic"framework exploits sensitivity and uncertainty analysis to identify and prioritize data gaps and to build knowledge infrastrucure (databases and QSARs). Mass balance exposure model hypotheses (i.e., predicted concentrations) can be evaluated ("ground truthed") with monitoring data following the incremental stages from emisisons to concentrations in the environment, to food sources and to humans. During this evolutionary process (model development, application, evaluation, refinement) there is a need to balance data availablility (uncertainty) and regulatory needs (timeframes). Various exposure and risk assessment frameworks can be established with different objectives and levels of model complexity. The general approach outlined here can be used to identify data gaps and build databases and predictive tools to reduce uncertainty in exposure and risk assessments and regulatory decision-making.

Platform abstracts

CS01 - Climate changes, biological invasions and pollution

CS01A-1

Effects of warming and cadmium on the feeding behaviour and growth of the aquatic invertebrate shredder Limnephilus sp.

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Metal contamination is still an environmental problem and can affect several species of invertebrates at environmentally realistic concentrations. Invertebrate shredders play an important role in plant litter breakdown, since they participate in the fragmentation of plant material and decomposition of coarse particulate organic matter. The Intergovernmental Panel on Climate Change predicts an increase in water temperature that may affect invertebrates, causing faster growth rates, shorter developmental time and smaller size at maturity and reduce the ability of invertebrate survival on poor nutrient diets. It is probable that the combined effect of metals and increased water temperature may have strong impacts on the processes in which invertebrate shredders are involved, further compromising the functioning of freshwater ecosystems. Therefore, we tested how leaf consumption by invertebrate shredders and their growth are affected by cadmium and whether increasing temperature modulates this relationship. A common species of invertebrate shredder was collected from an unpolluted stream and acclimated to the laboratory. In one experiment, the animals were allowed to feed on alder leaves, while exposed to increased Cd concentrations (3 levels, ≤ 10 mg/L) and to a temperature typically found in streams in autumn (15ºC) and to 21ºC to simulate a warming scenario. In another experiment, the animals were kept under starvation for 4 days while exposed to cadmium (10 levels, ≤ 35 mg/L) and then were released from the stressor and allowed to feed on alder leaves. Cadmium content on leaves and animals was analyzed by inductively coupled plasma-atomic emission spectrometry. The exposure to Cd decreased the consumption rate by shredders but not its growth rate. We also observed an increased invertebrate feeding activity at higher temperature. Cadmium accumulated more in the leaves than in the animals. In conclusion, results indicate that the increase in Cd concentration and an increase in temperature of 6ºC affected the feeding behavior and growth performance of the invertebrate shredders Limnephilus sp.. This may compromise at longer times the survival of sensitive shredder populations with direct impacts to plant litter decomposition and nutrient cycling in freshwater ecosystems.

CS01A-2

The effect of temperature on the toxicity of cadmium towards Caenorhabditis elegans

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Temperature has in the past few decades been shown to be an important factor in ecotoxicology. As both biological and chemical reactions are determined by the ambient temperature it follows that many processes involved in the toxicological profile of a chemical, such as biological degradation rates, growth rates and general metabolic rates, as well as abiotic degradation, absorption and diffusion rates, all are temperature dependant. It is therefore expected that the toxicity of a compound will also change with temperature. In this study we determined the effect of different ambient temperatures on the reproduction, lifespan and intrinsic population growth rate of nematode *Caenorhabditis elegans* exposed to cadmium, in an agar based test system. The total reproduction and lifespans of *C. elegans* were very similar for all cadmium treatments, so these endpoints on their own would suggest that there was no significant difference between the toxic effects of the concentrations used. Fertility, however, expressed as "Time to first egg", was a most sensitive endpoint. Hence, when all endpoints were combined in a three component population growth model the effect of the individual cadmium doses showed that the high doses have detrimental effects at high temperatures, while population growth can still take place at the low temperatures. This demonstrates the importance of evaluating the toxic effect of chemicals on population levels rather than on single endpoints determined from individuals.

CS01A-3

Low temperatures enhance the chronic toxicity of Cd and Cu through different mechanisms

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The effect of cadmium and copper on the reproduction of the enchytraeid worm Enchytraeus crypticus was investigated at temperatures representative of different climatic region of temperate areas. The aim of this study was to investigate the influence of temperature on metal toxicity. The effect of both metals was investigated at six different temperatures (11°C, 13°C, 15°C, 18°C, 21°C and 25°C). As differences in metabolic rate at different temperatures would likely result in different uptake and excretion kinetics of the metals, exposure durations were varied and experiments terminated when there was 100-200 juveniles in the control containers. EC10 and EC50 values for effects on reproduction were obtained. The EC50 values for both cadmium and copper toxicity towards reproduction declined in a linear pattern with temperature. Going from 25°C to 11°C this resulted in a two-fold decrease of EC50 for Cd and a three-fold decrease for the EC50 of Cu. Internal concentrations of Cd and Cu in worms at 11, 18 and 25 °C were measured over time. These results showed that tissue-Cd accumulated faster and to higher levels at 25°C compared to 18 and 11°C. However, in the Cu treated worms, tissue concentrations were lower at high temperatures. This indicates that the mechanisms behind the temperature induced changes in toxicity of the two metals are different. Possible causes behind these differences will be discussed.

CS01A-4

Ecotoxicity under impact of climate change: Temperature modulates toxicity of the fungicide pyrimethanil

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Mean temperatures will increase by 2.5°C in spring and summer till 2080 in Hesse (Germany). Additional climate change effects will result in generally warmer springs and moister summers. These altered climatic conditions may lead to higher agricultural consumption of pesticides, particularly of fungicides. The run-off of these pesticides into nearby water bodies will become enforced by more frequent extreme rain events. The aim of the study was to investigate if the ecotoxicological impact of the fungicide pyrimethanil on aquatic key species is altered under future temperature scenarios.

We examined the model substance pyrimethanil (fungicide) in standard ecotoxicological test designs with aquatic primary producers and invertebrates. Among 5 tested model species, *Daphnia magna* was the most sensitive organism. In the following we tested the response of *D. magna* to low doses of pyrimethanil in a multigenerational experiment simulating dynamic temperature regimes in Hesse´s waters today and in future ("cold year today", "warm year today", "warm year 2050 - 2080").

Most juveniles were detected under warm temperature conditions expected for 2050-2080. Surprisingly offspring was not positively correlated with rising temperature in "warm year"simulations. Instead we observed reproductive minima and maxima over experimental time course, just offspring under "cold year"conditions followed temperature gradient. Additional effects of low dose of pyrimethanil were often masked by temperature and only observed in generations being in an optimal reproductive state.

With increasing temperatures in the future the effects of the pesticide may occur at lower concentrations than today. Furthermore the result of an enhanced reproduction rate in the future temperature scenario indicates increased zooplankton abundances due to warmer springs. In consequence, this may also affect the phytoplankton as a result of enhanced feeding activity of the zooplankton. Finally the timing and duration of clear water phases in lentic systems may be altered.

Multigeneration experiments under predicted climate scenarios are a useful tool to assess the impact of currently used pesticides under future climate change conditions. The results of the study indicate that xenobiotics may have a stronger effect on the biocoenotic structure and finally on the productivity of limnic ecosystems in the future with the expected temperature increase.

CS01A-5

Combined effects of soil moisture and a fungicide on soil organisms - a study with Terrestrial Model Ecosystems -

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1. Introduction

The German Federal Environment Agency is expecting a decrease of summer precipitation of circa 16-19% in 2071-2100 and an increase by 10-20% in winter in comparison to the reference period of 1961-1990 for Hesse/Germany. The Intergovernmental Panel on Climate Change (IPCC) predicts substantial increase in the intensity of daily precipitation events in the Mediterranean region, while mean precipitation may decrease by 30-45%. Changes in precipitation may lead to changes in soil moisture content. When exposed to this climate-related stressor organisms may react differently towards chemicals (e.g. pesticides).

2. Materials and methods

Two studies were conducted. One took place in Portugal, the second in Germany. In both cases, the terrestrial model ecosystems (TMEs) consisted of intact, undisturbed soil cores. In Germany, the fungicide pyrimethanil was investigated at 11 concentration levels, each at three different moisture regimes in two replicates. In the Portuguese TME study the same endpoints were investigated, but with a Mediterranean soil and its community. The design differed from the German study, with only two fungicide concentration levels but four replicates per treatment. Two and eight weeks after application of the fungicide the sampling was conducted.

In this contribution, the effects of three moisture levels and different concentrations of pyrimethanil on the abundance and diversity of enchytraeids as well as the feeding activity of the soil organisms and their vertical distribution will be presented.

3. Results and discussion: Enchytraeids and feeding activity

Both study sites can therefore be considered as "rich"in enchytraeids. In Germany, the abundance of the enchytraeids was significantly affected by the fungicide as well as by soil moisture. Feeding activity differed between the nominal moisture levels and showed an opposed effect in depth profile. At high moisture levels the feeding activity had its highest values in the top 4 cm, while in dry soil the activity is higher in deeper layers.

4. Conclusions

For the abundance of the enchytraeids no interaction between soil moisture and pyrimethanil could be detected. In contrast, feeding-activity, responded to the interaction of climate and chemical stressor, at least at nominal values. Further statistical evaluation of actual moisture contents, taxon-specific abundance and analytical measurements of the exposure concentrations are in progress.

CS01A-6

Antioxidant defenses and oxidative damage in amphipods under multiple stressors

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In estuarine systems, bottom hypoxia and increasing pollution load are the main stress factors causing alterations in benthic communities, yet little is known about their combined effects on estuarine organisms. We tested the individual and combined effects of exposure to hypoxia and to polluted sediment at environmentally realistic concentrations on the Baltic amphipod Monoporeia affinis using a suite of biomarkers.

In the first stage, amphipod responses to different combinations of oxygen levels (hypoxia vs. normoxia) and contaminant (polluted vs. unpolluted sediment) were determined using antioxidant enzyme activities (SOD, CAT, and GST), lipid peroxidation status (TBARS), protein carbonyl content (PCC), and DNA integrity (DNA-SB), and acetylcholinesterase (AChE) activity. There were significant increases in CAT and SOD activities and TBARS levels in response to both moderate hypoxia and contaminated sediment, while GST increased and AChE decreased in response to the contaminants only. Significant positive correlations were observed among the antioxidant enzymes and between the enzyme activities and TBARS concentration, suggesting a complex response to the oxidative stress. Furthermore, the negative effect of hypoxia on DNA integrity was significant; with frequency of DNA-SB increasing in animals exposed to hypoxia in contaminated sediment. Stepwise discriminant analysis allowed 75-100% correct discrimination of amphipods according to their treatment group; this grouping aids in stress assessment in field-sampled animals as well as understanding general patterns in the data that may guide future research.

In the second stage, we hypothesized that amphipods exposed to naturally occurring fluctuating

hypoxia may develop adaptations to survive and recover upon reoxygenation, whereas exposure to contaminants would exacerbate oxidative stress in hypoxia-challenged animals and compromise the recovery. This hypothesis was tested using the most informative biomarkes identified in the first stage. The results support the hypothesized potential of xenobiotics to hamper ability of benthic animals to cope with changes in oxygen regime, such as fluctuating hypoxia and migrations from hypoxic to oxygenated environments. Thus, to predict the outcome of hypoxia effects in polluted coastal areas, it is crucial to understand the interactions between antioxidant responses to contaminants and hypoxia and physiological mechanisms causing and counteracting oxidative damage.

CS01B-1

Interactions between depletion of ozone and climate change: a tale of two pollutants

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Although the Montreal protocol is working, climate change will influence the recovery of stratospheric ozone and also cause significant changes in the environment that will affect organisms as well as processes in the environment. Photochemically produced tropospheric ozone (O_3) is projected to increase over the next 20-40 years in certain regions of low and middle latitudes because of interactions of emissions, chemical processes, and climate change. If emissions of anthropogenic air pollutants from combustion of fossil fuels, burning of biomass, and agricultural activities continue to increase, concentrations of tropospheric O_3 will tend to increase. Numerical models suggest that annual deaths due to ground-level oxidants such as O_3 alone could reach 2 $x10^6$ in 2050, while the cost of crop damage is predicted to be of the order of US $20x10^9$ pa by 2030 with additional effects on non-crop plants. Decreases in precipitation and increasing temperatures due to climate change are likely to restrict plant growth and compromise the ability of plants to re-distribute resources for protection from UV radiation and other environmental factors - especially important in harsh environments. Consumption of plants by herbivores usually decreases under elevated UV-B radiation because of increased synthesis of compounds that absorb UV-radiation and also deter herbivores by acting as antifeedants. Over the coming decades, rising concentrations of CO_2 in the atmosphere and increased planting density may counteract this beneficial effect of UV-B radiation. Acidification of lakes and oceans is a major stress factor closely related to climate change and solar UV-B radiation. Increasing acidification of marine waters due to increased atmospheric concentrations of CO_2 decreases the rate of incorporation of carbonate in many marine organisms and makes them more vulnerable to solar UV-B radiation. Pre-industrialization, the concentration of CO_2 in the atmosphere was about 280 ppmv. This corresponded to an average pH in the oceans of about 8.2. The current level of 390 ppmv CO_2 has lowered the pH of oceanic waters by about 0.1 units. A further decrease in pH of 0.3 - 0.4 units is expected by the year 2100. Increased melting of ice sheets will reduce the concentration of colored organic matter in waters and allow increased penetration of UV radiation with resultant increased damage to aquatic organisms.

CS01B-2

Are melting glaciers increasing exposure of Alpine wildlife to contaminants?

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Due to climate change, chemicals that accumulated in glaciers through evaporation from warm regions and subsequent condensation in cold regions are being released into alpine environments. To examine the role of glacial geography in determining contamination of wildlife in alpine ecosystems, we measured persistent organic pollutants and mercury in apex predators, ospreys (Pandion haliaetus) breeding in 16 watersheds in western Canada. Despite living in environments far from point sources of contamination, ospreys in Canadian alpine lakes had significant levels of DDT, PCBs, toxaphene and mercury. After accounting for proximate factors such as trophic level and lipid content, HCB, chlordane, mercury, PCBs decreased with watershed size and toxaphene, DDT, HCB and mercury decreased with watershed size divided by lake size. Ospreys feeding in large watersheds that drain into relatively small lakes had higher levels of contamination. Toxaphene, mercury, HCB, and DDT all decreased with the amount or proportion of glaciation while toxaphene and chlordane decreased with elevation. Thus, foraging ecology (trophic level) and geography explained significant portions of the large variation in osprey contaminant levels in western Canada, but the geographical variation was not consistent with the idea that the patterns in contaminants were determined by current long-range transport or glacial melt. Rather, we suggest that contaminants largely melt out during annual snow pack melt and bioaccumulate at lower elevation lakes, especially low elevation lakes with poor drainage. Our study highlights the importance of understanding how biological processes integrate physical patterns when studying the environmental chemistry of wildlife.

CS01B-3

Surface water quality and climate change issues: impact of hydrology and temperature on total organic carbon and nitrate in small scale water services

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The increase of mean temperature on Earth and its consequence on extreme meteorological events like floods and droughts have raised climate change issues to a major environmental and public health concern. These events induce very low or very high river flows that may impair the quality of surface water with some potential health impacts when used for drinking water production by small scale water services (SSWS). This study aims at assessing the impact of temperature and hydrology on surface water quality with particular emphasis on the amount of total organic carbon and nitrate. Data of 3 French rivers was gathered over a 26 year period, from 1983 to 2009. It appears that a rise of water temperature can be associated with a slight increase of total organic carbon concentration while the amount of nitrate becomes lower. Additionally, the flow rate seems to be a better determinant of water quality than temperature. Indeed, the concentration of total organic carbon decreases from very low to medium flow rate, but increases from medium to very high flow rate. However, the opposite pattern should be considered regarding nitrate. Consequently, crossing such data, the present study shows that the probability of exceedance of the French limit of quality for total organic carbon in surface water resources used by SSWS is much higher at low and high flow rates associated, respectively, with high and low temperatures. In the same way, the probability of exceedance of the French limit of quality for nitrate in surface drinking water resources is much higher for medium and high flow rates associated with low temperatures. These findings should be useful for water companies, water industry regulators, health authorities and policymakers. In fact, the present conclusions should allow anticipating on the adaptation of drinking water treatment based on the thermal and hydrological conditions.

CS01B-4

Multi-parametric approach to assess combined effects of pollution and climate change in West African aquatic ecosystems

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In the last decades, West African aquatic ecosystems were strongly impacted by climate change inducing increase of water salinity, and by anthropic activities such as industry, agriculture and intensive fisheries that discharge chemical pollutant in environment. To assess the combined effects of both environmental changes, a multiparametric approach was performed. For this purpose, Black chinned Tilapia (Sarotherodon melanotheron) were sampled during dry and rain period in 8 sites characterized by various contamination and salinity profiles. Levels of pollution by PAHs, PCBs, OCPs in sediments of eight sampling sites were characterized. A set of biochemical biomarkers including biotransformation enzymes (EROD activity and glutathione-S-transferase), oxidative stress indicator (lipoperoxidation), neurotoxicity end-point (brain acetylcholinesterase) and estrogenicity biomarker (circulating vitellogenin) was measured to understand the effects of contamination. Moreover, physiological parameters such as growth rate, condition factor and gonado-somatic index were measured in all tilapia to provide data on effect of environmental conditions on fish physiology. The results of this work shows that the health fish living in West African aquatic ecosystems is impacted by combined effects of chemical pollution and climate change and argue for a better assessment of these ecosystems to prevent furthers degradation.

CS01B-5

Interactive effects of multiple-stressors resulting from habitat degradation and climate change in a model reptile species.

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The ability to assess multiple-stressor effects that result from habitat degradation and climate change is crucial for maintaining viable populations and overall ecosystem health. Chemical stressor effects have been relatively well studied but effects have seldom been investigated in conjunction with common stressors that wild populations must endure. For example, field studies have indicated that malarial infections in reptiles positively correlate with increased temperature (a concern regarding global warming) leading to infection incidence as high as 50%, and the interactive potential of increased parasitism in conjunction with additional anthropogenic stressors is yet to be investigated. To approach this question, we utilized the Western fence lizard (WFL, Sceloporus occidentalis) as a model species to characterize multiple-stressor effects projected to be caused by climate change and habitat degradation including: increased malarial parasitism, decreased basic resource (food) availability and exposure to chemical contamination (trinitrotoluene, TNT) to determine the overall interactive-potential of these stressors. To accomplish this, we tested the null hypotheses: (1) Multiple ecosystem-level stressors characteristic of habitat degradation and climate change have no interactive effects on lizard health and fitness, and (2) Environmental stressors are uniquely identifiable via genomic signatures and these signatures can be used to identify predominant stressors in multiple-stressor scenarios. The first hypothesis was tested by evaluating stressor impacts on 29 toxicological endpoints investigated in clinical toxicology bioassays characterizing single-stressor and pairwise-stressor effects. Although few interactive effects were identified, three interactive effects of potential ecological concern were observed: (1) food limitation may eliminate hormesis of TNT on animal weights, (2) combined food limitation and malarial infection may impact immune response as well as (3) impact testes size and quality. To test the second hypothesis, we utilized next-generation DNA sequencing to develop a de novo bioinformatics infrastructure and high-density microarray to assess transcript expression for Western fence lizard. The tools are being used to determine the genomic signature for each stressor and stressor interaction utilizing observed mechanistic and metabolic impacts as the foundation for signature development.

CS01B-6

Climate change damage functions in LCA - (1) from global warming potential to natural environment damages

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Energy use often is the most significant contributor to the impact category 'global warming' in life cycle impact assessment. However, the potential global warming effects on the climate at regional level and consequential effects on the natural environment are not thoroughly described within LCA methodology. The current scientific understanding of the extent of climate change impacts is limited due to the immense complexity of the multi-factorial environmental changes and unknown adaptive capacities at process, species and ecosystem level. In the presentation we argue that the global warming impacts from a product system being studied in an LCA must be seen in context with the changing future background situation. This background situation is among other things affected by e.g. cumulative atmospheric greenhouse gas emissions of yet unknown magnitude. Here, we define climate change damage on the natural environment as climate change driven environmental changes. The man-made environment such as cultivated land, infrastructure and urban areas is not considered.

Hypothetical climate change damage functions representing both sensitive and robust responses were analyzed in relation to cumulative green house gas emissions. An attempt was made to link these hypothetical damage functions with current experimental evidence of biological and biogeochemical responses to a changing growth environment. Each LCA stage involves uncertainty due to e.g. choice, modeling, sampling and measurement errors apart from natural variation. Error propagation throughout the stages of the LCA is thus needed. The relative uncertainty (expressed as the coefficient of variation) of the product related emission, of the background situation and of the natural environment responses were compared. It seemed that the overall relative uncertainty of a characterization factor for climate change might be at least 64%-152% indicating a large variability around the unknown mean climate change damage.

CS02-1

Ship based oil spill monitoring, a new integrated system for thickness estimation and operational overview

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With an increasing number of offshore oil wells, oil spill prevention and recovery receives a growing focus amongst authorities and oil companies. When accidents happen oil spill spreads to form a thin layer at sea surface, and the disaster scene quickly turns extensive and over-complex. Reliable sensor capability and data acquisition systems are critical for an efficient recovery operation. Currently, there are several systems available for detection and monitoring of oil spill, however, it is acknowledged that there are still major shortcomings in terms of giving a comprehensive overview covering everything from on-scene recovery operations to strategic planning. Particularly is it a challenge to provide relevant information to the vessel crew that is operating the dispersant agents, oil booms and skimmers. The present paper outlines a commercially available system, named SECurus, designed to comply this. The system is built around a vessel mounted, motion stabilized sensor platform containing a cooled infrared (IR) camera, daylight camera and xenon searchlight. The IR camera serves two distinct purposes: 1) As a sensor technology for oil spill detection and 2) As a tool for operational overview in combination with the daylight camera for night and day vision. As sensor platform, IR is recognized for providing relative thickness evaluation of oil spill which is critical for prioritizing recovery operations. An open sensor communication interface is developed and tested with 3rd party radar oil spill detection systems. All sensors, including the camera video streams, are geo-referenced and integrated into an electronic chart system (ECS). Computer generated graphics can be transposed between sea chart and camera views with principals from augmented reality. In addition to operational usage at North Sea oil spill response vessels, the system has been extensively tested through planned exercises in collaboration with The Norwegian Clean Seas Association for Operating Companies (NOFO).

CS02-2

Effects of acute oil spills on the Norwegian marine environment

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A number of small-to-medium size acute oil spills have occurred in Norwegian waters in the latter years. The Institute of Marine Research (IMR) has performed damage assessment studies after several recent oil spills. Measurements of the levels of oil-related organic contaminants have been carried out in seawater and various biota samples after the spills, with follow-up investigations undertaken half a year to one year later if necessary. The objectives of the studies were to assess the degree of contamination in the marine environment, to monitor the contamination with time, and to quantify the possible damage inflicted on shellfish and commercially important fish stocks.
Oil spills taking place at three locations in Norway in the period 2007-2009 will be discussed:
1. Cargo vessel "Server", wrecked near Fedje, Hordaland in Western Norway in January 2007. The total amount of oil spilled to the environment was 380 tons.
2. The discharge of 4000 tons crude oil at the Statfjord A oil platform in the Tampen region of the North Sea was the second largest discharge till now in Norway after 40 years of exploration. A second, minor spill of 70 m3 oil occurred at the same platform in May 2008.
3. Cargo vessel "Full City"ran aground near Såstein, Telemark in Southern Norway in July 2009. The total amount of oil spilled was 300 tons. The spill has caused a considerable contamination of the nearby coastal area. The study of the consequences of the spill was one of the most detailed undertaken in Norway.
The results of these studies largely lead to the same conclusions. In all cases, an increase in oil-related contaminants (polycyclic aromatic hydrocarbons, PAH, and their metabolites) was demonstrated in various compartments of the environment (seawater; fish liver and in some cases muscle; fish bile; crab, shellfish or shrimp tissue). The levels in the water and mobile biota were not alarmingly high and went relatively quickly back to background levels. The highest levels were found in the organisms that could not escape from the contaminated area (trapped fish, mussels). The overall effect of the spills seemed also to be influenced positively or negatively by external factors, such as poor weather (in the case of "Server"and at Statfjord A in December 2007), or pre-existing contamination from other sources (in the case of "Full City").

CS02-3

Reported in the Gulf of Mexico following the Deep Horizon oil spill; what risk do alkylnaphthalenes pose?

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Alkylnaphthalenes (AN) are commonly present following spillages of crude oils and were reported in the Gulf of Mexico following the Deep Horizon platform disaster. They also dominate oil industry produced waters and consequently AN are commonly present as a major component within tissue extracts of marine biota contaminated by petroleum products. Surprisingly, little information concerning the toxicity of AN has been reported and monitoring of hydrocarbons has largely been focused on the USEPA priority pollutant list of 16 polycyclic aromatic hydrocarbons, even though within crude oils, alkyl derivatives are much more prevalent than the parent compounds. We now report the toxicity of a range of commercially-available and specially synthesised C_{2-8} substituted non-branched and branched AN to the mussel, *Mytilus spp*.
Acute (48 h) semistatic exposure tests were performed using test solutions of individual C_2-C_8 AN and as a mixture. Health effects were assessed using inhibition of mussel clearance (feeding) rate bioassays and critical body residue parameters (IC_{20} and IC_{50}) were derived. Depuration studies (5 d in clean seawater) were also performed. In addition, tissues from mussels exposed to aromatic fractions from a weathered and a biodegraded crude oil, and that from wild North Sea (UK) mussels, were extracted and analysed by tandem gas chromatography (GCxGC-TOF-MS), and the accumulation profiles of the bioaccumulated AN compared.
The lower molecular weight compounds (C_{2-3}) were found to have similar toxicities to each other, whether mono- or di-substituted. The C_4 compound 2,6-diethylnaphthalene was significantly less toxic ($P<0.05$). Tissue IC_{50}s for the C_{2-3} compounds were very close to the QSAR models for mussel clearance rates, but higher molecular weight compounds were far less toxic than model prediction. An exception to this was the synthesised C_8 branched AN, 2-(1',5'-dimethylhexyl) naphthalene, which reduced clearance rates to a similar extent to the lower molecular weight AN implying that the structure of the AN and not just the molecular weight or hydrophobicity, is important. Mussels were generally able to rapidly recover from exposure to AN. Profiles of accumulated AN were similar within oil-exposed and contaminated wild mussels.
Marine environmental monitoring should include measurements of AN but our results suggest that other compounds are perhaps more likely to bioaccumulate, persist and pose a longer term threat to marine biota.

CS02-4

Effects of oil and oil treated with dispergent on the arctic amphipod Gammarus setosus

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When oil is released in an area with ice we will face a complex interaction between oil, water and ice. The oil will be absorbed by snow on the ice edges, it may be trapped in the ice in brine channels and it may be moved underneath the ice. The ice field as such will also be under constant transformation driven by wind, currents and temperature. Some ice floes may be transported relatively far from their original position and relatively far from their original neighbors. Alltogether this may be a strong driving force for drift and spread of oil after oil has been released in an ice field.
These processes have been followed during an offshore field experiment in the marginal ice zone East of Svalbard in May 2009 [1] and basic field data have been used to accomplish a realistic and relevant exposure study in the laboratory. Chemical results from analyses of water samples of the laboratory exposure experimnt showed good correlation with identical data monitored during the offshore field experiment with oil in ice infested seawater. Due to the low input of energy during the exposure period in the laboratory study, there were no indications of oil droplet formation in the water fraction caused by the dispergent. However, levels of naphtalenes were relative high. Among the effect markers, general stress was indicated by a significant decrease in lysosomal stability of ampipod haemocytes in the oil+dispergent treatment.

CS02-5

Ecotoxicology and risk assessment of crude oil spill in a palm oil plantation

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This report is a quantitative/qualitative impact assessment of the effect of the massive crude oil spill that occurred in October 03, 2006 on a parcel of land having nine thousand, five hundred (9,500) matured oil palm trees. An assessment of the impact was based on the observed changes in relevant valued ecosystem components (VECs), using biochemical end point parameters from both laboratory and field study of the spill environment, multiple-lines-of-evidence approach and consideration of the relevant literature. The toxicity of the spilled oil was evaluated using lumbricus species (earthworms) and diazotrophic bacterium Azotobacter vinelandii found in ecosystem. Exposure of earthworms to the crude oil-contaminated soil samples taken from the palm plantation three months and one year after the spill resulted in 68 - 76 % death and significant loss in weight in those surviving after 96 hours. Relatively very low number distribution of earthworms was observed in the oil polluted plantation when compared with control (unpolluted) site and with significant bioaccumulation of the PHC in the earthworms. There was a significant reduction in nitrogen fixation in soil cores taken from the oil-contaminated oil palm plantation and also in population and distribution of Azotobacter vinelandii. There was a significant reduction ($p < 0.05$) in the chlorophyll content in samples of fresh palm leaves taken from the polluted palm plantation, between February 2007 and December 2007 when compared with the control site, thus suggesting that the toxic effects of the crude oil spill exerted and is still exerting a considerable impact on the yield capacity of the oil palm trees and the ecosystem productivity. There was a good correlation in chlorophyll content of the maize planted in simulated oil-contaminated soil and the yield in maize grain and this was made manifest in the yield of maize planted in parallel uncontaminated plots. Based on the above correlation, it was concluded that the crude oil spill in the plantation negatively affected the productive capacity of the palm trees and the plantation ecosystem in general.

CS02-6

Deriving disaster impact distances for 'Seveso'-companies in relation to protected nature areas

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After the disaster at Seveso Italy in 1976 legislation was developed to protect humans and the environment from industrial accidents. This ultimately resulted in the development of the so called Seveso II directive (Council Directive 96/82/EC on the control of major-accident hazards). EU-member states are obliged to bring this directive into force in the national laws. In the Netherlands this resulted in the Regulation to assess distances to nature areas, in short called Reban, which became active in 2006.
Reban states that there needs to be a "sufficient distance between heavy industry and vulnerable nature areas in case of an industrial disaster". To determine this distance there "needs to be an appropriate tool that can determine distances between the industry and nature". The Dutch government would provide such a tool to execute risk analyses. The National Institute for Public Health and the Environment (RIVM) was asked to develop this tool.
Seveso companies working with the most hazardous chemical compounds require a permit for new installations or expansions of facilities when positioned in the vicinity of protected nature areas. Risk analyses must make clear, that sufficient distance is kept between the facilities and the nature areas in case of an industrial accident.
A tool to assess this distance has to contain fate and effect assessment, to deliver critical distances when combined with regulatory acceptance criteria on maximum allowable exposures. Comparison of a critical distance with the geographical distance between facility and nature area provides insight in the matter of sufficient distance. The approach is to be applied in cases for so-called Seveso-facilities near nature areas specifically protected under the Nature protection act, including Natura 2000 nature areas.
This presentation considers a tool which has been designed to evaluate the ecotoxicological risks imposed by Seveso-facilities which can be positioned in the vicinity of protected nature areas. For those areas, the ecological vulnerability is explored with the tool. Through realistic disaster scenario's compounds, we explore the resulting distributions of impact distances, and compared these with realistic distances between Seveso facilities and protected nature areas on GIS maps. We show how the critical distance concept can be used together with ecological vulnerability analyses to conclude whether a facility can be considered to be at a safe distance or not.

CS03 - New approaches in the simulation of realistic multistress and multispecies scenarios

CS03-1

Sensitivity shift in Daphnia magna population response caused by size selective predation

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The quest for more realism in ecological risk assessment demands for looking beyond ecotoxicological standard tests by additionally considering e.g. population structure, competition or predation. Predation is considered to be a major factor controlling dynamics and structure of freshwater comunities.The outcome of predation varies with both prey size and predator size, thus mostly appears to be size selective. Moreover, body size affects an animal's sensitivity to toxicants, e.g. it is known for *Daphnia magna* that neonates in general are more sensitive than adults.

In order to analyse combined effects, we simulated the population dynamics of *D. magna* under predation and toxic stress and compared the model output to laboratory data. As a predator, larvae of the backswimmer *Notonecta maculata* were used. For modelling *Daphnia* population dynamics and *Notonecta* predation we applied two individual based models which were already shown to adequately predict different scenarios.

In the laboratory, we tested the effect of p353-Nonylphenol (Np) on populations of *D. magna* under predation pressure. Population dynamics, including size structure, were recorded for 4 different treatments during 11 weeks: {1} control populations, {2} populations under predation, {3} populations exposed to two peaks of Np and {4} populations under predation and exposed to two Np peaks. Single larvae of *N. maculat*a were placed in prepared aquaria after *D. magna* populations reached peak abundance. During test backswimmers grew from first to fith instar.

In both, modelling approach and laboratory study predation was found to significantly alter *Daphnia* population size and structure. Since size selectivity differs across *Notonecta* instars, the impact on *Daphnia* populations changed during development of the predator and was highest in large backswimmers. Under predation of larger backswimmers, size structure of *Daphnia* populations shifted towards higher number of neonates and lower numbers of adults compared to control. Thus the combined effect of predation and toxicant finally led to the extinction of *D. magna* populations, whereas total abundances of other treatments were similar to or above control level. We will demonstrate that multiple stressors may result in severe effects on population level, which are not predictable from single stressors and that mechanistic modelling might be an appropriate tool for predicting the combined effects in environmental risk assessment.

CS03-2

Daphnia under stress: a case study

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Natural populations worldwide are increasingly exposed to pesticides. This may not only impact target pest species but also a wide variety of non-target species, including keystone species that are crucial to the functioning of natural ecosystems. Pesticides impose strong selection pressures on natural populations. This may lead to the evolution of resistance, which itself may be associated with correlated responses, including potential costs of evolution. Populations are also exposed to natural stressors like parasites and predators, and these stressors may interact simultaneously with pesticide exposure. While studies on local genetic adaptation and costs of evolution are rare in non-target species, studies on direct interactions between stressors, especially when dealing with biotic stressors, are typically implemented in non-target species.

We used the water flea *Daphnia magna*, a non-target species often used as a model system in ecotoxicology, in an integrated research approach in which we focussed on three aspects that are of key importance to understand the evolutionary ecology of pesticide exposure: (1) the capacity of natural populations to genetically adapt to pesticide exposure, (2) the added complexity of synergistic effects caused by simultaneous exposure to natural stressors, and (3) the potential interference of evolutionary costs of adaptation to pesticide exposure. In a next step we tried to understand the mechanistic base of the observed responses using a cDNA *Daphnia magna* microarray.

CS03-3

Will climate change uncover low-dose effects of pesticides? A multigenerational study with the midge Chironomus riparius

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The potential risk of pesticides under climate change conditions is not yet accurately assessable for aquatic wildlife. We hypothesize that near-natural dynamic temperature regimes may uncover the ecotoxicity of low dosed pesticides due to the concomitant successive exposure to suboptimal and optimal temperatures, in particular under impact of climate change.

The hypothesis was tested by performing a bifactorial multigenerational study with the aquatic model organism *Chironomus riparius*, which has been chronically exposed to a low dose of the fungicide pyrimethanil under two dynamic present-day temperature simulations (cold year today: 11.0-22.7-18.6°C, warm year today: 14.0-25.2-21.7°C) and one future scenario (warm year in future: 16.5-28.1-24-1°C). During the 140-days-lasting multigenerational study covering spring and summer season, parameters related to survival, emergence, reproduction, population growth and genetic diversity of C. riparius were analysed.

In general, life-history-traits of *C. riparius* are highly dependent on temperature and generational time. In simulated spring, a ´no-effect-concentration´ (NOEC) of pyrimethanil provokes slightly adverse or hormetic effects on *C. riparius* in three temperature scenarios (P- to F1-generation) as expected from standard test. But an exposure of *C. riparius* to a NOEC of pyrimethanil at a thermal situation likely for a present-day or future warm summer (F2-generations onwards) uncovers considerable adverse effects on mortality and genetic diversity. Under the simulated climate change situation, pyrimethanil-treated C. riparius reveal the highest mortality, a substantially reduced population growth rate and a sizeable loss of genetic diversity. Although adverse pyrimethanil effects vanish until the end of the summer in both present-day scenarios, *C. riparius* sub-populations (control, pyrimethanil treatment) exposed to the climate change scenario break down after F3-generation.

The results gained so far highlight the importance of near-natural climate impact research to better understand and manage the ecotoxicological risk of agrochemicals today and in future. Not only the impact of climate change, but also considered safe concentrations of pesticides may pose a reasonable risk for aquatic insects in future.

CS03-4

Effects of imidacloprid pulses on macrozoobenthos in lentic microcosms

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Over the past decade the use of a new generation of insecticides, the Neonicotinoids, has been marketed strongly. Imidacloprid was a best seller thanks to its low application rates, high activity against pests and fast degradation. However, because of its high solubility in water it has the potential to enter surface waters. In the risk assessment tests were conducted under laboratory conditions, based on single species and continuous-exposure scenario. In agricultural areas, periods of peak application followed by rainfall events can lead to pulse contaminations, where concentrations can reach toxic levels for non-target invertebrates. The aim of this study was to assess the impact of Imidacloprid pulses on non-target aquatic macroinvertebrates. To achieve realistic exposure conditions the experiment was carried out using infield microcosms. This method is designed to simulate natural lentic ecosystems and enables the testing of many indigenous invertebrates developing within microcosms. During the experiment optimal conditions for Imidacloprid degradation prevailed, therefore this experiment has to be considered a 'best case' rather than a 'worst case' exposure scenario. Measurements on structural aspects of the macrozoobenthos showed some evident responses: At the greatest concentration tested (nominal concentration 40 µg/L) diversity and abundance of some taxa (e.g. Chironomidae) decreased; the emergence of some chironomids species and of Ephemeroptera declined at the highest concentrations tested (nominal concentrations: 17.3 and 40 µg/L). The results provide evidence that despite rapid dissipation short-term exposures to imidacloprid can significantly affect the structure of freshwater ecosystems, even at low concentrations. The microcosms method showed also to be a valuable tool in the risk assessment of contaminants.

CS03-5

Combined effects of pesticide exposure and predation risk: what can we learn from behaviour and physiology?

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To assess the impact of pollutants in field situations we need to understand whether their effects are magnified when combined with natural stressors like predation risk. Such synergistic interactions sometimes occur but we do not understand when to expect and how to explain these. One way to advance is to start unraveling jointly affected behavioural and physiological end points of combined exposure to pesticides and predation risk. To this aim we investigated in larvae of the damselfly Enallagma cyathigerum the joint effects of pesticide exposure (glyphosate) and predation risk (visual and chemical predator stimuli) on growth and associated behavioural (food intake) and five (sets of) physiological variables:.(1) expression levels of the stress protein Hsp70, (2) the activity of two key antioxidant enzymes in insects, superoxidedismutase (SOD) and catalase (CAT), (3) the degree of oxidative damage to lipids as quantified by levels of lipofuscine and TBARs, (4) the levels of energy reserves: proteins, sugars (glucose and glycogen) and total fat, and (5) the activity of acetylcholinesterase (AChE), an enzyme strongly linked to the efficiency of nerve signal transmission. There was a strong synergism on growth: the pesticide only caused a growth reduction in the presence of predation risk. This growth synergism was not behaviourally mediated as food intake was higher in the presence of the pesticide and not influenced by predation risk. All five (sets of) physiological variables were affected by isolated exposure to predation risk but only one by isolated exposure to the pesticide (upregulation of Hsp70). Pesticide exposure did, however, interact with predation risk for oxidative stress and oxidative damage: SOD was only influenced by the pesticide in the presence of predation risk and TBAR levels only increased in larvae that were jointly exposed to the pesticide and predation risk. Together, this suggests that under combined stress exposure the larvae were not able to react to the associated oxidative challenge by upregulation of SOD and CAT, and as a result encountered damage, namely the peroxidation of fat. This may have contributed to the observed synergistic growth reduction. Such combined behavioral and physiological approach is therefore a promising avenue to increase our understanding of when such synergisms are to be expected.

CS03-6

Assessment of soil contaminants bioavailability using a multi-marker approach in laboratory and field experiments

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Simultaneous contamination of soil by various substances (contaminant mixtures) presents a challenge for risk assessment. Chemistry analyses give the level and type of contaminants. Toxicity tests assess the effects of bioavailable compounds to selected species using standard important endpoints (survival, growth, fertility). Bioavailability can be defined as "the fraction of a substance that will exert an effect in a organism". This fraction is "toxicologically bioavailable". Conversely, bioaccessibility is referred to as "the fraction of a substance which is readily mobilizable by an organism in a soil". Bioavailability is critical for understanding effects that might result from exposure of biota to contaminated soils and sediments. Soils and sediments from military range and training areas (RTAs) as well as Munitions Experimental Test Center (METC) are contaminated principally by energetic materials (EM) and metals. Their chemical characteristics are relatively well known and toxicity assessment of soils from RTAs and METC are in some cases available. However, bioavailability on these sites needs to be comprehensively characterized. Conventional toxicity tests give limited toxicological information and do not consider variability (e.g., temperature, humidity) of current and future field conditions. Improvement of standard assays by adding alternative biological endpoints (cellular, biochemical, molecular biomarkers) can help to understand the toxicity observed and give the appropriate information for the selection of biological parameters for other tier assessment levels (e.g., field mesocosm assays, field studies) where standard chronic endpoints can not be used. Use of selected biomarkers alone gives appropriate information on chemical stress, range and class of contaminants, and health status. This paper presents case studies using laboratory and field bioassays and an integrated approach including earthworm (Eisenia andrei) toxicity tests and a suite of biomarkers and chemical analyses. Parameters such as lysosomal membrane fragility of cð'lomocytes (neutral red retention time) can be used to assess the chemical stress whereas the antioxidant system (catalase and superoxide dismutase activity) and detoxification metabolism (glutathione S-transferase activity, metallothionein), as well as the immune activity and the contaminant uptake are used to assess the bioavailability of contaminants and the health status of the exposed organisms.

EC01 - Advances in passive sampling and dosing techniques

EC01A-1

A decade monitoring of PCBs and PAHs by silicone rubber passive samplers in parallel with

deployed mussels

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Since the introduction of passive sampling in the aqueous environment two decades ago, many research papers have been published. However, most of the monitoring studies performed with passive samplers were one-off surveys and programs covering several years are very rare. Here a decade monitoring in autumn and winter with Silicone Rubber Passive Sampling (SRPS) in parallel with deployed mussels (Mytilus edulis) running from 2002 at eight Dutch coastal stations will be discussed. Results were processed using the latest partition coefficients and models for calculation of the concentrations in the water phase. Sampling rates were clearly lower in winter when the water temperature was lower. The average of the estimated concentrations of PCBs in the water phase ranged from 3 pg L^{-1} (PCB170) - 40 pg L^{-1} (PCB52). Concentrations of PAHs in the water phase ranged from ~10 pg L^{-1} (indeno[1,2,3-cd]pyrene) to 4000 pg L^{-1} (phenanthrene). Estimated concentrations were higher in winter compared to autumn, for PCBs as well as PAHs. Comparing amounts per mussel before and after exposure reveals that in about 20% of the cases contaminants were released during exposure. Although this is no evidence that equilibrium is attained it does mean that data are likely positioned on both sides of the equilibrium.The resulting lipid based bioaccumulation factors (LBAF) were evaluated and showed more variation in winter compared to autumn. Furthermore, also the LBAFs for PCBs were on average significantly lower in winter compared to autumn. Relating LBAF to the water temperature showed a decrease of LBAF with temperature (~0.04 log units). Such a decrease was not found for PAHs. Lower LBAFs in winter for PCBs were mainly caused of the higher concentrations calculated or the water phase and not, or much less, by changes in the concentrations in the mussels. In spite of the observed temperature effect, that only became significant because of the large dataset, the log LBAF values show an excellent relationship with logKow values. Further fine tuning of research that relates passive sampling to uptake by mussels should take the water temperature and salinity into account for the passive sampling as well as uptake by mussels.

EC01A-2

New developments in passive air sampling for current-use pesticides and other priority chemicals

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Passive air samplers are cost effective and simple tools that are invaluable for assessing risks associated with pesticide use. They can be used to monitor and evaluate regional and long range transport of pesticides and provide spatially resolved data that is required for developing transport and fate models. Under the Global Atmospheric Passive Sampling (GAPS) network, passive air samplers comprising polyurethane foam (PUF) disks are being used to deliver air concentrations of several organochlorine pesticides at more than 50 global sites. The value of these samplers has been recognized and there is a need to extend their application to more volatile and polar pesticides that are typically used these days. This study investigates a modified PUF disk sampler, the SIP (or sorbent-impregnated PUF) disk which used XAD powder impregnated into the PUF to enhance sorptive capacity. An eight-month field calibration study of the PUF disk and SIP disk samplers has been undertaken during 2010 to establish sampling rates and sorptive capacities for a range of current-use pesticides that are relevant to Canada. Preliminary results are also available from the first deployment of the SIP disk sampler at a subset of 20 GAPS sites alongside the conventional PUF disk sampler. This study provides the first global-scale survey of several classes of priority chemicals, including CUPs. The GAPS network also includes several urban sites (e.g. Toronto, Canada; Paris, France). These locations can be compared to background and agricultural areas and this may provide useful information about transport of CUPs from application areas.

EC01A-3

EU-wide environmental monitoring of Persistent Organic Pollutants using butter as biomonitoring matrix

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Lipids are good matrices to accumulate many persistent organic pollutants (POPs) due to the lipophilic nature of these chemicals. Dairy products are easily accessible and available world-wide and analysis of this matrix reflects the contamination level of the environmental compartment from which they derive.The Stockholm Conventions and the Global Monitoring Plan (GMP) encourage the production of monitoring data to effectively evaluate the presence of the POPs in all regions, in order to identify changes in levels over time, as well as to provide information on their regional and global environmental transport.

Suitable sample matrix is needed to measure the temporal and spatial distribution of the priority POPs, which are both intentional and unintentionally released to the environment. Passive sample techniques have been applied to the GMP monitoring activities to assess the global long range transport of POPs. The data produced by passive samplers have been shown to correspond to the more advanced active air sampler. The advantage with passive air sampling is that no advanced equipment demanding electricity is needed. The samplers can therefore be set up everywhere, even at remote areas. But still some education of the personnel is needed to operate the sampler. Aspects such as wind direction, temperature and sampling time are crucial to register and incorporate into the calculations.

In this study we demonstrate the feasibility to use another passive sample approach for screening purposes, i.e. animal lipids such as butter. The advantages to use butter as biomonitoring matrix are several; due to the lipophilic and persistent properties of POPs they are bioaccumulated in lipid rich matrix; butter reflects the contamination level of the environmental compartment from which they derive due to well known transfer factors; butter is available in almost all geographic regions and is cheap and easily accessible; moreover, this biomonitor is well buffered against temporal variations. The air-grass-cow transfer factors are available from controlled experiments. Hence it remains to verify the correlation in the field in order to assess ambient air quality. The POP levels of 160 samples from Europe have been analyzed and will be evaluated together with available air data to establish a correlation between air contamination and dairy products (assuming minimal commercial feed contamination influence).

EC01A-4

Determination of deployment specific chemical uptake rates for SDB-RPS Empore disk using a passive flow monitor (PFM)

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The use of the adsorbent SDB-RPS-EmporeTM disk in a Chemcatcher type passive sampler is routinely applied in Australia when monitoring herbicides in aquatic environments. One key challenge in the use of passive samplers is mitigating the potentially confounding effects of varying flow conditions on chemical uptake by the passive sampler. Performance reference compounds (PRCs) may be applied to correct sampling rates (Rs) for site specific changes in flow and temperature; however, evidence suggests the use of PRCs is unreliable when applied to adsorbent passive samplers. The use of the passive flow monitor (PFM) has been introduced for the assessment of site specific changes in water flow. In the presented study we have demonstrated that the Rs at which both atrazine and prometryn are accumulated within the SDB-RPS-EmporeTM disk depend on the flow conditions. Further, the calibration of the measured Rs for chemical uptake by the SDB-RPS-EmporeTM disk to the mass lost from the PFM has shown that the PFM provides an accurate measure of Rs for flow velocities from 0-16 cm s-1. Notably, for flow velocities > 16 cm s-1, a non linear increase in the Rs of both herbicides was observed which indicates that the key resistance to uptake into the SDB-RPS EmporeTM disk is associated with the diffusion through the overlying diffusion limiting membrane. Overall the greatest uncertainty remains at very low flow conditions which are unlikely to occur often in surface waters. Validation of the application of PFM has also been undertaken in a limited field study.

EC01A-5

Effective in-situ measurement of pore water PCB concentrations in sediment profiles using passive samplers

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Since their introduction, the use of passive samplers to measure freely dissolved concentrations of hydrophobic organic compounds (HOCs) in aquatic environments has become more prevalent. However, there are limited studies that have utilised passive samplers to assess in-situ pore water concentrations. Measurements of pore water concentrations are important as they are a more appropriate indicator of the bioavailable contamination in an ecosystem. This study focuses on measuring sediment pore water concentrations of polychlorinated biphenyls (PCBs) in an AC amended contaminated sediment site by: (i) measuring vertical pore water profiles of PCBs from 0-50 cm using both polyethylene devices (PEDs) and polyoxymethlyene (POM), (ii) dosing the passive samplers with a larger suite of performance reference compounds (PRCs), and (iii) interpreting PRC depletion to assess the development towards equilibrium. In August 2008, sampling rods with ultra thin POM (17 _m) and polyethylene (PE) were placed in a remediated sediment treatment plot located in a tidal mudflat area of the South Basin in San Francisco Bay. The sampling rods were deployed in a radial pattern at each location and were retrieved over time. In order to assess the approach towards equilibrium, the PE and POM passive samplers had been impregnated with PRCs. Three different methods were used to estimate the pore water concentrations: (a) assuming equilibrium has been achieved in the POM after 100 days of exposure, (b) depletion of PRCs from the PE and assuming a first-order process uptake model and (c) depletion of the PRCs from the POM assuming a first-order process uptake model. Average pore water concentrations ranged from 0.4-5.2 ng/l in the top 30 cm of sediment and from 0.9-9.2 ng/l in the deeper sediment layers (30-40 cm) depending on passive sampler material and the method used to interpret to depletion of PRCs. The close agreements of pore water estimates for the two sampler materials (PE and POM) and the different methods used to derive pore water concentrations demonstrate the robustness and suitability of the passive sampling approach. Better understanding of the performance of different sampler materials and uniformity in methodologies to calculate pore water concentrations considering the dissipation of PRCs will allow sediment managers and researchers to make a well informed choice for monitoring and study designs.

EC01A-6

Development and use of polyethylene passive samplers to detect triclosans and alkylphenols in an urban estuary

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To be able to use polyethylene passive samplers (PE) in the field, the partitioning constants between PE and water (K_{PEw}) of the compounds examined must be known. The K_{PEw}s of triclosan (TCS), methyl triclosan (MTCS), n-nonylphenol (n-NP), n-octylphenol (n-OP), and t-octylphenol (t-OP) into PE were measured as a function of pH, temperature, and salinity, and a salt effect was calculated for TCS, n-OP and t-OP. Log K_{PEw}s used for calculating dissolved concentrations were taken from 20 C studies taking salt into account: 3.42 (TCS), 4.45 (MTCS), 4.13 (n-NP), 3.68 (n-OP), and 2.87 (t-OP). As expected, the K_{PEw} of hydroxyl-group containing compounds were strongly affected by pH, whereas MTCS with its methylated hydroxyl-group was not. Measured K_{PEw}s could not be estimated from octanol-water partitioning constants due to the semi-polar makeup of the compounds investigated. Instead, a good correlation (K_{PEw} = 0.679 * K_{hdw} + 1.033, r^2=0.984, p=0.001) was obtained with hexadecane-water partitioning constants (K_{hdw}) predicted from COSMOtherm. During deployments in Narragansett Bay (RI) in the fall of 2009, concentrations of MTCS and t-OP in surface and bottom waters ranged from 50 - 270 pg L^{-1} and 3.5 - 11 ng L^{-1} respectively. These concentrations are far below EC_{50} values for rainbow trout. Surface/bottom and bottom/porewater activity ratios were calculated, which indicated surface waters as the main source of MTCS, while surface water as well as sediments were sources of t-OP.

EC01B-1

Air concentrations of current use pesticides (CUPs) in Tuscany and Lazio region, Central Italy, using passive air sampler (PUF disk)

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Very little is known regarding the use and levels of pesticides in Italian agricultural regions. Passive air sampler (PAS) comprised of polyurethane foam (PUF) disk were deployed at four sites in the agricultural areas of Montalcino (Tuscany region) and four sites in Terracina (Lazio region) to asses air concentrations of current use pesticides (CUPs). From twelve CUPs screened only 6 were routinely detected in samples (Chlorpyrifos ethyl, Chlorpyrifos methyl, Trifluralin, Clorothalonil, Pendimethalin, and Chlortal-dimethyl). At Montalcino, air concentrations (pg/m3) of Chlorpyrifos (ethyl and methyl) ranged from 3-80, and Pendimethalin ranged from 1-90 followed by Trifluralin 2-20, and Chlorotalonyl 1-5. Chlortal-dimetyl was not detected at this sampling site. These results are lower than those reported in other agricultural areas of the world. However, at Terracina air concentrations were higher than those detected at Montalcino. Air

concentrations (pg/m3) of Pendimethalin ranged from 30-1000 and Chlorpyrifos ranged from 70-10 000 (~10 ng). At Montalcino, seasonal patterns shows that Pendimethalyn was detected only during period 1 and 2 while Chlorpyrifos (ethyl and methyl) showed the uniform distribution during the whole year at all the sampling sites. The seasonal variation of CUPs were less defined at Terracina. Nevertheless, Trifluralin showed a similar patter of pesticide use at both sampling sites with highest concentrations during period 3. The differences observed at the sites might be related to the different agricultural practices. At Terracina, there is a prevalence of conventional agriculture and the use of a wide range of pesticides. However at Montalcino there is a long tradition for organic farming activities.

EC01B-2

Estimating community consumption of Illicit drugs using passive samplers (POCIS) to monitor municipal wastewater

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Monitoring with the Polar Organic Contaminants Integrative Passive Sampler (POCIS) in untreated municipal wastewater may be a tool for accurately estimating community consumption of illicit drugs and other drugs of abuse. We conducted an evaluation of the capacity of POCIS to monitor municipal wastewater for estimates of community consumption of cocaine and its major metabolite, amphetamine drugs, and prescription and illicit opioids. We estimated POCIS sampling rates between 0.1 - 0.3 L/d for the analytes in laboratory studies, and deployed POCIS in untreated wastewater at two wastewater treatment plants (WWTPs) in Canada to estimate community drug consumption. The results were generally consistent with data generated by monitoring 24-h composite samples of the untreated wastewater.

EC01B-3

Pharmaceuticals, personal care products, and agrochemicals in a rural Canadian watershed via passive and active sampling

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A suite of 44 pharmaceuticals, personal care products, and agrochemicals were measured in sewage lagoons and surface receiving waters of Dead Horse Creek, Manitoba, a rural tributary of the Red River draining into Lake Winnipeg. These chemicals were monitored via both active grab sampling and passive sampling using the Polar Organic Chemical Integrative Sampler (POCIS), to determine the capability of both types of sampling in characterizing releases from periodic pulse discharges of sewage lagoon waters to the watershed, and to delineate levels and post-release fate of both human point source and agricultural and veterinary non-point source inputs of these chemicals. Major agrochemicals found were atrazine and 2,4-D, consistent with the heavy agricultural land use in the watershed. These chemicals were not observed in wastewaters, indicating negligible inputs home garden and ornamental use. Trimethoprim was observed upstream of known wastewater outfalls, indicating non-point veterinary release from runoff. Carbamazepine and gemfibrozil were observed in surface waters at concentrations up to 200 ng/L only downstream of lagoon outfalls and only during sewage release, indicating human point sources only. Time-weighted-average concentrations from POCIS were consistent with concentrations from instantaneous grab sampling, indicating that POCIS was capable of adequately capturing pulse inputs of contaminants to surface waters in an integrative, continuous manner.

EC01B-4

Can monitoring for WFD and MSFD be undertaken using passive samplers?

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Contaminant monitoring for priority pollutants is an integral part of the UK obligations under the Water Framework Directive (WFD) and OSPAR monitoring programmes. Also, the Marine Strategy Framework Directive (MSFD) requires Good Ecological Status (GES) be maintained. Descriptor 8 of GES states that concentrations of contaminants are at levels not giving rise to pollution effects.

It is therefore important to establish whether environmental concentrations of chemicals on existing EC and OSPAR chemical priority lists (e.g. those used to determine chemical status under the WFD) are of toxicological significance, and whether there are additional substances with potential to cause harm in the UK marine environment.

In order to inform this process, passive samplers (silicon rubber and POCIS) were deployed to provide information on presence and freely dissolved concentrations of a wide range of potential target substances for monitoring programmes. The survey covered a wide range of locations around the UK, from industrial estuaries to relatively unimpacted offshore waters. Samplers were deployed for periods of 4 - 8 weeks during spring and summer 2009. In a smaller subsequent follow-up survey, samplers were also deployed alongside mussels. The mussels were analysed for priority substances as well as for effects such as stress on stress, DNA damage, neutral red and a number of biomarker via qPCR.

The presentation will focus on the value added by using these techniques over traditional monitoring techniques and what changes to current procedures or measures may be pertinent given the results obtained, in order to achieve good environmental status.

EC01B-5

Sampling of organic pollutants in marine waters using continuous flow integrative samplers and semipermeable membrane devices

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The determination of organic pollutants in the environment is relevant for the evaluation of the environmental status. The presence of organic pollutants in the water column has great fluctuations as a result of a combination of natural and anthropogenic effects. Indeed, the hidrofobicity of these compounds favours their sorption on particulate material and their bioaccumulation. For these reasons bivalves are commonly used in the marine international monitoring programs to evaluate the water column contamination. However as an alternative tool, last decades multitude of passive sampling devices and set-ups are being developed to get a representative water sample. Passive sampler accumulates contaminants as an organism from the environment, being proportional to concentration and time of exposition. The most commonly used device is the semipermeable membrane device (SPMD), that use low density polyethylene membrane for the determination of organic pollutants in surface, ground and marine waters. Recently other innovative integrative samplers have been developed with high sampling rates unaffected by turbulences and with negligible lag values, such as the continuous flow integrative sampler (CFIS), which use polydimethylsiloxane (PDMS) for the determination of polycyclic aromatic hydrocarbons (PAHs) and organochlorine compounds.

In this study, the efficiency of two integrative samplers (SPMD and CFIS) has been tested in the marine environment in spring and autumn. Concretely the study has been performed in Mar Menor Lagoon (SE of Iberian Peninsula, Spain), that is a hypersaline (42 to 47 psu) coastal lagoon with a mean depth between 3 and 4 m, that is one of the largest ones (135 km2) of the Mediterranean basin. Albujón Wadi is the main surface watercourse that flows into this lagoon from Cartagena Field, which is one of the most relevant horticulture areas in Europe. The samplers have been immersed in 4 sampling points for 1 week and the daily concentration of pollutants has been determined by SBSE/GC/MS. CFIS and SPMD systems are two useful tools as integrative samplers in the marine environment and results were similar to those obtained with daily sampling method. Their potential application is limited by the hydrofobicity of the considered pollutants, because they are not adequate for the more hydrophilic compounds as triazines.

EC01B-6

Evaluation of non agricultural pesticides air contamination: a field study using Tenax passive samplers

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Pesticides are not only used in agricultural activities, they are also used for different treatments like pest control or green way and public roads upkeep. To evaluate the influence of these latter activities on air contamination, the technique of passive air sampling has been chosen. This simple method is easy to carry out and cost-efficient, and it allows large scale sampling, essential for providing a specific description of the spatial and temporal variations in the atmospheric contamination levels. The passive air samplers (PAS) used in this study have been developed in our laboratory and consist of a Tenax resin tube protected by a specially designed shelter allowing an air flow. This system enables diffusion through the Tenax resin tube. Afterwards exposure, the tubes have been extracted by thermal desorption using an Automatic Thermal Desorber (ATD). After this first step, molecules have been analysed by gas chromatography and mass spectrometry. In this study, two sites of two companies and one of a private house located in eastern France were chosen to perform the sampling which occurred after a professional or non-professional pesticides application for the upkeep of the areas. In total, eight active substances (2,4 D, Dichlorprop, Diflufenican, Flazasulfuron, MCPA, Mecoprop-p, Picloram and Triclopyr) were used during the treatments and were therefore analysed. PAS were deployed outside but also inside, in the offices or in the house, in order to see if there is a possible contamination coming from outdoors. Results have showed that a part of applied pesticides were found mainly in the PAS corresponding to the day of the treatment. For example, following a treatment using 3 active substances, one pesticide was found, only outside, on the PAS put in place on the application day (Picloram: 1518 - 14326 pg.PAS-1.day-1). The second one was also detected on the treatment day but on following days too (Diflufenican: 1274 - 42941 pg.PAS-1.day-1), suggesting volatilisation process. 2,4 D was observed throughout the sampling, outside and inside (2,4 D: 200 - 19498 pg.PAS-1.day-1. This pesticide was also found in a blank made before the treatment what could be explained by another source, knowing that the sampling site is located in an agricultural area. But the highest values were nevertheless observed on the treatment day.

EC01C-1

Monitoring by LC-MS/MS of 28 endocrine disruptor compounds in surface water using passive sampling devices: comparison of POCIS and Chemcatcher

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Regarding to the growth of interest concerning the presence and the identification of human made pollutants and xenobiotics in the environment, multi-residue analysis techniques and representative water sampling methods need to be developed. Some of these micro pollutants of interest are known as Endocrine Disruptor Compounds (EDCs) due to their effects on the endocrine causing behavior disorders, decreased reproduction or birth malformations for example. As those molecules display large range of physico-chemical properties, most of the current studies are focused on one or two EDCs families.

This project is focused on the evaluation of the current pollution of surface water media threw the development of a multi-residue and multi-family of EDCs analysis method coupled to passive sampling devices.

28 EDCs of interest have been selected for this project among emerging contaminants listed by the European Union. This combination of different EDCs families representating agricultural, pharmaceutical and human pollution, has been chosen to be found in surface water and analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Two passive sampling devices have been selected to monitor those compounds: the Polar Organic Chemical Integrative Samplers (POCIS in Pharm configuration) and the Chemcatcher (in polar configuration). Both are still in development but already showed good efficiency to monitor some of the compounds of interest. Calibration of those systems have been realised with the Cemagref Lyon in 50L aquariums with continuous renewal of doped solutions for 28 days. Both showed good linearity for most of the compounds of interest up to 21 or 28 days but seem to have reached equilibrium after 14 days for some of the molecules. Calibration experiment has been validated by Cemagref Lyon with controled and stable temperature, conductivity and flow. Both systems showed same behaviour but calculated sampling rates were higher for the POCIS than for the Chemcatcher. The two systems have been exposed in the field for 4 weeks between june and july 2010.

Experiments confirmed linearity for somme of the molecules and enable us to calculate sampling rates for some that hasn't been tested yet. Those two passive samplers combined to an optimised multi-residue analytical method allow us to monitor a representative mixture of endocrine disruptors in surface waters.

EC01C-2

Calibrating passive sampling and passive dosing techniques to lipid based concentrations

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Equilibrium sampling into various formats of the silicone polydimethylsiloxane (PDMS) is increasingly used to measure the exposure of hydrophobic organic chemicals in environmental matrices, and passive dosing from silicone is increasingly used to control and maintain their exposure in laboratory experiments. Both these equilibrium partitioning approaches are normally calibrated to freely dissolved aqueous concentrations (C_{free}), which often are considered the effective concentration for partitioning, bioconcentration and toxicity. In the present studies we extend the calibration of such methods towards equilibrium partitioning concentrations in lipids ($C_{lipid,partitioning}$). The first approach proceeds in two steps; (i) the concentration in the PDMS (C_{P-DMS}) is determined and (ii) multiplied with recently determined lipid to PDMS partition ratios ($K_{lipid,PDMS}$). The second approach applies external partitioning standards in vegetable or fish oil for the complete calibration of equilibrium sampling techniques without additional steps.

Equilibrium in tissue sampling in three different fish yielded lipid based PCB concentrations in good agreement with those determined using total extraction and lipid normalization. These results support the validity of the in tissue sampling technique, while at the same time confirming that the fugacity capacity of these lipid-rich fish tissues for PCBs was dominated by the lipid fraction.

Equilibrium sampling of PCB contaminated lake sediments with PDMS coated vials and with Head Space Solid Phase Microextraction (HS-SPME) yielded lipid based concentrations that were in good agreement with each other, but about a factor of two higher than measured lipid-normalized concentrations in the organisms.

Passive dosing was applied to bioconcentration and toxicity studies of several PAHs with the terrestrial springtail *Folsomia candida*. Within the bioconcentration study, equilibrium partitioning concentrations in lipids served as a well defined reference for the evaluation of measured concentrations in the springtails. In the toxicity tests of naphthalene, phenanthrene and pyrene, lethal concentrations were determined also on a $C_{lipid,partitioning}$ basis and were in good agreement with the typical range of lipid membrane burdens for baseline toxicity (40-160 mM). This demonstrates that these new calibration principles also can be applied within a toxicological context.

EC01C-3

PWSDs-YES application as estrogenic chemicals screening and monitoring tool for STP effluents

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Commonly, STPs are regarded as point source of the EDCs in water that have inhibited human and animal endocrine activities [1]. Conventionally to monitor these compounds in water from point source, grab water sampling was used to analyze pollutants but by this method, only water soluble compounds' temporal level can be detected and long term effect caused by bioaccumulation cannot be predicted [2]. It is suggested that integrative passive water sampling devices (PWSDs) like semi permeable membrane device (SPMD) can detect organic compounds in water to monitor wide concentration variation with time and to conjecture bioaccumulation of organic pollutants for a longer period [3]. Also, with combining yeast estrogen screening (YES), SPMD can show estrogenicity of sample as a term of Estradiol equivalent quantity (EEQ) [2]. Passive samplers have been used globally as monitoring tool for organic contaminants in water [4] but in Korea, grab sampling method is only used. With the restoration project on four major Korean rivers these days, water quality, flood control, and ecosystem vitality are major issues in Korea. Therefore, improvement of current water monitoring system is needed to understand correct status of water quality and potential impacts to the ecosystem. The research goal of this study is to develop water quality monitoring system using SPMD-YES combining method for monitoring Korean waters to compensate limitations of current water grab sampling methodologies. As a preliminary study, in this paper, comparison between grab and passive sampling was conducted for estrogenic compounds to check possibility of SPMD-YES application on water quility monitoring. Also several sophisicated conditions including adsorbent type, deployment period were tested.

EC01C-4

Toxic pressure on algae: extraction tools for identification of contaminants in marine waters

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Toxic pressure on algae: extraction tools for identification of contaminants in marine waters

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Summary
Micro-algae as primary producers at the basis of the marine food chain are expected to be affected by anthropogenic contaminants and natural toxins in estuarine and coastal ecosystems. These chemical stressors are hypothesized to disturb algal growth and, therefore, the carrying capacity and sustainable development of estuarine and coastal ecosystems. In our study we aim to identify the chemical compounds that have an effect on algae in order to quantify the toxic pressures in the Dutch coastal zone.
In the present study, passive sampling of dissolved chemicals is performed and different extraction methods are tested in order to find a suitable method for further research with effect-directed analysis (EDA) to identify chemical compounds that affect algal life. Pulse amplitude modulated fluorescence (PAM) was used to test algae toxicity of concentrated samples and fractions thereof. We have compared different extraction methods that are suitable for the concentration of a broad range of compounds from marine water in order to identify unknown toxic compounds. Different passive samplers were compared with water extraction methods in terms of performance and response in the PAM assay.
Passive samplers seem to provide i) an increase in method sensitivity, ii) simplicity in use, and iii) relevance to ecological risk assessments not easily obtainable with extraction of water samples.
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EC01C-5

Sorption and desorption kinetics of polar organic chemicals on Empore disks deployed in the passive sampler Chemcatcher

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Sorption and desorption kinetics of 22 compounds were investigated with Empore disks as a receiving phase. The polymer used was poly(styrenedivinylbenzene) modified with sulfonic acid groups to make it more hydrophilic: SDB-RPS. The octanol water partitioning coefficients ($logK_{ow}$) of the compounds ranged between -2 and 4. Three sorption studies were performed to determine the partitioning coefficients between SDB-RPS and water ($K_{SDB-water}$), sampling rates (R_s), and the time needed for compounds to reach half the equilibrium state ($t_{½}$; a measure of integrative sampling capacity). The sorption experiments were performed in a channel with flowing river water (8 cm/s; ca. 15 °C) that was spiked with the set of 22 compounds to reach aqueous concentrations between 400 and 1000 ng/L. SDB-RPS disks were placed in a modified (rectangular) version of the Chemcatcher disk holder and positioned uniformly at the sides of the channel. Duplicate disks were removed over periods of 6 to 12 days and extracts analysed with LC-MS/MS. Also desorption of chemicals from SDB-RPS was studied in the channel. Disks were placed in the channel with spiked river water to passively load them with the compounds. Then, the channel was drained and refilled with unspiked river water and desorption was followed by removing duplicate disks over 12 days. In another experiment, passively loaded disks were placed in beakers filled with tap water. Thus the proportion of desorbed compounds (over 12 days) could be assessed by measuring both the disk and the water phase. In a third desorption experiment, passive loaded disk were placed in a circular tank with a flow through of deionised water, to maintain aqueous concentrations of desorbed compounds at 0. Sorption experiments produced fairly robust values of $K_{SDB-water}$, R_s and $t_{½}$ (the CV of the parameters ranged between 11 and 21%). Values of all three parameters tended to increase with the hydrophobicity of the tested compounds. Sampling rates ranged between 0.1 and 0.4 L/d, and t½ was fairly low, between 2 and 6 days. All compounds readily desorbed from SDB-RPS. More hydrophilic compounds showed a higher proportion of compounds desorbing in the channel and the beaker (up to 70%) compared to the more hydrophobic compounds (up to 10% desorption). The percentage desorption correlated linearly with hydrophobicity. Desorption reached very high levels >90% in the flow through system running with deionised water.

EC01C-6

Comparison of four passive samplers for the determination of anti-androgenic contaminants in surface waters

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Endocrine disrupting compounds (EDCs) are environmental contaminants that have the potential to disrupt the normal functioning of the neuroendocrine system in humans and animals[1]. Endocrine disruption appears to be particularly prevalent in the aquatic environment,possibly because aquatic animals are highly susceptible to EDCs effects as long-life exposed through wastewater effluents. Thus far, endocrine disruption has been mostly concerned with estrogenic compounds but recent surveys have indicated antiandrogenic activity in aquatic environments[2]. It is therefore crucial to identify the currently unknown structures of antiandrogenic chemicals in effluents in order to determine their contribution to the reproductive dysfunction in aquatic populations and their potential risk to human health. Most conventional environmental screening methods in water involve grab sampling and only provide a snapshot of the levels of pollutants at the time of sampling. Passive sampling can be an alternative sampling approach that overcomes these limitations[3]. Since anti-androgenic compounds in effluents are a complex mixture of polar and apolar chemicals [4], passive samplers able to sample the broadest range of log Kow must be used. Passive samplers can usually be suitable for hydrophilic or for hydrophobic compounds[5]. In this study, 4 different passive samplers were investigated for their ability to extract a variety of hydrophilic and hydrophobic contaminants and detect anti-androgenic activity present in contaminated surface waters. Two single-phase passive samplers were preliminarily tested to check sampling efficiency, showing good recoveries for a wide range of compounds. An extraction method was optimized and passive sampler anti-androgenic activity was tested before and after membrane precleaning to evaluate background contribution. Deployed passive samplers showed significant differences in the anti-androgenic activity profiles. Current work is focusing on the identification of key antiandrogenic contaminants present in passive sampler extracts using bioassay-directed fractionation and mass spectrometry.

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EC01D-1

Headspace POM-SPE as a novel and fast (48 h) passive sampling approach to determine PAH bioavailability in field sediments

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Bioavailability of hydrophobic organic contaminants (HOCs) in sediments and soils can be determined by measuring either the rapidly desorbing fraction (e.g., with Tenax or cyclodextrin) or the freely dissolved concentration in pore water (with passive samplers, such as SPME or POM-SPE). Recent comparison studies suggest that the second approach generally provides the most accurate assessments of uptake and subsequent effects of HOCs; however, the main disadvantages of passive samplers are that their application requires sampler-water partition coefficients and that equilibration takes several weeks. In terms of commercial applications, such long equilibration times are undesirable. The objective of the present study was therefore to develop a novel bioavailability method based on passive sampling, which should however yield results much faster than conventional passive sampling techniques. To this end, we applied headspace analysis with passive samplers at elevated temperatures. Focus was on polycyclic aromatic hydrocarbons (PAHs) in field-contaminated sediments. Out of 6 different headspace samplers, POM was selected as optimal phase, based on extracted PAH pattern and data variability. Optimal

exposure conditions were obtained in stirred systems at 120 degrees Celcius. Under these conditions, equilibrium was reached in only 48 hours. The mass of sediment, water, and POM, as well as the length of the cool-down period appeared not to be crucial, which indicates a robust (not operationally-defined) method. In order to match PAH concentrations in POM upon extraction of sediments during headspace analysis to PAH concentrations accumulated in aquatic worms (Lumbriculus variegatus) during bioaccumulation experiments, a correction factor was required. The application of such a factor does not turn the method into an empirical one by definition; rather the factor reflects the interplay of the (unknown) bioaccumulation factors, Henry constants, and the POM-air partition coefficients at 120 degrees Celcius. Interestingly, the correction factor appeared independent of the PAH and the sediment. Its application resulted in a close relationship between measured and predicted PAH concentrations in worms (predictions within a factor of 5). Headspace POM-SPE therefore appears to be a simple, fast, and robust method to determine PAH bioavailability in sediments. Its advantages over conventional passive sampler use will be discussed.

EC01D-2

Investigation of dioxin-like activity in water from the Three Gorges Reservoir

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The Yangtze River is the third largest river in the world and the Three Gorges Dam (TGD) is the largest dam in the world. The closing of the TGD may result in drastic environmental alterations. Persistent organic pollutants (POPs) are ubiquitous environmental contaminants. Trace POPs in water of TGR were only reported sporadically, their ecotoxicological effects have never been studied there. EROD assay is generally regarded as being an early warning signal for the Ah-receptor-related toxic effects of PCBs, PAHs, and related compounds. EROD with H4IIE rat hepatoma cells bioassay is a bioassay established to measure CYP1A1 induction caused mostly by dioxin-like chemicals in environmental samples.

In this study, triolein SPMD technology was applied to sample and concentrate the priority organic pollutants in water of TGR. EROD-bioassay was used to assess the potential toxicological effects of water in TGR. The aim of this study was to investigate the levels and distribution patterns of Ah-agonists effects in surface water of TGR by combining SPMD sampling technology with EROD bioassay.

After 24 h incubation the TEQ value is related to all the compounds able to elicit a response (persistent and non-persistent compounds). Compounds that still elicit a response after 72 h incubation in the EROD bioassay are defined as persistent. EROD of 7 and 24 d exposure ranged from 27-990 pg TCDD/sample. However, the TEQ of PCB only ranged from 0.04-2.5 pg TCDD/sample. Therefore, PCB contributes very little to the EROD activity in the samples from water of TGR. The TEQ of PAH ranged from 6.4-42.3 ng TCDD/sample. Therefore, PAH in SPMD may be main contributor to EROD activity of 24 h incubation. In case of 72 h incubation, other persistent compounds not targeted in the study may exist to cause EROD induction. Further study warranted to clarify the differences.

EC01D-3

Determination of high partition coefficients from diffusive mass transfer in the boundary layer

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In many areas of environmental sciences and engineering, partition coefficients (K) are widely used for the characterization of the fate of chemicals. Experimental determination of partition coefficients between two phases, however, becomes very difficult as the absolute value increases. In a conventional shaking method, time required for equilibration between two phases increases exponentially with increasing log K. This often results in many potential experimental artefacts. Thus, an innovative method using the diffusion in the boundary layer has been developed to measure partition coefficients between two phases. According to a film diffusion theory in the boundary layer, the rate of diffusive mass transfer is a function of diffusion coefficient, the thickness of the boundary layer of a fluidic medium, and partition coefficient between the two phases. Partition coefficient can be correlated with experimentally determined mass transfer coefficient by a simple relationship because diffusion coefficients in a well-characterized medium such as air and water can be easily predicted and the thickness of the boundary layer is a dynamic property of the medium. This principle is substantiated in three model systems - partition coefficients between poly(dimethylsiloxane) and water (K_{PDMSw}), 1-octanol/air partition coefficient (K_{oa}), and Henry's law constant (H). Kinetically measured partition coefficients were well correlated with literature data without only a few chemicals. The range of partition coefficients that can be determined within 30 h was 10^3~10^6 for K_{PDMSw}, 10^5~10^{10} for K_{oa}, and 10^{-4}~10^{-7} atm m^3 mol^{-1} for H.

EC01D-4

Does the kinetic resistance on the sampling medium side affect passive air sampling rates? Experimental evidence and modeling.

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The mechanism of passive air samplers (PASs) is based on molecular diffusion from air to the passive sampling medium (PSM). According to the curret model for the PAS, the kinetic resistance for the chemical transfer is mainly contributed by the boundary layer between air and PSM. Within the PSM, the chemical is assumed to distribute uniformly and the kinetic resistance is negliable. The air concentration (ng/m^3) can be derived from the sequestered chemical amount (m_s, ng) and the passive sampling rate (R, m^3/d). At the initial uptake stage, when surface evaportation is negliable, mS linearly increases with the PAS deployment time and R is proportional to the chemical's molecular diffusivity in the air, which is a weak function of temperature and molecular size. However, recent field calibration studies on PAS observed a larger variations of R on temperature and molecular size than can be explained with the model. In this study, we developed a hypothesis that the chemical sequestered on the PSM is not uniformly distributed. To test the hypothesis, uptake kinetics within PSM was investigated by using cylindrical XAD- (and PUF-)PSM concentrically segmented into three layers. PCBs uptake kinitics on each of the layers were measured in duplicates at seven time points from 1 (0.5) week to 24 (12) weeks. Results indicate PCBs sequestered are non-uniformly distributed within the PSM. Even for the mono- and di-PCBs which are more volatile (lower sampling medium-air partitioning coefficient), over 80% distributed in the outter layer of the PSM. Based on the experimental results, a model considering the chemical transfer and kinetic resistance within the sampling media was developed to describe the passive sampling process.

EC01D-5

Sorption behaviour of polar organic compounds on passive sampling material - Which functional group governs sorption?

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Pharmaceuticals are expected to become a growing problem.[1] Very often, these compounds can be found in waste water, or enter directly in the environment. In general, they are polar or even charged molecules and their concentration are low, which makes their detection often very difficult. Concentration of these compounds with passive sampling is promising way to allow for the analysis with standard techniques such as MS. Many materials have been employed for this purpose,[2-4] but not much is known about the exact sorption mechanism of the compounds on these materials.

Aim of the research is to get a better insight into the influence of various functional groups in selected chemicals on the sorption to frequently employed passive sampling materials. Therefore, we decided to employ the commonly used OASIS polymers (HLB, WCX, WAX, MAX and MCX). These polymers carry a-polar moieties, hydrophilic part as well as charged groups, which should allow the adsorption of the target compounds. Various polar chemicals were tested on the different OASIS polymers to determine which functional group in the compound is crucial for a successful sorption on the OASIS material. This knowledge is crucial to taking a grounded decision which sorbent can be employed as a passive sampling material.

The results of this research indicate that especially apolar functionalities have a great impact on the sorption, whether the compound is charged or not. The more pronounced the apolar moiety is, the better the compound can interact with the sorption material. Furthermore, it emerged that the conditions of the aqueous phase, such as salt concentration, influence the sorption behaviour of charged molecules dramatically. The higher the salt concentration the lower the adsorption of charged compounds, hence the Kd values.

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EC01D-6

Application of passive dosing to study the biotransformation and biodegradation of hydrophobic compounds

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Achieving well-defined and constant dissolved concentrations of hydrophobic compounds is challenging due to volatilization or sorptive losses. With passive dosing, continual partitioning into the test medium of compound(s) loaded in a polymer compensates for losses, and provides defined and constant dissolved concentrations. Passive dosing can be used for studying biotransformation/degradation. Here, the polymer HOC reservoir also compensates for losses due to the bio-transformation/degradation process itself. Furthermore, a large mass of test compound is introduced so that compound turnover is significant even at low dissolved concentrations thus facilitating measurement of the relevant endpoint (e.g., metabolic products in biotransformation or growth in biodegradation). This study details two applications of passive dosing for studying bio-transformation/degradation. A format has been developed to study the biodegradation of phenanthrene and fluoranthene by the bacterial strain EPA 505, allowing degradation rates to be quantified at defined freely dissolved concentrations from mg/L down to ng/L levels. Passive dosing was also applied for quantifying the mutagenicity of benzo(a)pyrene metabolites produced after activation by the liver S9 mix in the in vitro Ames II assay. Compared to the case with spiking, responses from passive dosing were shifted by a factor 100-1000 to lower concentrations, and were also more reproducible between repeated tests. This difference in apparent sensitivity cannot solely be explained by partitioning, and is due to slow dissolution kinetics as well as mass-depletion of the spiked benzo(a)pyrene. Therefore, passive dosing is a useful tool for the study of hydrophobic compound bio-transformation/degradation at well-defined dissolved concentrations down to very low levels. Important advantages include studying process kinetics at precisely defined dissolved concentrations and allowing increased compound turnover even at constant and low concentrations.

EC02 - Atmospheric chemistry, transport and deposition

EC02A-1

The global fate and transport of organic contaminants: concepts and misconceptions

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Following the detection of synthetic organic chemicals in remote and sparsely populated regions of the global environment in the 1960s, there has been a steady increase in the understanding of the processes that control a chemical's spatial range in the environment. Measurements of the occurrence of contaminants in remote environments have been complemented with conceptual and numerical models of varying complexity that seek to explain, quantify, and predict a chemical's ability to undergo long range transport and to accumulate in remote regions. In particular, the concepts of global fractionation and global distillation that we formulated in two papers in the mid-1990s have captured the imagination of many with a concern for the global fate and transport of organic contaminants. While reviewing the literature we frequently encounter misconceptions, especially surrounding the use of terms such as the global chromatography, global fractionation and distillation, grass-hopping, and cold condensation. We have encountered a number of misconceptions, such as "cold condensation results in the precipitation of pure chemical", "the bulk of a persistent organic contaminant's global inventory will eventually migrate to reach the poles", "global fractionation is driven by temperature gradients"or "low temperatures will cause higher concentrations in air". It is perhaps inevitable that misconceptions arise because the phenomena are complex and there is often a lack of reliable emission, monitoring and physical chemical property data. In this presentation we describe and discuss the concepts or present state of the science surrounding global transport of chemicals, especially over considerable latitudinal differences with their inevitable differences in temperature.

EC02A-2

Global cycling of PCBs - intercontinental and northward migration of distributions predicted by multicompartmental modelling

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Global distribution and fate of contaminants depend on physical-chemical properties, geo-spheric transports, effectiveness of exchange between the atmosphere and ground compartments and degradation kinetics. A comprehensive global multicompartmental model (MPI-MCTM, [1]) is used to study how substance properties together with environmental conditions propagate into PCBs global distributions. This is the first time a fully coupled global atmosphere ocean general circulation model is used for studying PCBs. A special focus is given to intercontinental transport To discern the impact of the substances physico-chemical properties on fate, identical primary emissions (i.e. PCB 153 emissions were input to study four PCB congeners, i.e. 28, 101, 153, and 180. The period 1950-1995 was simulated under present-day model-generated climate with spatial resolutions of 3.75° (T31) in the atmosphere and approx. 3° (GR30) in the ocean. The substance large-scale mobility in meridional direction is compared with the predictions of a global, zonally averaging model, GloboPOP [2], run with similar input parameters (emissions, besides others).

The centre of gravity (COG) of soil and vegetation burdens shows a different migration pattern for Eurasia and North America and amongst the PCB congeners. Whereas for Eurasia the migration is following the main wind direction (westerlies), for North America the formation of a secondary burden maximum in Alaska shifts the COGs to the northwest. This secondary maximum was found to be caused by trans-Pacifc transport of PCBs, rather than local emissions. Trans-Pacfic transport of PCBs is shown to be related to an amplified wave pattern in the North Pacific region, with a lower than usual Aleutian Low and higher than usual surface layer pressure over the north American continent leading to events of effective transport.

The results proof that atmospheric transport is very effective for large-scale re-distribution of PCBs. The meridional re-distribution is more effective, but relaxes slower to long-term changes in emissions than predicted by a zonally averaging model. On the decadal time scale trans-Pacific transport constitutes a significant secondary PCB source to North America.

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EC02A-3

Can clouds enhance long-range transport of low volatile, ionizable and surface-active chemicals?

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Atmospheric partitioning and transport of low volatile organic compounds is strongly influenced by the presence of water (e.g. clouds) and its deposition velocity (e.g. rainfall, snow). It was identified that the assumption of continuous rainfall underestimates the residence time and the transport potential of non-volatile substances. The liquid water content of clouds and the high specific surface of frozen or liquid cloud droplets can significantly contribute to the total activity capacity (i.e. the capacity to sorb chemicals) of the atmosphere for non-volatile, ionizable and surface active substances.

A modified version of the regional multimedia activity model for ionics MAMI, including two-layered atmosphere with atmospheric boundary layer (ABL) and lower/middle troposphere (LMT), interface partitioning, intermittent rainfall and variable cloud coverage was applied to a selection of ten low volatile or ionizable chemicals to investigate the potential of clouds to enhance the atmospheric transport potential.

Probability density functions were derived for input substance properties and environmental parameters to quantify uncertainty and variability and probabilistic simulations at steady state were run for a constant emission to the atmospheric boundary layer to identify key model inputs.

The degradation rate, the duration of dry and wet periods and the parameters describing air-water bulk partitioning (K_{AW} and T) and ionization (pK_a and pH) determine the residence time in the ABL. In the LMT, however, the residence time depends also on the water content of clouds and on interface partitioning. In some cases the residence time and its variability range is similar in the two compartments, while some compounds (e.g. diazinon, 2,4-D, perfluorooctanoic acid) are more persistent in the LMT. The longer residence time predicted for some compounds in the LMT is due to the capacity of clouds to sorb non-volatile molecules in the liquid water and at the interface of cloud droplets.

The efficiency of wet deposition to remove low volatile organic pollutants from the atmosphere is limited primarily by the duration of the dry interval between precipitation events. During dry periods persistent non-volatile chemicals can be transported to the troposphere. Here, the high capacity of of tropospheric clouds to sorb non-volatile and surface active chemicals limits oxidation and wet deposition rates and increases the potential for long-range transport

EC02A-4

Organic tracer compounds of ambient air particulate matter in the Western Mediterranean Basin and influence of natural and anthropogenic emission sources

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Monitoring and chemical analysis of atmospheric particulate matter (PM) is important due to its health impact and influence on climate change (1,2). The air quality in the urban area of Barcelona in the Western Mediterranean Basin is dominated by traffic related emissions and characterized by high levels of particulate matter and reactive chemical species due to emissions, the weak synoptic conditions and high solar radiation (3,4). Nevertheless, occasionally emissions from natural sources, such Saharan dust from Northern Africa and wildfires on the Iberian Peninsula, substantially influence the levels of the PM (5). Selected filter samples from 2009, representing different 'scenarios', were analyzed on organic tracer compounds, e.g. polycyclic aromatic hydrocarbons, hopanes, alkanes, anhydrosugars and primary sugars, as well as secondary organic aerosols tracer compounds, e.g. carboxylic acids. The results are discussed in terms of their relation to emission sources and influence of meteorological conditions in order to get an insight on the complex organic aerosol.

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EC02A-5

Sources, Transport and Deposition of Polycyclic Aromatic Hydrocarbons: China and the Western U.S.

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Polycyclic aromatic hydrocarbons (PAHs) emissions have been increasing in parts of Asia due to increased combustion and China is now the world's largest emitter of PAHs. Size fractionated particulate matter (PM) samples (including PM2.5 and PM10) were collected in Beijing before, during and after the 2008 Olympics, during both source control and non-source control periods, and analyzed for a wide range of PAHs, nitro-PAHs, oxy-PAHs, and high molecular weight (MW 302) PAHs. These data were used to understand the effect of source control measures on reducing PAH concentrations in a Chinese megacity. In addition, using a series of remote sites located at different elevations in the Pacific Northwestern U.S., the episodic atmospheric transport of PAHs from Asia to the U.S. west coast was identified. The deposition and accumulation of PAHs was also studied at remote, high elevation sites in western U.S. national parks. PAH sources to these remote ecosystems included local aluminium smelting activities, urban areas, and natural gas flaring.

EC02A-6

Contaminant profiles of air and soil around Casey station, Antarctica; discerning local and distant contaminant sources

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The primary input of Persistent Organic Pollutant (POP) contamination to the Antarctic is expected to be via Long Range Atmospheric Transport (LRAT) from emissions in neighboring Southern hemisphere nations. In addition to LRAT, system input of POPs must increasingly consider alternate pathways. Human activity in the Antarctic represents a potential direct source of both legacy and current-use chemicals.

It has been two decades since the organic chemical composition of air masses arriving in the Australian Antarctic Territory (AAT), which spans the majority of the eastern Antarctic sector, was last investigated. Here we present the first atmospheric measurements made as part of a new continuous monitoring effort at Casey station (66°17' S 110°31' E), one of Australia's all-year research stations. The results are evaluated alongside POP contamination data of soil samples collected around the Casey station perimeter and the respective sample profiles are assessed for clues as to local and distant contamination sources.

Results suggest a potential local source of the currently produced, involatile, deca-brominated PBDE congener 209 which contributed substantially to PBDE profiles of all samples. Profiles of polychlorinated biphenyls (PCBs) and organochlorine pesticides on the other hand primarilly support LRAT as the primary input pathway of these contaminants, whilst a dominance of endosulfan in air samples evidences its ongoing application in the southern hemisphere.

EC02B-1

A trace element approach for determining the geographic sources of semi-volatile organic contaminants in an alpine ecosystem in New Zealand

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Semi-volatile organic contaminants (SOCs) are known to be transported thousands of kilometres in the atmosphere. Ultimately, these SOCs end up in cold remote ecosystems far from where they have been used. The transport mechanism of SOCs to these pristine locations occurs via a series of volatilisation-transport-condensation steps, however little is known about the SOC contribution of different geographic sources to a specific remote site. Previous studies have used local wind observations and/or air-mass back-trajectory modelling to gain information about the geographic sources of SOCs to remote sites. However, these modelling-type approaches have limitations. Local wind observations can be highly variable, especially in complex mountainous terrain, and do not adequately describe long-range sources. On the other hand air-mass back-trajectories tend to ignore localised synoptic-scale winds in an alpine area in favour of global-scale winds.

The objective of this study was to investigate a new geographic source apportionment method for SOCs that uses trace element profiles in atmospheric particulate matter (PM) to determine SOC origin. Daily samples of PM and SOCs were simultaneously collected using two high-volume air samplers from 16 January to 16 February 2009 at Temple Basin, a remote alpine ecosystem in New Zealand's Southern Alps. For each sample day, the relative contribution of regional New Zealand sources versus longer-range Australian sources was determined using a binary mixing model of the source trace element profiles. In addition, local wind observations were used to determine the percent of time the wind came from each source region, while HYSPLIT air-mass back-trajectory modelling was used to determine the percent of time the air mass had spent in each source region before reaching the sampling site. The trace element, local wind observation and air-mass back-trajectory approaches were used in correlation analysis with SOC concentrations. Correlation analysis indicated that the organochlorine insecticide endosulfan I tends to be transported from Australia, while the broad-spectrum organophosphate insecticide chlorpyrifos is largely influenced by regional transport from the agriculturally-rich Canterbury Plains of New Zealand. In addition, the high molecular mass polycyclic aromatic hydrocarbons (PAHs) tend to come from longer-range Australian sources, while the low molecular mass PAHs tend to come from more localised New Zealand sources.

EC02B-2

Comparison of lichen, conifer needles, passive air sampling devices, and snowpack as passive sampling media to measure semi-organic volatile organic compounds in the atmosphere

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Passive sampling media, including lichen, two-year old conifer needles, resin-based passive air sampling devices (PASDs), and snowpack were used to monitor semi-volatile organic compounds (SOCs) in the atmosphere at pristine, high elevation sites in western United States national parks. The magnitude of accumulation and relative sorption affinities for SOCs were compared in

all four sampling media to determine the affect of media properties and SOC physical chemical properties on accumulation. Lichen and two-year old conifer needles preferentially accumulated endosulfan sulphate, PASDs preferentially accumulated HCB, and snowpack preferentially accumulated dacthal, pyrene, benzo[a]anthrancene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]perylene, dibenzo[ah]anthracene, and benzo[ghi]perylene. Regression analysis confirmed snowpack and lichen showed a similar composition of the atmosphere compared to conifer needles and PASDs. The influence of air-water partition coefficient (K_{AW}) , log octanol-air partition coefficient (log K_{OA}) , and the estimated SOC fraction in the particulate phase (Φ) on accumulation was evaluated. All four passive sampling media accumulated more SOCs with K_{AW} values up to 0.05, log K_{OA} values between 8 and 10, and Φ up to 20. Of the total measurements made for lichens, conifer needles, PASDs, and snowpack, 4, 0.7, 0, and 22% corresponded to SOCs with Φ > 60% indicating lichen and snowpack had a greater affinity for particulate phase SOCs compared to conifer needles and PASDs.

EC02B-3

Influence of the longe-range transport of POPs in the Antarctic ecosystem

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Polar territories play a very significant role in global environmental processes as they can give useful information about the influence of long-range transport processes, compound partitioning and fate processes. The most significant findings on the presence of persistent organic pollutants (POPs) in the terrestrial ecosystem of Deception, Livinsgtone Island and Byers Peninsula (Antarctica) are presented. Soil and antarctic vegetation (liquens and mosses, which in fact are important bioindicators of atmospheric pollutants deposited by wet or dry deposition), samples were collected in many sampling sites located in the large area of the Islands during a Spanish expedition in the Antarctica (Jan-Feb 2009) within the ATOS project. Air samples were also collected with active samplers deployed in 3 different altitudinal sites of Livingstone Island (from the Spanish Juan Carlos I research facility to the glacier). Only low-medium MW PAHs and medium chlorinated PCBs and HCB were detected in most soils, vegetation and air samples, with exception of those samples taken close to the research facility, in which the higher MW PAHs were also detected. This finding highlights the importance of the long-range transport of POPs to the pristine ecosystems. Higher POPs concentrations were also detected in soils of penguins colonies, suggesting that apart of long range transport of POPs, antarctic penguins may also redistribute POPs at local scale.

EC02B-4

Polycyclic aromatic hydrocarbons in air over Central Europe: what can we learn from diagnostic ratios for source apportionment and reactivity?

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Despite numerous uncertainties, diagnostic ratios (DRs) of parent polycyclic aromatic hydrocarbons (PAHs) are frequently used for source apportionment of these pollutants. However, due to the simplicity of the method, it is attractive especially for countries, where (e.g. due to lack of measurement data) sophisticated source apportionment methods cannot be applied.
The following five commonly used PAH DRs were investigated: anthracene / (anthracene + phenanthrene) ANT/(ANT+PHE), fluoranthene / (fluoranthene + pyrene) FLT/(FLT+PYR), benzo(a)anthracene / (benzo(a)anthracene + chrysene) BAA/(BAA+CHR), indeno(123cd) pyrene / (indeno(123cd)pyrene + benzo(ghi)perylene) IPY/(IPY+BPE) and retene / (retene + chrysene) RET/(RET+CHR).
PAH DR values derived from a literature survey on suitable PAH emission factors were applied to study their ability to distinguish between PAH sources at sites with well described source categories (road traffic, residential heating, industry) in the Czech Republic, Serbia and Bosnia and Hercegovina. Later, seasonal changes in source characteristics at the background receptor site Košetice, Czech Republic, were examined using ambient PAH data from long term monitoring (1996-2008). Then a mass balance model of PAHs in air was applied and uncertainties of PAH reaction rate coefficients were narrowed down. The suitability of PAH DRs for distinguishing between various characters of sampling sites (urban, industrial, rural, background) in regions with very limited information on ambient PAHs (15 countries in Africa) was also studied using data from a passive air sampling campaign conducted in 2008.
A good agreement between some literature based PAH DRs and ambient DRs from sites with a dominant influence of a local source was observed. However, the current knowledge on PAH reactivity was found to be insufficient for source apportionment of atmospheric PAHs at receptor sites far from sources. DRs were used to narrow down ozone and OH radical reaction rate coefficients´ uncertainties: kO3(2) of PYR and BPE in the particulate phase seem to be _10% of the highest rate coefficient measured using model aerosols in the laboratory and kO3(2) IPY in the gas phase could be higher than previously estimated by three orders of magnitude.

EC02B-5

Background atmospheric concentrations of PAHs are controlled by ubiquitous emissions from soils

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Soils are the major reservoir and one of the major sinks for POPs due to their strong affinity to soil organic matter (SOM). The fate and toxic effects of POPs are strongly affected by the quality and quantity of SOM which controls POPs availability for biological degradation, burial or revolatilization from soil. The main objectives of this study are to i) study the temporal and spatial variability of soil PAH fugacity and soil-air partition coefficients in rural and semi-rural areas of the Ebro river basin (Spain), NW England and urban Barcelona; ii) determine the soil/air fugacity quotients and the variables affecting them, in different soil types, under field conditions; iii) consider the field evidence for possible in-situ production of lighter PAHs in temperate areas to improve the understanding of PAH cycling and their implications as soil emissions controlling atmospheric concentrations of PAHs. Large soil fugacities were found compared to ambient air fugacities for PAHs with 2-3 aromatic rings and their alkyl derivatives at all the sites. The values of fs/fa were larger in early and late summer than in early winter. Daily ln (fs/fa) for sampling sites in the UK decreased with increasing KOA, indicating that the soil is an important source of lighter PAHs. This means that in agreement with previous works, the air concentration of the low molecular weight PAHs in the temperate areas is now being mostly controlled by the re-emissions of these chemicals from the soil. In order to study the influence of soil characteristics and temperature on gradients, the soil to ambient air fugacity ratios (fs/fa) were regressed against the different parameters describing the soil properties by equation: fs/fa= a + b (1/T) + c (log redox), thus indicating that the fugacity ratios increase at higher temperatures, and higher soil redox potential. The influence of the temperature is consistent with the seasonal variability and demonstrates that during warm periods soils increment their strength as a source. The influence of redox potential can be related to SOM quality or to soil microbiology, an important factor that could be related to in-situ production of PAHs from degradation of organic matter. The potential influence of soil emissions of PAHs as a factor controlling atmospheric concentrations of PAHs is discussed.

EC02B-6

Atmospheric deposition fluxes of contaminants close to a municipal solid waste incinerator

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European committee for standardisation approved on October 2009 EN 15841, the Standard method for determination of arsenic, cadmium, lead and nickel in atmospheric deposition. It recommends the use of bulk collectors, as Italian National Institute of Health (ISS) had already indicated. In this study atmospheric depositions were collected with bulk samplers, with the same features indicated in the new standard method. The goal of this study is to assess the contribution of a Municipal Solid Waste incinerator to the area's total contamination with polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDDs and PCDFs), polychlorinated biphenyl (PCBs), and heavy metals (HM). New generation plants, built after European Directive 2000/76/EC and the implementation of Best Available Techniques (according to IPPC Directive 96/61/EC), reduced the emissions of pollutants. However the locally contribution of POPs and HM in the flux depositions could be not negligible. The incineration plant studied is situated in a suburban area, not far from a tourist town (Riccione), an important Italian highway (A14), and the Adriatic coast. This plant is authorized to burn 127,600 t per year of urban, hospital, and cemetery solid waste. Samples were collected at five sites, three situated in the area most affected by plant emissions (according to the results of the Calpuff air dispersion model), one in an external site, considered as a reference, and one in an urban site. From 2006 to 2010, bulk atmospheric deposition samples were collected monthly for heavy metal analysis and at intervals of about 6 months for PCDD/Fs and PCBs analysis.
Results show that the studied area is subject to low contamination, as far as these compounds are concerned. Deposition flows show no significant differences among the monitored sites. However some temporal trends can be observed. In conclusion, the incineration plant is not the main source of pollutants in the studied area, which is apparently characterized by a homogeneous and widespread contamination situation, typical of an urban area. This is confirmed by the observation that deposition flows are not significantly lower than the other years, even though the plant was shut down for 6 months.

EC03 - Biodegradation and bioremediation of organic pollutants

EC03-1

Assessing bacterial diversity and its influence on biodegradation potential

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Biodegradation - and its corollary, persistence - is an important but poorly understood fate process that is central to all mitigation strategies, both at pre-manufacture stage of chemicals, and when they are eventually present in the environment. It represents one of the greatest scientific uncertainties in assessments (e.g. life-cycle, fate and exposure models) that underpin EU directives affecting their control (e.g. REACH, IPPC, WFD). Biodegradation is often measured experimentally by observing the degradation of a chemical substance in the presence of a bacterial inoculum, and relies on the probabilistic inclusion of specific degraders in the test system. They usually rely on one or a few inoculum sources and chemical substances. Ready biodegradability tests (RBTs) have long been the central foundation for understanding the biodegradation of chemicals in regulatory frameworks, but a recent shift in emphasis towards identifying persistent chemicals has resulted in REACH guidance advocating the use of longer duration tests with environmentally-relevant inocula concentrations.
Little is kown about the natural variation in biodegradation potential of different environmental compartments or how bacterial diversity influences such variation. We have recently been investigating how inoculum source, concentration, bacterial community composition and diversity (and the factors thought to impact it) affect biodegradation outcome. To achieve this we have been conducting inocula sample surveys, applying the latest molecular methods to characterise their diversity and subjecting them to novel miniaturised high-throughput biodegradation tests. One of our aims is a better understanding of biodegradation for the development scientifically sound screening tests for persistence.

EC03-2

The biodegradation of para-nitrophenol in river water: a functional gene approach

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OECD chemical biodegradation tests are conducted in the dark using unrealistically high concentrations of test chemicals. We investigated the effect of light on the biodegradation of *para-* nitrophenol (PNP) and the associated impacts on bacterial community composition. The biodegradation of PNP was followed over 100 days in water collected from the River Dene (Wellesbourne, UK). Total degradation of PNP at the concentration of 2mg/L occurred within 7 days in the dark, while degradation under light conditions was inhibited with only one replicate degrading (93%) after 56 days. Cultivable PNP degrading bacteria were not detected under light conditions. However, under dark conditions after 100% degradation of PNP, 10^5 cells/mL of cultivable PNP degraders were detected. Terminal Restriction Fragment Length Polymorphism of the16S rRNA gene indicated that bacterial community structure was differentially affected by dark and light conditions and that an isolated PNP degrader, *Pseudomonas syringae*, proliferated in the dark bottles but was absent from the light bottles. In addition, a fragment of PNP degrada-

tive gene *pnpA* (~267bp) was amplified from *P. syringae* which confirmed that this strain was a key PNP degrader *in situ*.

EC03-3

Impact of cell concentration methods on the performance of enhanced biodegradation tests within REACH

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Regulatory emphasis has shifted recently towards hazard identification and prioritising chemicals which are persistent, liable to bioaccumulate and are toxic, since chemicals with these properties have previously been shown to be most harmful to human health and the environment. Biodegradation is one of the most important fate processes determining persistence. Ready biodegradability tests (RBTs) have formed the core protocol for developing regulatory guidelines for persistency and environmental exposure assessments. They are highly prescribed, very stringent standardised tests that measure the biodegradability of chemicals. Due to the stringent nature of the tests and their high false negative rate, they offer little potential for prioritising on the basis of environmental persistence. This has been recognised in REACH guidance which advocates the introduction of a new tier of enhanced tests to enable efficient and effective identification of persistent chemicals. Reliable extrapolation from any small-scale systems to predict effects at local and regional levels are dependent upon test systems truly being representative of the real environment, including the nature of the microbial populations present. Enhanced tests allow increases in inoculum density to environmentally-equivalent concentrations, thereby incorporating increased and realistic microbial diversity. This presentation will describe the application of the latest molecular techniques and ecological models to quantify the distribution, abundance and diversity of bacteria in different environmental compartments and their influence on biodegradation outcome, with the eventual aim of developing robust screening tests for persistence.

EC03-4

Degradation of PFCs by anaerobic bacteria

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PFOS, PFOA and other PFCs are considered to be persistent compounds in the environment due to the highly stable C-F bond. Nevertheless, there are examples of microbially catalysed defluorination reactions, such as those observed for fluorinated acetates and benzoates. Reductive defluorination reactions under anaerobic conditions are exothermic. Such reactions could potentially serve as a terminal electron accepting process for anaerobic bacteria, as is the case for many reductive dechlorination reactions. A large number of anaerobic bacteria have been identified that use reductive dechlorination as an electron accepting process, often referred to as chlororespiration. Since the dehalogenases present in reductively dechlorinating bacteria may also be active towards other organohalogen compounds, we have tested a number of reductively dehalogenating bactera with PFCs. Several Desulfitobacterium strains, including Desulfitobacterium sp. PCE1, D. hafniense DCB-2, D. hafniense TCE1 and D. chlororespirans Co23 were incubated with PFOA and PFOS under anaerobic conditions. Culture samples were analysed by LC-MS/MS over a period of 14 months for PFOS, PFOA and their putative metabolites. First results from these experiments indicate that some of these strains are indeed able to dehalogenate PFCs.

EC03-5

Monitoring the transport and degradation of triclosan in field soils receiving sewage sludge

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The anti-microbial substance triclosan has widespread use in home and personal care products. It is moderately hydrophobic and can, therefore, partition to sewage sludge following waste water treatment. A field experiment was conducted over a 12 month period between November 2009 and October 2010, in order to track the fate of triclosan in three different soils (a sand, a sandy loam and a clay soil) in Eastern England following the application of sewage sludge. Three replicate plots were established on each soil. Dried sewage sludge pellets with a native triclosan concentration of 15-38 mg kg-1 were manually incorporated into the top 10 cm of each plot at an application rate equivalent to 50 tonnes per hectare. Monthly soil samples were taken at three depths (0-10, 10-20 and 20-30 cm). Triclosan concentrations were measured using GC/MS following accelerated solvent extraction (ASE) and solid phase extraction (SPE). Concentrations in the top 10 cm were initially very high in all plots (850-900 _g kg-1) but decreased progressively. Relatively rapid methyl-triclosan formation was measured in the sand and the loam soils between March and June, suggesting that triclosan biodegradation accelerated over this period. Methyl-Triclosan is a known methylated metabolite which is more lipophilic and is believed to be more environmentally persistent than the parent compound. Degradation was less pronounced in the clay soil and there was triclosan translocation to the lower horizons (20-30 cm). This probably reflects an increased retention of triclosan in the soil solid phase due to a higher organic matter content in this soil. Most triclosan movement was observed in the sandy soil, reflecting better drainage and associated leaching of solutes and colloids. After 12 months, triclosan concentrations had decreased to 15-20% of initial concentrations in the top 10 cm of soil. However, triclosan and methyl triclosan concentrations in the lower soil horizons (10-30 cm) were detectable. The results of this study provide evidence for triclosan degradation and leaching in different field soils and highlight the role of soil properties in controlling environmental fate.

EC03-6

The effect of non-UV light on the microbial degradation of crop protection products in an aerobic soil system

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Crop protection products (CPPs) are subject to strict regulatory processes prior to approval for commercial use. Laboratory studies are routinely used to study the environmental fate of CPPs, however systems may not provide an accurate representation of field conditions, and therefore extrapolation of data to a field environment is questionable. It is therefore essential to add complexity to laboratory studies to create a more environmentally realistic system. The following study investigates aerobic degradation studies of CPPs in soil which are typically conducted under dark conditions. The effect of phototrophic organisms on the transformation of CPPs was investigated by the inclusion of non-UV light to standard laboratory systems. A preliminary experiment compared the time it takes for 50%/90% of a compound to degrade (DT50 and DT90) for the experimental fungicide "fungicide A"and the herbicide chlorotoluron. A second experiment compared the transformation of several CPPs at a single sampling point; namely, prometryn, cinosulfuron, imidacloprid, lufenuron, propiconazole, and fludioxonil.

The DT50 of fungicide A was approximately halved from 373d to 183d under dark and light conditions, respectively. The DT90 of chlorotoluron was similarly halved from 79d to 35d under dark and light conditions, respectively. Experiment 2 showed a significant reduction ($p \leq 0.05$) in extractable parent compound under light for prometryn (4%), imidacloprid (8%), and fludioxonil (24%). Cinosulfuron behaved atypically to other compounds with a 14% increase in extractable parent compound under light ($p \leq 0.05$). There was no significant difference in CPP transformation under light for propiconazole and lufenuron. Chlorophyll α was significantly higher under light treatments for all compounds tested ($p \leq 0.05$) indicating the presence of phototrophic organisms in these systems. The enhanced rates of degradation observed for the majority of compounds may be due to direct degradation by phototrophic organisms or an increase in viable biomass/microbial activity from the addition of C produced by photosynthesis.

Light treatments displayed significantly higher bound residues for six of the compounds tested ($p \leq 0.05$), which may be due to; (i) 14CO2 assimilation by phototrophs; (ii) Degradation by photosynthetic or non-photosynthetic organisms, or; (iii) Sorption of CPPs to soil particles.

EC04 - Environmental fate and bioaccumulation of organic pollutants in aquatic systems

EC04A-1

Atmospheric transport and bioconcentration in plankton of chlorinated persistent organic pollutants in the Greenland current and Arctic Ocean

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Occurrence of POPs in the North Atlantic and Arctic Ocean is one to most important issues in the actual Global Change Scenario and during the last Decades. The Transport and subsequent deposition of this Pollutants to the Arctic has been studied extensively in the literature from the North American and Eurasian plateau but there is a lack of Knowledge about the transport of pollutants trough the North Atlantic and Arctic (Gioia et al., 2008; Sobek et al., 2004 and 2006). Several studies reported smaller latitudinal gradients and decreasing concentrations, from Europe to the Sub Arctic Ocean and Ice Covered Regions. Published results in this area did not report particulate, aerosol and Phytoplankton concentrations this study is the first showing this concentrations at the same time in the studied area. No residence times are published for pollutants in this area, this work is the first showing residence times in a latitudinal gradient, that could be compared to models published before (Jurado and Dachs., 2008). Fugacity results are shown for the first time in North Atlantic and Arctic-SubArctic area. This is an important standpoint for future research in order to give a better perspective in the study of the transport and cycling of these pollutants in the area.

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EC04A-2

LC-MS/ MS analysis for the identification and quantification of endocrine disrupters in surface water and in water for human consumption: monitoring of italian water supplies

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The presence of endocrine-disrupting compounds (EDs) in influent and effluent water samples from six waterworks located in Italy was studied. EDs may exhibit physiological effects at very low concentrations.

An advanced analytical method was developed using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous identification and quantification of selected Endocrine Disrupters (EDs) in raw and treated waters.

Analytes were pre-concentrated from 1-L water samples by SPE on HLB cartridges and analysed by reversed-phase LC-MS/MS. The mass spectrometer was operated in multiple reactions monitoring mode with ionspray interface in negative ionization mode using 17β-estradiol-d3 as internal standard. Moreover, the analytical protocol was modified in order to make it suitable to treated waters. A collaborative trial was conducted among the waterworks with the aim to evaluate the reliability of the analytical protocol for analyses of surface waters. The method was validated in term of sensitivity, selectivity, trueness, repeatability and inter-laboratory precision. Data on both the state of contamination of water for human consumption and on the efficiency of the treatment processes in removing EDs were obtained as a result.

The maximum concentration value in the influent water samples was 806 ng/l (nonylphenol), being all these values are comparable with the concentrations reported to be found in European surface waters.

Finally, the treatment processes used to make potable water destined to human use was evaluated in terms of ability in removing selected EDs: a complete or significant reduction of EDs contamination were always observed, with residual concentration level in the range of ng/L.

EC04A-3

Modelling the coupling of biodegradation with air-water exchange and its influence on POP concentration in plankton

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Atmospheric deposition, and particularly diffusive air-water exchange, is the main entrance and driver of the concentrations of many persistent organic pollutants (POPs) in the surface waters

of open oceans and Lakes. Once in the water, partitioning processes influence the transport pathways, degradation processes, residence times and the final fate of the compounds. Indeed, it is known that biogeochemical cycles, especially those related to organic carbon, affect the POP transport and sinks in the water column. The biological pump has received a lot of attention during the last decade, but it is not clear the role of biodegradation on air water exchange and accumulation of POPs in planktonic food webs. Therefore, the main objective of this estudy is to clarify the interactions of atmospheric inputs of POPs and the biogeochemical processes occurring in the surface ocean mixed layer emphasizing the implications of the biological pump and degradation in such processes.
Air-water-plankton coupled model has been developed modifying the approach proposed by Dachs and coworkers (1) to calculate air-water, water-plankton and settling fluxes. Additional modeling exercise has been used to include the potential degradation process occurring in the water column. Field measurements have been used in the validation of the model. All samples were obtained on board of RV-Garcia del Cid research vessel during two Mediterranean sampling cruises on June 2006 and May 2007.
Air-water-planton model for more persistent POPs (PCBs and HCB) obtains not only good agreement for measured plankton concentrations, but also has been able to reproduce the seen dillution effect. On the contrary, more labile compounds such as low molecular weigh PAHs and HCHs require to integrate the biodegradation processes occurring in the water column to reproduce more accurately field measurements. The trends observed in the Mediterranean provide important clues on the processes driving POPs in other oceanic regions, where the gradients in biomass and other environmental variables can be larger than in the Mediterranean.
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EC04A-4
Vertical eddy diffusion as a key mechanism for removing perfluorooctanoic acid (PFOA) from the global oceans
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The objective of this work is to estimate the importance of vertical (eddy) diffusion as a major removal process of POPs from the surface ocean. We focus on PFOA as a tracer as it represents a stable, highly water-soluble molecule and has recently been proposed as a novel chemical tracer of ocean circulation. As the ocean is generally vertically stratified, there are large concentration gradients with depth, so turbulent vertical eddy diffusion becomes a key process in removing compounds from the surface layer. Vertical eddy diffusivities are highly variable both in time and space, ranging from 10-2 m2 s-1 in the upper mixed ocean to 10-5 m2 s-1 in the open deep ocean. In this paper, we use such a three-layer vertical eddy diffusion turbulence model to study removal of PFOA from the upper ocean. Values of E are estimated using measured vertical profiles of PFOA, and compared to literature values. These E values are next extrapolated to obtain values for the global ocean and then used to obtain global vertical fluxes of PFOA. We attempt to (i) explain observed vertical PFOA profiles in the Japan Sea and Mid-Atlantic Bight solely based on transport through vertical eddy diffusion, (ii) derive the flux of PFOA at 100 m depth from measured global surface ocean concentrations of PFOA, (iii) estimate historical global ocean vertical diffusion fluxes of PFOA, and (iv) compare the importance of deep water formation and vertical eddy diffusion in removing PFOA from the surface water. The North Atlantic emerged as the main sink for PFOA, with accumulated removal fluxes over the last 40 years of 220 - 890 t, with the South Atlantic contributing another 90 - 360 t. The Pacific Ocean is estimated to have around 260-1,100 t PFOA below a depth of 100 m, while the Indian contributes only between 15 -60 t in total. To put the vertical diffusive flux values in the global oceans in perspective, we estimated the flux of PFOA entering the deep ocean via deep water formation. Total amounts moved by deep water formation are estimated to be 50 - 250 tons in the Norwegian Sea and 30 - 130 tons of PFOA in the Labrador Sea over the last 40 years. Thus, vertical eddy diffusion accounts for losses of PFOA to depth on the order of 620 - 2,400 t, while 80 - 360 tons have been lost from the surface oceans via deep water formation.

EC04A-5
Latitudinal distribution of polyfluorinated organic compounds in the air and surface water from the Arctic to the Antarctic
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Per- and polyfluorinated organic compounds such as perfluorocarboxylic and sulfonic acids, perfluoro alkyl sulfonamide and N-substituted sulfonamides (PFASs), and fluorotelomer alcohols (FTOHs) are substances that have been extensively used and applied in industrial and consumer products in the past 50 years due to their surface-active properties and thermal as well as chemical stability Scientific concern about PFCs increased due to their global distribution and ubiquitous detection in the environment and biota. Recent studies conducted in the presence of PFCs in wildlife from the Arctic have demonstrated that these chemicals are widespread in these regions and accumulated in the food chain. More studies are required to resolve how PFCs transport from the sources to the coast and marine environment.
The objectives of this study are to characterize the distribution of PFCs in the atmosphere and sea water of the Atlantic, Arctic and the Antarctic. Data will be used to estimate the transport path of PFCs from high contaminated region to relatively low contaminated region, and evaluate the air-sea gas exchange intervening in the transport process of PFCs into the Open Ocean and Polar Regions.

EC04A-6
Polycyclic aromatic hydrocarbons (PAHs) atmospheric concentrations and deposition over the open Mediterranean and Black Seas
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Polycyclic aromatic hydrocarbons (PAHs) are a group of semi-volatile organic compounds (SOC) which are ubiquitous in the environment, bioaccumulate in planktonic food webs and may cause a wide range of toxic effects in biota and humans. The atmosphere is their major pathway of transport and deposition for PAHs, being partitioned between the air gas and aerosol phases. There is little information on the PAH occurrence and deposition in marine environments far from shore areas (open sea). PAH atmospheric concentrations, spatial distribution and deposition across the Mediterranean Sea and in the Marmara and Black Seas are presented in this work. Data were collected during two sampling cruises performed on June 2006 and May 2007 on board of the oceanographic vessel B/O García del Cid (CSIC). In both campaigns, Barcelona was the initial and final port, with Istanbul and Alexandria being the intermediate stops respectively. A total of 44 integrated air samples (particulate + gas phase) were collected mostly along the Mediterranean but also in the Marmara and Black Seas by using high volume samplers. PAH atmospheric levels over the Mediterranean Sea were driven by air gas phase concentrations. ∑16 PAHs gas phase concentrations ranged from 2 to 4 ng m-3 whereas particulate phase concentrations varied from 0.1 to 0.3 ng m-3. The transect encompassing the Marmara and Black Seas exhibited a slightly highest PAH concentrations of 6 ng m-3 and 0.5 ng m-3 for gas and particular phases, respectively. Phenanthrene dominated the average gas phase congener pattern in the Mediterranean Sea accounting for 50 ± 15% of the sum of PAHs, whereas PAH congeners were more evenly distributed in the particulate phase being Benzo(b)fluoranthene and Benzo(e)pyrene the more abundant (12 ± 2% of the sum of PAHs). Dry atmospheric deposition fluxes in the Mediterranean Sea open waters ranged from 20 to 50 ng m-2 d-1, whereas in the Marmara and Black seas a value of 95 ng m-2 d-1 was calculated. Back trajectories analysis, diffusive air-water exchange and day and night concentration variations were also investigated in the present study.

EC04B-1
Fate of hydrophobic persistent chemicals in the pelagic system of Lake Maggiore during an algal bloom
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Modelling studies have supported the hypothesis that carbon-rich/biogenic particle dynamics control environmental fate of hydrophobic chemicals such as POPs in pelagic ecosystems. In particular algal blooms represent highly dynamic scenarios with enhanced organic carbon synthesis and grazing. Studying these un-steady systems can provide useful information on the dynamics of pollutants entrance into the pelagic food web, and the organic carbon control on the chemical inter-compartment exchange. Polychlorinated biphenyls (PCBs) (as a model for hydrophobic accumulative substances) were measured in the dissolved and different particulate matter fractions of the pelagic ecosystem of Lake Maggiore (Italy) during the spring algal bloom between March and May 2009. In order to assess: i) the effects of biomass development on the air-surface exchange of chemicals; ii) the effects of biomass growth on the concentration of chemicals in the dissolved phase; iii) the distribution of PCBs across the different particle fractions; and iv) the state and the evolution (with time) of the POC-water partitioning. The majority of PCBs were associated with the dissolved phase followed by the colloidal fraction and the 0.5-10 um fraction, while less than 1% of the PCBs was associated with fractions 10-95 um, 95-200um and >200um. PCB concentration in the dissolved phase decreased with increasing chlorination and with time during the algal bloom. The plot between Log KOW and the LogKOC/water (namely the partitioning between the OC in a given fraction and water) showed low values of the slope in the central part of the campaign, suggesting conditions far from the partitioning equilibrium or variability of parameters controlling the partitioning.

EC04B-2
Spatial and temporal dynamics of POPs in the Morava river
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Spatial and temporal dynamics of polyaromatic hydrocarbons (PAHs) in river sediments were a subject of the large scale study in the river shed of Morava river in the Czech Republic, the industrial area with frequent occurrence of floods. Following the studies focused on behavior of PAHs during floods (transfer of these compounds from bottom sediments to alluvial soils) and in the periods following floods (building up new burdens in sediments), the long-term study reported here was designed. Samples of river sediments from five sampling sites have been sampled for one year with the sampling period of four weeks. In addition to surficial bottom sediments, the samples of suspended material collected in the sedimentary traps, silicon rubber based passive sampler providing information on truly dissolved concentrations of chemicals of interest, and bulk water samples were taken every four weeks. All samples were analyzed not only for PAHs but a number of other legacy and emerging groups of persistent organic pollutants (POPs), they were characterized for their abiotic parameters and correlations of the POP levels with such parameters as well as with the meteorological and hydrological conditions were studied. Distribution of PAHs among the phases of aquatic ecosystem was also assessed, including spatial and temporal variability of such distribution. Such sampling design covered various seasons, temperature conditions, and hydrological situations and allowed for detail assessment of PAH behavior in highly dynamic river system. The results were further correlated with the results of toxicological assessments.

EC04B-3
Distribution and fate of musk compounds in a river: field study and model calculation
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Synthetic musk compounds are widely used as ingredients in personal care products. They have been recognized as important organic pollutants in aquatic ecosystems, and are in general introduced into the environment via waste water treatment plants (WWTPs). This contribution describes a comprehensive study on the distribution and fate of 13 different musk compounds and 3 further compounds (triclosan, diphenylsulfone, benzophenone). The study was conducted at the river Bilina (discharge < 5 m^3/s; Czech Republic) from headwaters to its mouth. Main goals of the study were to gather data on the distribution and fate of compounds originating from personal care products, to collect data on bioaccumulation of the targeted compounds especially in fish and to assess their impact on the riverine ecosystem.
Water samples (bulk and passive sampling), suspended particulate matter, sediments and fish were collected during several sampling campaigns at different seasons and analysed. Simple model calculations were used to calculate mass fluxes between the compartments and to get a better understanding of the targeted compounds' fate.
Freely dissolved water concentrations reached values of up to 152 ng/L for diphenylsulfone and 106 ng/L for the musk galaxolide. Most compounds showed elevated concentrations downstreams of the WWTPs. By and large, patterns of distribution of the compounds along the river

did not differ at different seasons, pointing at a constant composition of the waste water throughout the year. Remarkable differences in sediment concentrations were determined in dependence of sampling depth, indicating different sedimentation history and temporal changes in pollution of the river. The results clearly showed that explaining sediment-water distribution of compounds should take the spatial heterogenity (horizontal and vertical) of sediments into account. Field data confirmed that musk compounds bioaccumulate in fish. No bioaccumulation in fish was observed for the bactericide triclosan.
A comparison of measured concentration data with acute and chronic toxicity values for organisms from three trophic levels (algae, daphnia, fish) revealed that all compounds except triclosan and galaxolide were at least three orders of magnitude below EC50-values. The concentrations of triclosan, and to a lower extent that of galaxolide, raise concerns about possible adverse effects on the ecosystem, and further monitoring of this compound is advisable.

EC04B-4
Chiral signatures of selected pharmaceuticals as markers of biological attenuation processes in rivers
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The capacity of rivers for biological attenuation processes of trace polar organic pollutants are probably significant but poorly understood because the many factors (i.e., dilution, sorption, phototransformation, biotransformation) that control naturally attenuation are interrelated and difficult to study in isolation. Although it is possible to estimate in-stream attenuation rates from monitoring data, accurate estimates require large data sets. To overcome these limitations, chirality has been exploited in this work since only biological processes might change the enantiomer composition of chemicals. The main aim of this work was to investigate the chiral signature of the antidepressant venlafaxine and the β-blocker metoprolol to get insights into biological attenuation processes in river environment. This work includes laboratory-scale experiments to assess the contribution of sorption, phototransformation and biotransformation processes to the overall attenuation of venlafaxine and metoprolol as well as field observations to validate laboratory results. During base flow conditions (summer), enantiomeric fractions (EF) of each compound remained constant while they fluctuated during rainfall events (fall). High flow conditions probably prompted the release of chemicals accumulated during the dry season in sediment, where enantioselective biotransformation probably took place. Investigating chiral signature of selected pharmaceuticals is a promising tool not only to discriminate between abiotic and biotic transformation processes in river but also to assess the spatial and temporal variability in biological attenuation processes.

EC04B-5
Deconstructing complex aquatic systems: identification of active components and the metabolism of fludioxonil by phototrophs
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Assessment of the environmental fate of crop protection products (CPPs) incorporates a range of standardized laboratory studies designed to assess hydrolysis, photolysis, sorption and microbial degradation. In such studies complex, natural processes are separated in order to provide simple, standardized laboratory based systems that conform to legislative guidelines. In the case of freshwater microbial degradation, CPP degradation is examined in darkness. Clearly, such studies differ greatly from real world conditions.
Previously, we demonstrated that the incubation of such systems under non-UV light, to enhance system complexity and real world relevance by including phototrophs,, increased the degradation rate of CPPs when compared to standard, dark incubated, systems (presented at SETAC, Seville 2009).
Although such work demonstrated the enhanced degradative capabilities of more complex, phototrophic inclusive systems, our understanding of the factors involved was limited. In phototrophic inclusive systems there are a range of metabolic processes and community effects that are absent in the standard laboratory systems: metabolism by macrophytes, algae and periphyton, and enhancement of bacterial and fungal communities by macrophyte root structures, algal biofilms and planktonic algae.
In order to assess the metabolic capabilities of the various community factions within these complex systems, and in an attempt to understand the role of phototrophs in enhanced degradation, we isolated sub-communities and individual species from complex, phototrophic inclusive test systems and investigated their capacity to degrade fludioxonil. We were thus able to demonstrate that algae and macrophytes are metabolically competent, in isolation from bacterial and fungal communities.
In this way we have demonstrated the intrinsic metabolic significance of Elodea canadensis as well as algae from three phyla (Chlorophyta, Cyanophyta and Bacillariophyta (diatoms)) in the fate of the fungicide fludioxonil. Thus, it seems that current laboratory test systems are failing to consider the role of active, competent organisms that are likely involved in the fate of crop protection products in surface water environments.

EC04B-6
Determining air-water exchange, spatial and temporal trends of PAHs in an urban estuary using passive polyethylene samplers
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Passive polyethylene (PE) samplers were deployed at six locations within Narragansett Bay (RI, USA) to determine sources and trends of freely dissolved and gas-phase polycyclic aromatic hydrocarbons (PAHs) from May to November 2006. Freely dissolved concentrations of PAHs were dominated by fluoranthene, pyrene and phenanthrene, at concentrations ranging from 10s - 1,000s pg/L. These were also the dominant PAHs in the gas-phase, at 100s - 1000s pg/m3. A general north-south gradient was observed, with higher freely dissolved concentrations near the urban center of Providence. By and large, all stations followed the same temporal trends, with highest concentrations (up to 7,300 pg/L for sum PAHs) during the 2nd of 11 deployment, coinciding with a major rainstorm. PAH concentrations declined gradually through deployments III, IV and V, and could be explained by an exponential decay due to flushing with cleaner ocean water during tides. The estimated residence time (tres) of the PAH pulse was 24 days, close to an earlier estimate of tres of 26 days based on salinity gradients. Air-water exchange gradients indicated net volatilization of most PAHs at Conimicut Point. Further south at Quonset Point, gradients had changed to mostly net deposition of the more volatile PAHs, but net volatilization for the less volatile PAHs. Based on characteristic PAH ratios, Mount Hope Bay and Poppasquash were affected by fuel spill-derived PAHs. Dissolved PAHs at the other sites seemed to originate mostly from the combustion of fossil fuels.

EC04C-1
Stable isotopes and persistent organic pollutants patterns in an Antarctic food web
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Samples of several Antarctic organisms in different trophic levels (invertebrates, fishes, birds and pinnipeds) were analysed for persistent organic pollutants (PCBs, DDTs, HCHs, HCB, PBDEs, mirex, aldrin, dieldrin, endrin and chlordane-related compounds) as well as for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Collections occurred in King George Island (62º05'S, 058º23'W), Antarctica in the austral summers of 2004/05 and 2005/06, for the following species: krill (whole *Euphausia superba*), limpets (soft tissue from *Nacella concinna*), fishes (muscle from *Notothenia rossii* and *coriiceps*), birds (egg and liver from *Pygoscelis antarctica*, *papua* and *adeliae*, *Catharacta* sp and *Larus dominicanus*; only liver from *Daption capense* and *Macronectes giganteus* and only egg from *Sterna vittata*) and pinnipeds (fat from *Lobodon carcinophagus*, *Leptonichotes weddelli* and *Arctocephalus gazella*). In a general way the PCBs (from 6.82 up to 1821 ng g^{-1} ww), HCB (from 0.060 up to 136 ng g^{-1} ww) and DDTs (from 0.410 up to 524 ng g^{-1} ww) were the prevailing compounds within the species averages. PBDEs were one to two orders of magnitude lower than the organochlorine groups. Limpets presented results about three times quantitatively higher than krill, which could be mainly due to larger life spans. Fishes showed similar results in comparison to literature, which are reflected in superior trophic positions. Bird samples, influenced by several ecological factors, showed quantitatively higher concentrations and qualitatively heavier PCBs profile as the northernmost the species reaches and the more opportunistic/scavenger feeding habit it has. The stratification in pinnipeds results can be interpreted in a similar way. Carbon stable isotopes allowed the identification of two clearly distinct origins for organic matter: marine phytoplankton more depleted in ^{13}C, as seen in krill ($\delta^{13}C$ of -25.66"ð) and the more ^{13}C enriched microphytobenthos, as seen in limpets ($\delta^{13}C$ of -16.10"ð). Some effects account for intermediate values: diversified diet, $\delta^{13}C$ latitudinal variation for migrating species and trophic level (an evidently lower enrichment than nitrogen, but yet existent). Nitrogen isotopes ($\delta^{15}N$ from 4.51 up to 14.53"ð) showed patterns in accordance to literature and significant Pearson correlation preliminary results between $\delta^{15}N$ and several pollutants concentrations, which reinforces the biomagnification processes of such compounds.

EC04C-2
Environmental monitoring of HBCD in Europe
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Hexabromocyclododecane (HBCD) is a brominated flame retardant applied mainly in polystyrene foams as well as in flame retarded back-coats, for instance for upholstery textiles. During recent years, emission control programs have been implemented by the HBCD producers and users in order to reduce the environmental footprint. To assess the impact and relevance of these reduction measures, an environmental monitoring project was initiated in 2007. The project is designed to assess temporal trends over a period of up to ten years at different sites across Europe. The study focuses on compartments which are expected to be sinks for HBCD based on its physico-chemical properties and the life cycles of products containing HBCD. Samplings cover fish (every year) and suspended particulate matter (SPM; every second year). Selected sites are at the rivers Tees/UK, Western Scheldt/Netherlands, and Rhone/France as well as at Lake Belau/Germany. The later represents a site with little anthropogenic influence. At all sites bream, a bio-indicator for accumulation also used for the German Environmental Specimen Bank, were caught. In brackish water (Scheldt, NL), both sole and bream were collected. After sampling and dissecting the fish, muscle tissue was immediately frozen over liquid nitrogen. Annual samples of usually 15 fish per site were grinded and homogenized by cryomilling (pooled annual sample for each site). To assess variability, muscle tissue from individual fish were analysed for selected sites. SPM was sampled with passive sedimentation traps in the vicinity of sites where fish were sampled. SPM was collected from the traps every three months, freeze-dried and combined to annual samples for each site. HBCD analyses of annual pooled fish and SPM samples were performed applying a validated LC/MS-MS method that allows quantification of the major HBCD diastereomers (alpha-, beta- and gamma-HBCD). Results from the first three years of fish monitoring suggest that at three sites with diffuse exposure characteristics the concentrations of HBCD are decreasing. For example, the HBCD concentration of bream from the Rhone declined by about 60 % (from 1420 µg/kg lipid weight in 2007 to 531 µg/kg in 2009). Decreasing environmental HBCD concentrations seem to be consistent with the implemented emission control measures. However, at one site impacted by a former point source (i.e. Tees) no decreasing concentrations in fish could be observed over the indicated observed period.

EC04C-3
Influence of gender and age on PCBs accumulation in Chelon labrosus
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Chelon labrosus is an important commercial species and has been studied worldwide. However this species has never been studied for polychlorinated biphenyls (PCBs). Due to that, the concentration of 13 PCBs were measured in muscles and livers, of males and females of C. labrosus, of different ages, allowing the estimation of PCBs bioaccumulation along the life span, in the Mondego estuary. The Mondego estuary presented a relatively low environmental contamination of PCBs, with levels of total PCBs (tPCBs) in the sediments, ranging from 0.28 to 2.0 ng g^{-1} (DW). tPCB concentration in male muscle samples concentrations ranged from 7 to 14 ng g^{-1} (DW) and in females from 4 to 8 ng g^{-1} (DW). In male liver samples tPCB concentration ranged from 28 to 46 ng g^{-1} (DW), while females concentration ranged from 25 to 38 ng g^{-1} (DW). In males, liver and muscle PCBs concentration increase with age, with significant differences ($p=0.005$; $p=0.029$; $p<0.05$, respectively). In female samples, no significant differences were found along the life span (for both tissues), although liver concentration had a tendency to increase with age. Concerning the muscle samples, gender is the major factor contributing for bioaccumulation, while in liver samples is age. The most abundant congeners in all samples were CBs 138, 149 and 153.

EC04C-4

Persistent chlorinated organic pollutants and the decline of Atlantic eels

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There are concerns that chemical contaminants may have been a major contributing factor leading to the precipitous decline of Atlantic eels since the 1980s. In particular, polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and dechlorane plus (DP) were investigated due to their persistence, bioaccumulation and toxic potential. American eel (*Anguilla rostrata*) were captured at reference and contaminated sites in rivers of eastern Canada tributary to the Gulf of St. Lawrence, including the St. Lawrence R., and from the Hudson R., New York, USA. European eel (*Anguilla anguilla*) from Belgium were also collected for comparative purposes. Extracts of whole fish homogenates were analysed by gas chromatography-high resolution mass spectrometry (GC-HRMS) to determine the concentration of PCDD/Fs, non-ortho PCBs, PCNs, and DP. The remaining PCBs were measured with GC-quadropole ion-trap MS/MS. The GC-HRMS and GC-MS/MS instruments employed electron ionization and were operated in selected-ion-monitoring mode and selected-reaction-monitoring mode, respectively. Concentrations of dioxin-like compounds were used to calculate 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalents (TEQs) using fish-specific toxic equivalency factors. Reference American eels had values ranging from 0.36-1.70 pg TEQ/g ww (geomean = 0.75 pg TEQ/g ww) and others ranged from 0.48-8.95 pg TEQ/g ww (geomean = 2.10 pg TEQ/g ww). About 10% of American eels had measured TEQs > 4 pg TEQ/g ww, which has been suggested as the threshold for impairment of embryonic development. The TEQs for the majority of samples, however, indicate that the current risk of toxicity related to dioxin-like compounds is low.

EC04C-5

Study of bioaccumulation and metabolization of fluoxetine in benthic invertebrates by Micro-QuEChERS-NanoLC-Nano-ESI-MS/MS

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Fluoxetine is a widely used antidepressant, frequently found in aquatic ecosystems, which presented dissimilar effects on the reproduction of two gastropod species, P. antipodarum and V. piscinalis. Recently it has also been measured in fish downstream of effluent discharges indicating a high bioaccumulation capacity in vertebrate aquatic wildlife. However no data is available on fate and metabolism of pharmaceuticals in invertebrates. To better understand the interspecific sensitivity the bioaccumulation of fluoxetine and its in vivo metabolization into norfluoxetine have to be explored in P. antipodarum and V. piscinalis.

This kind of study requires the development of analytical tools for extraction and analysis of traces of fluoxetine and norfluoxetine in biotic environmental matrices like water's benthic invertebrates. These gastropods weigh just a few milligrams. With such a small sample size, extraction step and analysis are more difficult. So, advanced technologies are required to seek drugs traces in complex matrices like gastropods.

In this aim, we have established an analytical strategy as which consists in one single extraction for fluoxetine and norfluoxetine based on QuEChERS method, followed by a nano-LC-MSMS analysis. Indeed, nano-chromatography coupled with mass spectrometry (nano-LC-nano-ESI MS/MS) increases sensitivity, reduces the required initial sample amount and is a good tool to answer this ecotoxicological issue.

Both gastropods highly bioaccumulated fluoxetine. However, P. antipodarum bioconcentrated more fluoxetine than V. piscinalis, which can only be partly explained by their lipid content. However, N-demethylation into norfluoxetine was similar in both snails, and far less important than in fish. It implied the occurrence of this metabolic pathway in mollusks. The higher sensitivity of P. antipodarum to fluoxetine than V. piscinalis was at least enlightened by its higher bioaccumulation capacity, but not by higher metabolization into an active metabolite.

EC04C-PS

Poster spotlight: Revisiting bioconcentration and bioaccumulation in aquatic ecosystems

Miscellaneous

Poster spotlight highlighting abstracts TU 066, TU 067, TU 068, TU 069:

- Equilibrium sampling of environmental pollutants in fish - Comparison with lipid-normalized concentrations and homogenization effects on chemical activity
- Bioaccumulation of micropollutants in an estuarine food chain
- Bioaccumulation and trophodynamics of emerging contaminants in tropical mangrove food webs
- Accumulation of butyltin compounds in minke whales and long-beaked common dolphins from the Korean coast

EC05 - Environmental fate and exposure of Pharmaceuticals and Personal Care Products (PPCPs)

EC05A-1

Analytical approaches for the investigation of emerging contaminants in sources and treatment systems for drinking water production

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For their need of drinking water, millions of Europeans depend on surface waters, such as the rivers Danube, Meuse, Po and Rhine. In these waters, rapid improvements in chemical and bioanalytical techniques have led to the discovery of all kinds of so-called emerging contaminants at very low concentrations, including pharmaceuticals, illicit drugs, sweeteners, endocrine disrupting compounds and perfluorinated compounds. Some studies also reported the presence of traces of emerging contaminants in drinking water samples. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes. A combination of analytical approaches is applied for this purpose, including chemical screening techniques, hyphenated target analyses, biological early warning systems, bioassays and effect-directed analysis (EDA) approaches. This presentation gives an overview of chemical and bioanalytical approaches applied to investigate Dutch drinking water sources and treatment systems. Examples are discussed of the use of sensitive and specific techniques such as CALUX reporter gene bioassays for the analysis of hormone-like activities and the development and application of a UPLC-MS/MS method for the determination of (sub) ng/L concentrations of new pharmaceuticals. As an example of pharmaceutical analysis employing direct injection a study will be presented investigating the removal efficiencies of several emerging contaminants in a pilot-scale advanced oxidation treatment process. Presented studies show that quality assessment of drinking water sources requires the use of the most suitable techniques: biological, chemical or combinations thereof. Due to ongoing analytical improvement, our knowledge on contaminants in drinking waters sources steadily increases and also our responsibility to act accordingly.

EC05A-2

Pharmaceuticals and transformation products in surface water and drinking water

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Numerous studies describe the presence of pharmaceuticals in the water cycle, while their transformation products are usually not included. The current study is based on a monitoring campaign of ~30 common pharmaceuticals and transformation products in 14 surface waters, 10 pretreated surface water, 7 river bank filtrates and the 17 corresponding produced drinking waters. Various pharmaceuticals and transformation products were observed at concentrations of <10 to >100 ng/L in the water cycle. Concentrations were generally highest in surface water, intermediate in raw water and river bank filtrate and lowest or not even detected in produced drinking water. However, the concentrations of phenazone and its environmental transformation product AMPH exceeded surface water concentrations by almost one order of magnitude. Their high levels in river bank filtrate are likely a result of historical contamination thatare still present in river bank filtrate. Additionally, the concentration ratios of transformation products and parent pharmaceuticals were studied. Since some of these products have similar (pharmacological) activities as their parents, and they were observed at similar concentrations as their parents (i.e. phenazone, tramadol, venlafaxine, carbamazepine), monitoring transformation products is relevant. However, it was observed that 'product/parent' ratios varied less than a factor 5 over all samples (when quantifiable). This suggests that concentrations of transformation products might be estimated from concentrations of parents instead of beeing monitored. This should, however, be studied in more detail.

EC05A-3

Pharmaceuticals and personal care products in urban receiving waters

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Pharmaceuticals and personal care products (PPCPs) and other organic micropollutant from wastewater sources pose a potential threat to aquatic ecosystems and the human health. Urban areas are prone to PPCP contaminations since here large amounts of wastewater are produced transported and treated. However, the spatial and temporal complexity of urban water and wastewater flow makes the assessment of PPCPs a challenging task. In this paper we quantify the contribution of different PPCP pathways (wastewater treatment plant effluents, combined sewer overflows and groundwater discharge) out of a mid-European urban drainage catchment and assess the temporal variability of this contamination. The study area is a catchment of wastewater (sewershed) which is in a corresponding position to the groundwater catchment and therefore allow for a joint analysis of water and matter fluxes. We conducted a monitoring over the course of 13 months including samples from untreated and treated wastewater, surface water and groundwater. The resulting samples were screened for six PPCPs and micropollutants: Bisphenol A, technical 4-nonylphenol, caffeine, galaxolide, tonalide and carbamazepine. The annual water flow to and from the sewershed was quantified using the data of the wastewater flow and results from a numerical groundwater flow model. The combination of annual water flow with the median contaminant concentration yielded the mass flow out of the sewershed by treated wastewater, combined sewer overflow and by groundwater discharge. The results demonstrate that the release of micropollutants from the sewershed to the receiving waters is not a result of the effluents of the municipal wastewater treatment plant alone. For caffeine, bisphenol A and nonylphenol, the input of untreated wastewater by combined sewer overflow to the surface water can make 8% to 89% of the total mass release. A significant portion of bisphenol A and nonylphenol is released by groundwater discharge out of the sewershed boundaries. Surface water concentrations and mass fluxes of caffeine, galaxolide and tonalide correlate negatively with the water temperature. Therefore, these substances are characterized by a pronounced seasonal occurrence with rapid removal in times of higher water temperature despite the higher input by combined sewer overflow events in this period. The results of this study underline the complex interplay of release and fate of PPCPs in urban areas.

EC05A-4

Attenuation and dynamics of pharmaceuticals in a small German stream

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Pharmaceutical residues are commonly detected organic micropollutants in the aquatic environment. Their fate in rivers and the importance of individual attenuation processes (photolysis, biotransformation, and sorption) is still incompletely understood. Previous studies indicated that in rather deep and turbid rivers these potential processes not always result in a significant attenuation of pharmaceuticals. Therefore, we performed experiments at a stream with less turbid water and an expected increased exchange of river water and sediments to check dynamics and attenuation in such streams.

Experiments were carried out at the stream Gründlach, near Nuremberg, Germany. Composite samples were taken at both ends of a river stretch of approximately 12 km length located downstream of a sewage treatment plant. Moreover, pore water samples were taken and in-situ photolysis experiments at several sites within the river stretch were performed. The concentration of 15 pharmaceuticals was analyzed with HPLC-MS/MS after solid phase extraction.

Pharmaceutical concentrations varied at the first sampling site due to variable proportions of sewage water in the river. Concentrations and loads at the downstream sampling site were lower for most pharmaceuticals. In comparison to carbamazepine which was persistent, metoprolol was attenuated within the river stretch. This attenuation can be attributed to sorption and/or biotransformation since it was not susceptible to photolysis. For diclofenac, photolysis in unshadowed parts or the river stretch could also be relevant as we determined an in-situ photolysis half-live of a few hours.

Preliminary results of this study suggest that attenuation of certain pharmaceuticals in small streams can be relevant. Even within short river stretches pharmaceuticals can be eliminated due to the more intense exchange of river water with the sediment compartment and due to a higher efficiency of photolysis compared to large rivers.

EC05A-5

Tracing micropollutants during riverbank filtration under restored and non-restored conditions at the River Thur

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Riverbank Filtration (RBF) is a widespread, low-cost method to achieve high quality drinking water. Since little is known about the fate of organic micropollutants during RBF and the influence of river restoration on the processes in the hyporheic zone and the aquifer, we investigated the fate of micropollutants from the river through different groundwater transects. The field site was located in the lower part of the Swiss river Thur catchment, which was partially subject to river restoration. Two piezometer transects were installed: one is located in a channelized part where drinking water is produced by RBF and the other in a restored part of the river. First a screening was carried out for over 200 polar organic micropollutants using LC-HRMS, later 78 compounds were analyzed with an online-SPE-LC-MS/MS-method. Highly spatiotemporally resolved sampling of river water and groundwater was conducted between 2008 and 2010. During the screening we found 98 compounds in concentrations between 0.1 and 400 ng/L. The concentration patterns of the compounds over the two transects indicate that the behavior of different compounds in the transects is highly diverse at one point in time. During low flow conditions the pharmaceutical transformation products N-Acetyl-4-aminoantipyrine and Atenolol acid showed fast decreasing concentrations indicating biological degradation whereas Carbamazepine and Sulfamethoxazole persisted. Benzotriazole concentrations remained high even after a travel time of over 10 days and reached the drinking water well at a concentration of 200 ng/L. During a high discharge event the pesticide MCPA was found in the river at elevated concentrations up to 650 ng/L. Concentrations in the restored transect were one order of magnitude lower in the first two wells without any notable retardation. In further wells and in the transect in the channelized part, no MCPA was found. These observations implicate a strong degradation of MCPA in the first meters of the aquifer. The difference in the two transects is consistent with known travel times, which are shorter for the restored transect indicating that the travel time is an important influence parameter on biological degradation. Micropollutants from urban sources, such as the pharmaceutical carbamazepine, exhibited decreasing concentrations in the river during high discharge events. This caused decreasing concentrations in the groundwater transects with some retardation in the channelized transect.

EC05A-6

Mechanisms controlling the transport of carbamazepine and other trace organic compounds in a sand aquifer receiving wastewater discharge

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In North America, approximately 35% of wastewater currently is discharged to the subsurface using on-site wastewater disposal systems. As water demand increases, the volume of wastewater discharged to the subsurface also is expected to increase. While abundant information is available on the fate of wastewater macropollutants in the subsurface, there is limited information available on the fate of trace contaminants, including pharmaceutical and personal care products. Field investigations were conducted at a well characterized septic-system located in southern Ontario. At this site, wastewater is discharged to an anaerobic septic tank followed by infiltration through the unsaturated zone to the shallow water table. The disposal is seasonal, from May to October of each year. A detailed groundwater sampling network previously installed at the site was sampled both temporally and spatially for analysis of major water chemistry parameters and a suite of pharmaceutical compounds. In the groundwater zone, elevated concentrations of carbamazepine (up to 4000 ng L^{-1}), sulfamethoxazole (2000 ng L^{-1}) and ibuprofen (1,800 ng L^{-1}) were observed over 30 m from the wastewater disposal area. Concentrations of other pharmaceuticals, including gemfibrozil, naproxen and caffeine, were much lower and more variable. Laboratory column studies conducted to evaluate transport parameters of the pharmaceutical compounds under controlled flow conditions showed little correlation to the transport observed at the field site. Predictions based on the physicochemical properties of the compounds were in close agreement to the transport observed in the laboratory experiments. Predictions of field behaviour were less successful. Additional studies are required to further delineate the transport behaviour of pharmaceuticals in groundwater at wastewater disposal sites.

EC05B-1

Micropollutant plume in Lake Geneva

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To date, most research on environmental occurrence and fate of pharmaceuticals has focused on the presence of these compounds in rivers and streams, often at sites close to discharges of wastewater treatment plant (WWTP) effluent. However, only scarce data exists concerning the environmental fate of pharmaceuticals directly discharged from WWTPs into lakes. We assessed the spatial and temporal distribution of 39 priority pharmaceuticals and pesticides in Lake Geneva's Vidy Bay, which serves as the receptacle for the effluent of the WWTP of the city of Lausanne. Depth profiles of these substances were established between April 2010 and December 2010 in key locations in the Vidy Bay. These included the outlet of the WWTP, and two reference sampling sites located ca. 1.5 km upstream and downstream of the discharge point. Concentrations were measured using an analytical screening method based on ultra performance liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS).

A plume with significantly elevated pharmaceutical concentrations was detected in the water column above the point of wastewater discharge into the bay. The depth of the contamination hotspot varied with season, following the thermocline which decreased from a depth of 10m in April to 25m in October. The cold season led to straight temperature profiles, and allowed mixing of the water column. Explicitly, this caused the disappearance of the concentration peak above the WWTP discharge point and thus homogeneous vertical concentration profiles. Conductivity profiles were well-correlated to pharmaceutical concentrations, indicating that in the particular situation of wastewater discharge in Lake Geneva, it is possible to track the plume and estimate the micropollutant concentrations therein using conductivity measurements. To our knowledge this is the first reported micropollutant plume.

EC05B-2

PPCPs in recycling of dairy wastewater in a reconstructed mixed forest - forage formation

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The Golan Heights is a significant segment of the watershed of the Sea of Galilee, which comprises ca. 30% of the Israel's water supply. The alternatives for safe disposal of dairy farm sewage water are limited and sewage water might overflow into local streams, resulting in the uncontrolled dispersal of sewage water.

We have examined recycling of dairy effluents via irrigation of a mixed pasture - planted tree ecosystem. The effluent is stored in lagoons and used following minimal treatment.

We hypothesize that under deficit irrigation the migration of pollutants may be greatly retarded. We examined the partitioning of these contaminants between the: soil, soil solution, run-off, woody plant parts and grasses. This was carried out in two similar size drainage basins - one irrigated with wastewater effluent from a reservoir that received both dairy and human wastewater, and another non-irrigated basin. The latter basin was still under forage by ca. 1000 cows. Altogether 67 water samples were analyzed by LCMS.

The data show that PPCPs of both domestic and veterinary origin can be found in the soil extracts of the fields regardless of the irrigation regime. One compound (carbamazepine) was present in most of the water samples collected during 2009/2010 winter from the DWWE irrigated basin and also in 6 samples collected from the non-irrigated basin (that was under forage cows). The occurrence is a bit surprising owing to the almost strict domestic use of this chemical (and furthermore, one inhabitant only was prescribed to take the medication). Caffeine was the second most abundant compound under DWWE irrigation, yet it was first (7 out of 24 samples) in the irrigation-free basin, which is again quite unexpected. These two products are rather resistant in the environment and ubiquitous. Most all other PPCPs tested occurred in a small number of samples (1-8). The veterinary antibiotics were either missing altogether or occurred in 1-4 water samples from the irrigated site. At any event, the concentrations of those products that were detected were in the low range of 0.5-35 μg/L.

The research showed that recycling dairy wastewater through irrigation of a pasture - forest system under deficit irrigation poses little threat to PPCPs emission into water resources. Yet, this conclusion has to be reevaluated at this and other sites. In addition, it will be interesting to reveal the origin of PPCPs in the not-irrigated basin.

EC05B-3

Dynamics of sulfadiazine in manure-amended soil under field conditions

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In the last years, environmental fate and effects of the sulfonamide antibiotic sulfadiazine (SDZ) have been investigated in a series of studies. Förster and co-workers quantified the concentration dynamics of SDZ and metabolites in soil by using a sequential extraction procedure (Förster *et al*, 2009, ES&T); and the respective fractions could well be described by a conceptual kinetic model (Zarfl *et al*, 2009, Chemosphere). The next step towards an advanced understanding of the environmental behaviour of SDZ was to investigate the fate dynamics of SDZ under field conditions. Respective experiments were performed and the conceptual model developed for batch experiments was tested for its ability to simulate the observed fate dynamics of SDZ under field conditions. The experimental field plots were cultivated with maize and grass. During the vegetation period, the plots were fertilized three times (day 0, 49, and 133) with manure originating from SDZ-treated pigs. Soil samples were taken from the top soil layer during the vegetation period; occasional sampling was continued until the following spring. The soil samples were sequentially extracted and measured following published methods. For modelling, $CaCl_2$ extractable and high-temperature extractable parts of SDZ were assigned to an 'easily extractable fraction' (EAS) and to a 'residual fraction' (RES), respectively. Reference values of kinetic rate constants from laboratory batch experiments at controlled temperature and moisture conditions were used for simulations. Sink rate constants (k_1) were corrected for actual temperatures by an Arrhenius-like function. Substance availability in EAS was scaled by the fraction of dissolved SDZ in EAS, depending on actual daily soil water contents. Experimental values of EAS and RES fractions showed elevated concentrations directly after application of manure to soil and decreasing concentrations in the following. In model simulations, concentration dynamics of the EAS fraction were fitted quite well by using temperature and availability corrected rate constants. However, the concentrations in the RES fraction were clearly overestimated. The simulation fit to the RES fraction could be improved by increasing the rate constants k_1 and k_3 by factors of 1.5 and 4.0, respectively. Identical rate constants could be used to describe two different trials where maize or grass was cultivated.

EC05B-4

Generating screening-level scenarios of personal care products in China using GIS

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The development and application of models to predict in-river concentrations of down-the-drain chemicals (i.e. those used in household and personal care products and pharmaceuticals) are important components of the environmental risk assessment process. While such models (e.g. EUSES and GREAT-ER) exist in Europe, there are currently no available models to estimate exposure of personal care products in China. To this end, work was performed to create a GIS-based system that develops scenarios used to predict the fate of "down the drain"chemicals into freshwater ecosystems. Currently, many of the available inputs for the generation of environmental concentrations to be used in the exposure assessment are at a very coarse spatial scale, in some cases only a single value for the entire country is available. In this study, Predicted Environmental Concentrations in surface water (PECsw) were generated at the county-level (ca. 3,000 counties in China) based on both user supplied product information, as well as other geographically-linked socio-economic and environmental information from official census and other data sources. Product information such as category (e.g., hair, skin, etc.), composition (e.g., ingredient fraction) and "take off"values (GDP threshold under which the product would not be purchased) were used to distribute total tonnes of individual ingredients used in China. These data were combined with county-level economic information, population density (including urban and rural separation), dilution factors, and disposal mechanism (e.g., STP, septic, direct discharge to river, etc.) to estimate ingredient-level PECs in surface water.

Results show that local PECsw varies considerably across the country and that economic information ("take off"values and population GDP) can have a significant influence on the resulting ingredient distributions. The method presented incorporates the inherent spatial variability of the model inputs so that patterns can be identified and used in the risk assessment. In other

words, the ability to identify areas where existing combinations of model inputs may yield greater exposure estimates, i.e., the identification of realistic "worst case"scenarios. In addition, an understanding of where these "worst case"scenarios fit within the overall country-wide distribution (i.e., 90th percentile) is achieved.

EC05B-5

Predicting the fate and behaviour of cyclic volatile methyl siloxanes in two contrasting lakes

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The behaviour of three cyclic volatile methyl siloxanes (D4, D5 and D6) in lakes was explored using a fugacity-based steady-state non-equilibrium multimedia fate and transport model (a modified version of QWASI) in order to ascertain their likely environmental persistence and the relative importance of different loss processes (volatilisation, hydrolysis, burial in sediment and advection in outflow). Predictions were made for two contrasting North American lakes: Lake Ontario and Lake Pepin. Partition coefficients and the hydrolysis rate constant were adjusted for the mean annual temperatures of each lake. Half lives in sediment were calculated from partitioning theory, assuming that hydrolysis occurs only in the dissolved phase of the interstitial water. Estimates of substance-specific emissions were obtained by combining current per capita approximations of usage and fraction lost to domestic waste water, the population of the lake watershed and typical cVMS removal efficiency during waste water treatment. Predicted concentrations were generally lower and chemical residence times longer in Lake Ontario than in Lake Pepin, owing to greater depth, a much longer water residence time and a higher degree of dilution. Overall persistence in Lake Pepin was significantly influenced by the high rate of sediment burial assumed in the model, as well as by a relatively high rate of water discharge. Despite the many similarities of the compounds considered, the dominant loss mechanisms varied significantly and were not the same in each lake system. Hydrolysis was much more important for Lake Ontario than for Lake Pepin, especially for D4. Hydrolysis in sediment was not a significant loss process for any compound in either lake. Instead, the main net loss process for sediment was burial, which was especially important for D6. Although all three compounds are very volatile (log KAW > 2.5), the relative contribution of volatilisation to total losses was predicted to be limited in both lakes by the relative magnitude of other processes, sorption to organic phases in the water column and lake depth. Nevertheless, volatilisation is still predicted to be the most important loss process for D6 in Lake Ontario and is also responsible for more than 20% of all losses of D5 in both lakes. Model outputs were compared with observed concentrations in surface sediments and reasons for discrepancies discussed.

EC05B-6

Continental scale inverse modeling of European River contaminants

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We use a pan-European data set of measured river water concentrations of 16 common organic water contaminants, namely: Naproxen, Ketoprofen, Bezafibrate, Ibuprofen, Diclofenac, Gemfibrozil, Benzotriazole, Caffeine, Carbamazepine, Sulfamethoxazole, Methylbenzotriazole, Nonylphenolethoxy-1-carboxylate (NPE1C), Nonylphenol (NP), Bisphenol A, Estrone, Octylphenol (OP). We perform a back analysis of emissions and chemical half lives through a simple two-parameter inverse model. We identify for each chemical a set of Pareto-optimal or non-dominated pairs of half lives and emission factors that compare favorably with observations. Moreover, we derive log-linear regression equations to predict loads from an indicator of catchment population for each chemcial. On the basis of these equations it is possible to map continuous distributions in space of river concentrations and loads, therefore generalizing the results of a sampling campaign from discrete points, and enabling calculation of the total chemical loads to European seas, by considering the range of variability of emission factors and half lives.

EC05C-1

Determination of nine native steroid hormones in biological (e.g. blood and tissues) and environmental (e.g. manure, soil and sediment) samples by GC-MS/MS

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The vertebrate steroidogenesis is a metabolic pathway responsible for production of important biological process (e.g. growth and reproduction). Many substances are today known as endocrine disrupting chemicals (EDCs), i.e. interfering with hormone homeostatics and the steroidogenesis [1]. Key steroid hormones are; pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, dihydrotestosterone, estrone, 17α-estradiol and 17β-estradiol.
Many (eco)toxicologists seek to investigate the effects of EDCs on a range of vertebrates, and thereby identify the need for detailed and systematic studies on interactions between the individual steroid hormones expressed in the steroidogenesis. A prerequisite for this is a reliable analytical methodology for the simultaneous determination of several steroid hormones in the investigated biological sample (e.g. blood, gonads, brain).
When steroid hormones are released into the environment, predominant from animal wastes and human waste water, they have an environmental impact, e.g. the feminisation of fish, amphibians and others [2-3]. Consequently, there is a need among environmental chemists and engineers to measure steroid hormones in environmental samples (e.g. manure, soil, sediment and water) to describe the fate of these compounds in nature and in waste treatment optimization processes.
This presentation outlines for the first time the development and application of an analytical method to determine nine steroid hormones in biological and environmental samples. The methodology has been applied to toxicological studies (e.g. in-vitro studies, in-vivo frog studies, and seal and polar bears blood plasma) and environmental studies (e.g. soil sorption, manure content, manure separation technologies, activated sludge studies). Attempts will be made to present some of these cases.

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EC05C-2

The application of direct hollow fiber liquid phase microextraction for determining sludge adsorption of non steroid anti-inflammatory drugs during sewage treatment

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Non-steroid anti-inflammtory drugs (NSAIDs) are all consumed in large amounts in many countries and also belong to the pharmaceuticals most frequently detected in surface waters. Although they posses great similarities in physicochemical properties, studies show that ketoprofen are removed to a much lesser extent than ibuprofen during sewage treatment. This raises a number of interesting questions: Why do substances with such similar properties behave so differently under the same conditions? Ibuprofen is efficiently removed from the sewage water, but which are the main removal mechanisms? Is it totally degraded to carbon dioxide and water or non-toxic products or does a significant adsorbtion to the sewage sludge - which is often used as a fertlizer in crop production - take place during sewage treatment? How much of the total removal, in numbers, can be attributed to adsorption to sludge and where in the sewage treatment plant (STP) does the major adsorption in that case occur? The main purpose of this study is to investigate and quantify the removal due to sludge adsorbtion of the four aformentioned NSAIDs throughout the STP. This is performed by measurements of the NSAIDs in water as well as in sludge samples from the different steps of an STP. Analysis of such such complex samples poses high demandes on the analytical procedure. In this study hollow fiber liquid-phase microextraction (HF-LPME) is employed. The technique has several advantages over more classic extraction techniques, such as high enrichment, excellent clean-up, negligible consumption of organic solvent and simplicity in equipment and practical performance. It has also previously been successfully employed on digested sewage sludge slurry samples. Final analysis of extracts is performed by liquid chromatography quadrupole time of flight mass spectrometry (LC-MS Q-TOF). The method yields high enrichment factors (1 700 - 2 500 times) for extraction of the NSAIDs from influent water samples with apropriate sample dilution, giving overall MDL values of 0.04-0.11 ug/L for the different analytes and has also been successfully employed to biosludge samples. The study provides quantification of the sludge adsorption of four anti-inflammatory drugs throughout the sewage treatment process as well as a simple and environmentally friendly analytical method applicable for complex aquoeus as well as semi-solid samples.

EC05C-3

Analysis of personal care products in German fish tissue by GC-MS/MS: a nationwide study

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German Environment Specimen Bank (GESB) fish tissue samples, collected from fourteen different GESB locations, were analyzed for the twelve PCPs including fragrance materials, alkylphenols, photo initiators, and triclosan. The analytical method utilized pressurized liquid extraction (PLE) with in-cell silica gel clean-up, gel permeation chromatography (GPC), and gas chromatography ion-trap tandem mass spectrometry GC/MS-MS. Statistically-derived method detection limits (MDL) ranged from 1.2-37.9 ng/g wet weight. Galaxolide and tonalide, both synthetic polycyclic musk fragrances, were the most frequently detected PCPs. Galaxolide was detected in 13 out of 14 fish tissue samples whereas tonalide was detected in 9 out of 14 fish tissue samples. The maximum concentration of galaxolide (447 ng/g ww) and tonalide (15 ng/g ww) were measured in the Saar River at Rehlingen. Galaxolide and tonalide concentrations were positively correlated with percent lipid in fish tissue (r2= 0.711, p=0.001 and r2=0.769, p: less than 0.001, respectively). Triclosan was measured at or near MDLs only in the Elbe River at Blakensee and in the Donau River at Kelheim. PCPs were not detected in Lake Belauer.

EC05C-4

The uptake of pharmaceuticals into aquatic organisms; the importance of species traits and exposure route

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A wealth of information is now available on the occurrence and effects of pharmaceuticals in the environment, but our understanding of the uptake dynamics of pharmaceuticals through aquatic and terrestrial food webs is limited. The present study aimed to address this by:
- Comparing the uptake of pharmaceuticals into aquatic invertebrates with different traits, including the freshwater shrimp (Gammarus pulex), the water boatman (Notonecta glauca) and the freshwater snail (Planobarius corneus)
- Exploring the relationship between pharmaceutical physico-chemical properties and pharmacological properties and uptake into aquatic invertebrates
- Assessing the importance of the exposure route (water/dermal or food) in the uptake of pharmaceuticals into aquatic invertebrates
- Studying the trophic transfer of pharmaceuticals through a simple aquatic food chain

Using the radiolabelled pharmaceuticals carvedilol, fluoxetine, 5-fluorouracil, moclobemide, diazepam and carbamazepinebioconcentration factors (BCFs) for G. pulex ranged from 4.55-185900 and increased in the order moclobemide < 5-fluoruracil < carbamazepine < diazepam < carvedilol < fluoxetine. In N. glauca BCFs ranged from 0.13 - 1.60 and increased in the order 5-fluorouracil < carbamazepine < moclobemide < diazepam < fluoxetine < carvedilol. For P. corneus, the BCF for carvedilol was 57.3. The differences in uptake across the three organisms may be due to differences in organism size, mode of respiration and behaviour and the pH of the test system.
The relationships between VD and BCF were weak, strong correlations were found between Log Dlipw and BCF for both G. pulex and N. glauca.
When looking at the importance of exposure, the G. pulex data showed that the tissues concentrations in the G. pulex were significantly different for each exposure route for carvedilol and fluoxetine. The results indicate that uptake from dissolved chemicals from the water may be more important for accumulation in G. pulex than uptake from the food alone. However for N. glauca, the data show that the route of exposure of fluoxetine made a significant difference to the body burden of N. glauca. N. glauca took up less from the water compared to that assimilated from the food. Preliminary results show that fluoxetine can be transferred through three trophic levels of an aquatic food chain.

EC05C-5

Environmental fate of pharmaceuticals: dentification of fish bile metabolites and photo-transformation products of anti-inflammatory drugs

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EC05C-6

Investigating the chlorination and by-products of pharmaceuticals by liquid-chromatogra-

phy-tandem mass spectrometry

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The fate of pharmaceuticals in the water cycle has received a strong attention during the last few years and particularly during wastewater treatment and drinking water preparation. However, most studies have considered just the elimination of these compounds without accounting for possible transformation processes. Indeed, this may result in an underestimation of possible environmental and health hazards, as transformations may in some cases even lead to the production of more toxic compounds. An example is the chlorination of the widely used analgesic acetaminophen, which produces the toxic benzoquinone. Thus, it is crucial to investigate the degradation routes of pharmaceuticals during chemical oxidations that are often employed for drinking water preparation. To identify the formed by-products, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is particularly useful.

In this work, fifteen pharmaceuticals and metabolites have been treated with chlorine, the most common water disinfection chemical. After 24 h of reaction, it was quenched with ascorbic acid and the remaining pharmaceutical concentrations measured by LC-MS/MS. Under these relatively strong oxidation conditions, six of them (ibuprofen, ketoprofen, clofibric acid, fenoprofen, carbamazepine and bezafibrate) were found to be non-reactive. Subsequently, the reaction kinetics of the nine compounds that reacted with chlorine (phenazone, propyphenazone, atenolol, salbutamol, propanolol, diclofenac, naproxen, salicylic acid and indomethacine) were investigated in detail at different chlorine dose, pH and bromide concentrations by means of experimental design methodology. Also, several transformation products were tentatively identified by positive and negative electrospray LC-MS, in the scan mode, and LC-MS/MS, operated in product ion scan, with a hybrid quadrupole-time of flight instrument (QTOF). Finally, the by-products were investigated in real samples (surface and waste water) by SPE-LC-MS/MS.

EC06 - Fate and exposure modelling including scenario analysis

EC06A-1

MACRO in FOCUS versus PEARL - do both models adequately describe groundwater exposure to pesticides under Swedish conditions?

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Groundwater exposure is one of the critical endpoints in the risk assessment of plant production products, regulated in the European Union by the Directive 91/414/EEC. In Sweden, simulations with the model PEARL, one of the four pesticide leaching models applied in the EU, and the implemented scenario Hamburg is accepted unless the predicted environmental concentrations in groundwater (PECgw) exceed 0.01 µg/L, i.e. a threshold by a factor of ten below the regulatory limit. For higher PECgw, simulations with the model MACRO and three national Swedish scenarios are requested, which are more representative for the Swedish hydro-geological characteristics with macroporous soils and the cold climatic conditions. Aim of this study is to investigate deviations between the model predictions in dependence of the physical-chemical properties of the pesticides, and to evaluate the appropriateness of the safety factor of ten for acceptance of PEARL Hamburg simulations. Comparing simulations with both models have been performed for the treatment of cereals and pome fruits for 25 non-volatile hypothetical chemicals with varying combinations of half-lives (DT50) and organic-carbon partition coefficients (Koc) covering the relevant range of physical-chemical properties of pesticides. For the simulations with PEARL, which also considers volatilization as a dissipation pathway, additional 25 volatile chemicals with the same DT50- and Koc combinations were defined. In general, model simulations with PEARL yielded lower PECgw than simulations with MACRO and the Swedish scenarios. In particular volatile compounds showed by up to three order of magnitude lower concentrations. High macropore flow in the scenario Näsbygård resulted in a strong fluctuation of the magnitude of PECgw in dependence of the weather conditions and thus application time. For the protection of human health, conservative values should be considered as far as possible in the regulatory risk assessment of pesticides. Considering this approach, the simulation results suggest that for Swedish conditions, the model MACRO and the Swedish scenarios should be preferred for chemicals with a Koc above 100 L/kg. Moreover, for low DT50 (< 50 days) - low Koc chemicals (< 500 L/kg), model simulations for the scenario Näsbygård should be performed for several dates of initial application in order to avoid an underestimation of the leaching potential as a result of its sensitivity to weather conditions.

EC06A-2

Modelling approach to estimate emission of Plant Protection Products from protected crop systems to surface water in Mediterranean countries

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A sector with relatively unknown impact on the environment is the horticultural sector. Greenhouse production is considered by the Regulation (EC) No 1107/2009 to prevent release of PPPs into the environment, contrary to field conditions. However water boards frequently measure the occurrence of PPPs and biocides in surface water near and in protected crop areas. In this study the FOCUS PEARL model was parameterized to perform simulations for Plant Protection Product (PPP) emissions from greenhouses to surface water, after application to a tomato crop. A comparison with the open field was made in order to investigate how the climatic conditions, which are more controlled under greenhouse conditions, influence the PPP emissions to surface water. Although only a limited number of runs were performed, the results show the existence of two hypothetical substances (subsequently indicated as CC and CC M) in the water drained from greenhouses, contrary to what is stated in the European Regulation (EC) No 1107/2009. The irrigation amounts given during the crop cycle and the temperature conditions within the covered structure have an important influence on PPP's emission as increasing the irrigation volume and/or decreasing the temperature result in increased concentrations of CC and CC-M in drainage water whereas the opposite occurs when the temperature is increased. Flushing the soil at the end of the crop cycle and irrigating with minimum water excess during the crop cycles, as an alternative to over-irrigation for the entire cultivation period, resulted in lower CC and CC-M leaching/drainage. However to generalise these findings, additional runs for other substances and other crops than tomatoes are required.

EC06A-3

FROGS (French Refinement Of Groundwater Scenarios)

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French higher-tier national scenarios were developed for field crops in order to support the registration of Plant Protection Products (PPPs) in France. The scenarios cover the most representative agricultural conditions in France and are implemented in a ready-to-use tool.

The scenarios consist of combinations of weather, soil, and crop parameters. France is divided into 31 Agricultural Units (AU). One MARS-tile is assigned to each AU, consisting of daily weather data. The selected tile represents the largest agricultural area within the AU.

Relevant soil types were identified and their area within each AU was calculated by INRA Orléans. In total 18 major representative soil types are considered with profile depths ranging from 40 cm to 140 cm.

The field crops sugarbeet, winter wheat, winter oil seed rape, grain maize, fodder maize, winter barley, potato, and sunflower are considered in the scenarios. They are not calculated as monoculture, but as realistic crop rotations (1-3 years). The selected rotations as well as the crop characteristics (such as emergence and harvest dates) are AU-dependent.

Depending on the occurences of the crops within the AUs and the possible combinations of soils and crops, different numbers of scenarios are available for the crops, ranging from 49 scenarios for potatoes to 289 scenarios for fodder maize.

The final endpoint used in the evaluation is the 90th overall percentile concentration, resulting from the combination of the 80th temporal percentile of each single scenario and the 80th spatial percentile from all scenarios together.

The scenarios are implemented in an ACCESS®-database, so that all parameters are stored in a transparent way and are fully accessible. A FROGS user interface is also available in order to enter the substance parameters and application scenarios, and prepare the relevant input files for PEARL 3.3.3 simulations, and process the PEARL 3.3.3 results for evaluation.

All the available options of PEARL 3.3.3 regarding substance and application parameters are also applicable in the FROGS tool. In addition, FROGS allows for input of the applications relative to BBCH growth stage, in full adequation with the intended use of the PPP. Output summaries, distribution graphs, and the final PECgw value for the parent substance and all metabolites are derived automatically by the FROGS-tool. Mitigation by location or soil properties is possible without the necessity of further scenario calculations.

EC06A-4

Short-term air-concentration variability: benefits of model integration

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Several monitoring campaigns from different locations have shown how air concentrations of persistent semivolatile chemicals (SVOCs) can exhibit short-term (less than 24-hour) variations. In some cases, the observed patterns were ascribed to the variability of planetary boundary layer (PBL) height and structure. This aspect, which is widely known to influence the dispersion of pollutants emitted to atmosphere, is well accounted for in air dispersion (AD) models, which are well suited to describe single episodes of pollutant release and transport. Integrating the capabilities of such models into a multimedia fate box (MFB) modelling approach could thus offer important insights into the key processes that govern the short- and long-term behaviour of SVOCs. In the present work, a coupled meteorological preprocessor-MFB model previously developed by our research group is run to test its ability to capture the short-term variations observed in the PCB air concentrations measured in Zurich during a three-day period; a good model performance, with an agreement wich is generally within a factor of about 2-3, was observed. The fully-integratedy version of the model, which also incorporates an AD model based on the Gaussian equation, was then run for two PAHs, in order to assess the impact of an incinerator-like point source on the concentrations in air and soil. Results stressed the large influence exerted by PBL meteorology in determining the frequency and amplitude of the short-term variations in air concentrations and showed the magnitude of the potential increase in concentrations due to the point source. Further simulations showed the increased contamination of the soil environment due to the combination of low PBL height, precipitation and point source contribution and the extent of the consequent degassing episodes due to favourable atmospheric conditions (such as increased PBL heights and wind speed), which have the effect of "recharging" the lower air compartment.

EC06A-5

Modelling the cycling of persistent organic pollutants in shelf seas with a combined hydrodynamic and fate and transport ocean model: the North Sea system

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The environmental fate of selected persistent organic pollutants (POPs) in the North Sea is modelled with a combined hydrodynamic and Fate and Transport Ocean Model (FANTOM). Large amounts of POPs enter the North Sea system from the surrounding countries. Major entrance pathways of POPs to the North Sea are through atmospheric deposition and river inputs, with additional contributions coming from bottom sediments and adjacent seas. POPs in the ocean are subject to a wide range of processes including mechanical, chemical, physical, and biological processes. The fate of POPs in the oceans is not yet completely understood, though oceans are generally considered to act as ultimate sinks for POPs. Budgets of POPs are calculated to determine whether the North Sea can act as a reservoir of POPs. In this study, the POPs PCB 153 (almost insoluble in water) and lindane (gamma-HCH) (very soluble in water) are modelled for the period 1995 - 2005. A 3-D hydrodynamic model is a necessary prerequisite tool for modelling the fate of POPs in the ocean. For this purpose, we have developed a very high resolution version of the Hamburg Shelf Ocean Model (HAMSOM) for the North Sea region. The impact of climate variability on POP levels in the North Sea is investigated by performing future scenario model runs in 10 year time slices to the year 2100 using plausible POP input levels. Hydrodynamic variables are calculated in the HAMSOM which is forced at the surface and open boundaries. POP processes are calculated with the FANTOM model. Evolution of the total concentration of a pollutant at a fixed point is calculated with a simple Eulerian, advective-diffusive model

with sources and sinks. Atmospheric concentrations of POPs are provided by output from the atmospheric model of the MSC-E, EMEP. The net flux of a pollutant to the sea surface from the atmosphere is calculated as the net value of gaseous air-sea flux, dry particle deposition flux and wet deposition flux. POP concentrations in river estuaries have been calculated from available datasets. Results show that gamma-HCH concentrations are highest in summer but PCB 153 concentrations appear to be greatest during the winter months. Concentrations of PCB 153 and gamma-HCH decrease in the North Sea over the period 1995-2005. Preliminary results show that sediment concentrations also decrease during this period, suggesting that perhaps the North Sea cannot act as a reservoir of POPs for the oceans.

EC06A-6

Development and validation of environmental fate model for herbicides of paddy fields using grid-catchment integrated multimedia modeling system (G-CIEMS) for all Japan area

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We developed high accuracy model which could calculate daily concentration variation for many herbicides in all rivers in Japan. Monitoring data were actively used for representative chemical concentrations in environment for estimation of risk analysis. However, observed values have temporally-spatially specific information for limited numbers of chemicals. It is important to cover temporally-spatially variable concentration for many chemicals. So, model calculation should be applied, especially for ecological risk analysis. Pesticides are absolutely necessary. However, pesticides should be carefully controlled because of its original purpose of herbicidal, insecticidal, or fungicidal actions. Rice is a most major crop plant in Japan. Since schedules of rice transplanting collect to a specific period in each region, pesticides are also intensively used and run off to rivers. Herbicides are especially need to be regarded because these are thrown in paddy water and that why have high run-off ratio. For ecological risk analysis, it is important to calculate daily variation of pesticide concentrations in all rivers in Japan for many kinds of pesticides.
We had developed the multimedia environmental fate model G-CIEMS (Grid-Catchment Integrated Multimedia Modeling System) and Japanese GIS data set used for this model. In this study, we developed the method to predict daily variation of concentrations of many herbicides to paddy fields in all area in Japan. For developing the method, we collected, analyzed, and make a database of various kinds of relative information. We calculated daily emission amount of 26 kinds of herbicides for each river segment and air mesh, which data suitable for G-CIEMS model. In order to validate this model, we investigate herbicide concentrations in seven rivers in Japan. In total 182 pairs (= 7 river sites x 26 herbicides), herbicides were detected in 171 pairs that usable to the validation of this model. To evaluate the reliability of this model, peak concentration and peak days were compared between predicted variations and observed variations for 171 pairs of sites and herbicides. Peak concentration differences between predictions and observations were less than one order of magnitude in 113 pairs which reached 66% of total 171 pairs. Peak day differences between predictions and observations were less than two weeks in 136 pairs which reached 80% of total 171 pairs.

EC06B-1

Guidelines for good modeling practice in environmental assessment of chemicals

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Multimedia models are increasingly used as scientific tools and in a decision-making context. One of the obstacles to more rapid and widespread adoption of models in risk and hazard assessment for chemicals is the lack of clear guidelines for applying them. Without such guidelines, model users may remain unaware of limitations, uncertainties, omissions and subjective choices in models. The risk is then that models are used for purposes different from those intended, making untenable conclusions possible. In order to stimulate the correct and careful use of models, the SETAC Exposure Modeling Advisory Group has taken the initiative to provide a set of clear *good modeling practice (GMP)* guidelines. The guidelines aim to serve as a reference for applying existing models for the environmental assessment of chemicals in a decision-making context.
The selection of an appropriate modeling tool is an essential requirement in the modeling process. Models under consideration should be examined for appropriate scope and domain of application, and their constraints and major assumptions. The proposed GMP guidelines emphasize the need to clearly document the identity and version number of the model that is used, as well as the values and sources of all input data. Any changes made to the default parameterization or model equations must also be clearly specified in the report. A comprehensible and complete documentation of the model results is crucial. Each result must be clearly associated with a corresponding set of input data. Model results should be clearly separated from interpretation and discussion. One of the goals of the GMP guidelines is to ensure that an interested third party can reproduce the modeling result. This will increase the transparency of decision-making processes that rely on model results. Recommendations for sensitivity and uncertainty analysis are also provided.
A hazard assessment of decamethylcyclopentasiloxane has been carried out as a case study to illustrate application of the good modeling practice guidelines.

EC06B-2

Transfer of PCB from sediment to biota: development of a bioaccumulation model in a risk assessment perspective

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Many chemical, physiological and trophic factors are known to be important in PCB bioaccumulation processes and trophic transfers in biota. Understanding the primary factors influencing PCB contamination of fishes is critical for predicting and assessing the risks to upper-trophic levels consumers including humans. We propose here (1) to identify PCB contamination pathways that could explain between and within species variability in fish concentrations; and (2) to describe PCB transfer along fish food chains. Three freshwater river fishes (Barbel, Bream and Chub) were sampled in three sites along the Rhone river (France), where fish consumption is partially prohibited for exceeding the current regulatory threshold. By combining stable isotope mixing models and stepwise regression, we showed that fish body size, PCB concentration in sediment and foraging habitat (exploitation of detrital carbon sources) explained around 80% of the within- and between species variability observed in PCB concentrations. Spatial gradients of contamination are more important than the type of food consumed and its trophic status. A bioaccumulation food-web model, based on physiological processes, was then developed in order to describe PCB transfer along the food chain of these fish species. Variation of environmental conditions (water temperature), individual physiological traits (growth rate) and physico-chemical properties of PCBs (Kow) are considered. We used Bayesian Inference to calibrate the involved functions, and we thus were able to pass on data variability and parameter uncertainty to model predictions and to provide a credibility interval around them. The predictions of our bioaccumulation model describes seasonal variations in fish PCB concentrations (due to environmental conditions) and appeared particularly efficient in a risk assessment perspective. By linking sediment to fishes contamination, our model could help in determining sediment management guidelines in the future.

EC06B-3

Prioritising chemicals used in PCPs in China for environmental risk assessment: application of the RAIDAR model

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Chemicals used in personal care products (PCPs) represent a significant fraction of chemicals used in commerce in China. Recently, there has been emerging concern regarding the use of a number of substances classified under this category, including the nitro- and polycyclic musks, UV blockers such as methylbenzylidene camphor, and preservatives such as the parabens. Unlike pharmaceuticals, PCPs, enter wastewater and the aquatic environment after regular use during showering or bathing. The environmental fate and effect datasets of many cosmetic ingredients, however, are limited. Methods for assessing environmental fate and prioritising chemicals used in PCPs are thus needed. In an effort to address this knowledge gap we have identified the chemical ingredients used in 2500 PCPs across China, and estimated the annual emission of these chemicals. An initial list consisting of approximately 1000 chemical ingredients from these products is identified and characterized. Next, the physical-chemical property data for these substances have been estimated, and used as model inputs in the Risk IDentification And Ranking (RAIDAR) model, which has been parameterized for the Chinese environment. The result from this ranking exercise is a list of chemicals for which risk assessment could be prioritized.

EC06B-4

Cross-sectional trends in human body burdens with age? The role of atmospheric emissions.

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Body burdens from the cross-sectional biomonitoring of persistent bioaccumulative contaminants in a human population are often plotted against age. Typically in the literature, such cross-sectional relationships have been interpreted to provide information on longitudinal trends in human exposure. We used longitudinal trends in human exposure simulated with the CoZMoMAN model to calculate cross-sectional body burden versus age trends (CBATs) and to investigate the factors that control them. Our calculations indicate that CBATs can be categorized into three general shapes: dual maxima, adolescent minima, and rapid escalation. Each CBAT shape occurs at a characteristic time relative to the emissions profile. A population cross-section will exhibit the dual maxima profile during periods of increasing emissions. The adolescent minima will be observed during periods of decreasing emissions, and rapid escalation corresponds to periods after emissions have ceased but contamination of the environment remains. Many model input parameters, including chemical partitioning properties, metabolic degradation half-lives and the length of the emission period, have surprisingly little influence on the CBATs. The most important factor controlling the shape of cross-sectional time-trends is the amount of time elapsed since emissions have decreased. We propose that what many cross-sectional biomonitoring studies have interpreted as an increase in body burden with age is actually a result of sampling many decades after the peak in emissions occurred. As such, we propose that CBATs can be used as an indicator for the temporal trend of the emissions experienced by a given population. In other words, human biomonitoring studies can be used to reconstruct emission timelines. In some instances, the emission peaks reconstructed from human biomonitoring data occur earlier than current historical emission estimates suggest.

EC06B-5

Increase of contaminant levels in the Arctic due to future climate change

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We have applied the Danish Eulerian Hemispheric Model (DEHM) to study the impact of climate change on contaminant levels in the Arctic. DEHM is a 3-D atmospheric chemistry-transport model developed over the last 20 years for studying the long-range transport of SO2, SO4, and Pb to the Arctic. The model covers the Northern Hemisphere and all important source regions for the Arctic are included in the model domain. This model has been developed further to include four chemical groups: a group related to ozone chemistry, a group related to primary particulates, a group with mercury species/chemistry, and a group with Persistent Organic Pollutants (POPs). The model has a spatially detailed 3-D atmosphere up to 15 km over the surface. In addition, it has four surface compartments: a 75 m thick ocean layer, a 15 cm thick soil layer, and dynamically evolving vegetation and seasonal snowpack compartments.
The model system has been run with meteorology obtained from ECHAM5/MPI-OM (SRES A1B scenario) for three different decades: 1990-1999, 2090-2099 and 2190-2199. In this climate scenario the global temperature is predicted to increase continually with 3 °C by the end of 2100 and 4.2 °C by the end of 2200 relative to the period 1971-2000.
The model system was run initially for a period in order to spin-up the concentrations of POPs in the ocean and soil compartments. The concentrations in air, ocean water and soil of all the species from this simulation where used as initial concentrations for three different model runs with constant emissions (from year 2000) for the decades: 1990-1999, 2090-2099 and 2190-2199 using input from the ECHAM5/MPI-OM climate model. The differences between the results for the three decades are hence only due to changes in the meteorological as predicted by the ECHAM5/MPI-OM model system.
The results produced with the DEHM model system indicates that the atmospheric concentrations of the studied POPs will increase due to increased atmospheric transport to the Arctic in a future warmer climate. This study has shown that it is useful to use meteorological output from climate models to investigate the exposure levels of contaminants under future changed climate conditions.

EC06B-6

Assessing the potential implications of global climate change on human exposure to contaminants in the Arctic: opportunities & limitations

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The influence of global climate change on the transport of organic contaminants to the Arctic and the long-term implications for human exposure is a complex issue. There are at least five broad categories of change to consider; i) chemical use and emissions ii) extent of contaminant delivery to the Arctic environment iii) processing of contaminants in the physical environment iv) processing of contaminants in the human food chain and v) changes in exposure due to alterations in the lifestyle of Northern communities. Climate model-based projections describing changes to the physical environment in the Arctic are available and were used as guidance in scenario development. The main goal of the study was to estimate/constrain the factor of change associated with potential alterations to the physical environment and other aspects identified above (e.g. dietary transition). Global-scale fate and transport simulations were undertaken using Arctic Contamination Potential ($eACP_{10}$), a model-derived output integrating long-range transport and accumulation in surface media (i.e. excludes mass in atmosphere), to estimate factors of change associated with different scenarios. Simulations coupling output from fate/transport models with human food web bioaccumulation models were also conducted to examine the influence of diet. Compensatory behaviour was found to be a recurrent theme throughout this investigation whereby a single parameter (e.g. temperature) or combination of parameters (e.g. temperature + primary productivity) can exert antagonistic effects that tend to dampen the response to alterations in the physical environment with respect to human exposure potential. For hydrophobic contaminants (i.e. octanol-water partition coefficient > 100 000), shifts in diet appear to represent the greatest potential for change in contaminant exposure. While long-range atmospheric transport potential and air concentrations in the Arctic may be enhanced under global climate change, contaminant amplification and exposure potential in surface compartments most relevant for humans may actually be reduced in comparison to contemporary conditions. However, the realism/representativeness of the modeling tools used is a major uncertainty underlying these conclusions. A more detailed and dynamic treatment of the cryosphere would be a particularly useful undertaking in the overall context of this study.

EC07 - Integrated chemical and biological approaches for toxicant identification

EC07-1

Effects-directed analysis of contaminated sediments from the Sava River, Croatia

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The application of toxicity identification and evaluation (TIE) protocols for the toxicological characterisation of contaminated sediments showed that the majority of the observed adverse ecological effects were associated with toxic organic chemicals. Considering enormous number as well as chemical and toxicological diversity of organic contaminants, effects-directed analysis (EDA) represents the best available tool for the comprehensive assessment of hazardous chemical contamination in aquatic sediments. The aim of this paper is to present the results obtained in a case study carried out in the Sava River basin, Croatia. The analytical approach used for screening included a detailed characterisation of the collected samples, based on the combination of gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/time-of-flight mass spectrometry (LC/Q-TOF). Ecotoxicity profiling of the investigated samples was performed using a battery of bioassays, including cytotoxicity, chronic toxicity, EROD activity; inhibition of the multixenobiotic resistance (MXR), genotoxicicity and estrogenic potential. The most pronounced effect, detected in the Sava River sediment, was CYP1A induction potential. It was predominately associated with the nonpolar fraction and polycyclic aromatic hydrocarbons were indicated to be responsible for the observed effect. However, other endpoints, such as algal toxicity and estrogenic potential, indicated comparatively higher importance of polar contaminants.

EC07-2

Integrated bioassay and chemical analysis of glucocorticoid and estrogenic activities in the rivers Rhine and Meuse

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Effect-directed analysis (EDA) has shown to be a valuable approach to investigate the nature of biologically active compounds. Various research groups have succesfully applied EDA and identified compounds responsible for endocrine disrupting, especially estrogenic, effects. However, many other hormone-like compounds are excreted naturally or are used as pharmaceuticals and thus might enter the environment via similar routes as estrogens. Indeed, using novel CALUX bioassays for progestagenic, androgenic estrogenic and glucocorticoid receptor activation, the presence of especially glucocorticoid activity in Dutch surface waters and glucocorticoids in waste water was recently reported. However, data on glucocorticoid activity remained scarce and its chemical identity in surface water unknown.
We investigated the presence and identity of glucocorticoid and estrogenic activities in the Dutch catchments of the main rivers Rhine and Meuse. GR and ER CALUX measurements were done throughout the year and at five different locations to investigate spatial and seasonal differences. Having experienced that natural and synthetic hormones often explain the majority of endocrine activities in aquatic samples, we decided not to perform a full EDA, but first to develop and apply specific and very sensitive target analysis methods on LC-LTQ-FT-orbitrap MS and UPLC-tQ-MS for a large number of natural and pharmaceutically used steroid hormones (estrogens, glucocorticoids, androgens, progestagens). The methods were used to analyze bioactive samples and to calculate the extent to which natural and synthetic hormones were responsible for the measured activity. GC-screening was applied to obtain a more integrated picture of the contaminant composition of the samples.
Glucocorticoid and estrogenic activities were detected at all locations and followed a seasonal pattern. Estrogenic activity was found in higher concentrations in the Meuse than in the Rhine. Glucocorticoid activity in waste water ranged between 0.01 and 0.6 µg dex-eq/L. Analytical methods were developed for over 30 steroid hormones. In waste waters, glucocorticoid activity was predominantly explained by cortisol, cortisone, prednisone, prednisolone and triamcinolone acetonide. The results in surface waters will also be discussed. GC-screening demonstrated the presence of complex and location specific mixtures of e.g. pesticides, pharmaceuticals, industrial solvents and flame retardants.

EC07-3

Structure elucidation of mutagenic contaminants in blue rayon extracts of river water

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We elucidated the structure of mutagenic compounds in water samples using effect-directed analysis (EDA) based on a combination of (i) chemical fractionation, (ii) mutagenicity testing, and (iii) chemical analysis. Blue rayon passive samplers preferentially adsorbing planar aromatic compounds were exposed in the Elbe river downstream of discharges from dye industries. The blue rayon extracts were separated into acidic, neutral and basic fractions using cation and anion exchange cartridges and fractionated further by LC using a polymeric C18 column and subsequently a phenyl-hexyl column. The Ames fluctuation assay with strain TA98 was used to screen samples for mutagenic compounds with and without metabolic activation. Fractions showing the highest mutagenicity were analyzed by LC-MS/MS using a high resolution/high accuracy LTQ Orbitrap. Chromatograms were deconvoluted using the software MZmine and full scan and product ion mass spectra of intense peaks were submitted to molecular formula generation using the software MOLGEN-MSMS. To assign a structure to the molecular formulas, a database search in ChemSpider was combined with structural classifiers such as MS/MS fragmentation interpretation. High mutagenicity was found predominantly in the neutral and acidic farction. Based on the accurate mass measurement and isotope abundances a unique molecular formula could be assigned to almost all of the peaks of interest in the two LC-sub-fractions of the neutral fraction showing the highest mutagenicity. The structure elucidation from molecular formulas was the most critical step, but a range of compounds could be tentatively identified and confirmed by reference standards. Many other polyaromatic compounds yielded a low number of characteristic MS/MS fragments. Furthermore, often a large number of possible structures for the same molecular formula were retrieved from the Chemspider database. Thus, refined analytical approaches to include additional structural classifiers were investigated: A. comparison of ionization efficiencies in ESI and APCI in positive and negative ion mode for a range of reference standards suggests that these may be a useful classifier to suggest the presence of certain functional groups The use of deuterium exchange experiments and chemical derivatization was studied as a complementary approach to selectively confirm or rule the presence of functional groups.

EC07-4

Characterisation of dioxin-like compounds in road-side snow

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Snow is a useful tool to measure the accumulation of contaminants because it scavenges pollutants from the air due to its large specific surface area. In the current study volatile and non-volatile dioxin-like contaminants were identified in snow samples using an effect-directed analysis (EDA) approach.
Snow was collected in Oslo, the capital of Norway and one background sample was collected in a mountain area. The samples were filtered and extracted using solid phase extraction. The particles on the filters were extracted using accelerated solvent extraction, cleaned-up on a multilayer silica gel column and separated in 2 fractions: the hexane fraction F1 and the DCM fraction F2. In addition a subsample of the particles was analysed for black carbon (BC) content. Both the water fraction and the two particle fractions were analysed for AhR agonists using the CALUX assay and by GC-HR-ToF-MS for broad-spectrum analysis and to target for PAHs.
The AhR agonist levels in the water fractions from the city samples were between 15-387 pg/l. An elevated AhR activity of 221pg/l TEQCALUX was detected in the mountain sample. In the F1 particle fraction, where dioxins, furans and PCBs elute, TEQCALUX was <LOD. In the F2 particle fraction, where PAHs elute, TEQCALUX was between 1354-7389 pg/l.
One possible explanation for the elevated levels in the water fraction of the mountain sample could be the occurrence of BC in the samples collected in the city. All samples except the mountain sample were filtered before analysis. Dioxins and other contaminants are known to bind to BC and BC could possibly trap part of the contamination originally present in the snow, this could result in lower AhR agonist levels in the water fractions of the filtered city samples.
With targeted analysis of PAHs 0.0008-0.04% and 2-9% of the TEQCALUX could be explained in the water fractions and the particle F2 fractions respectively. This highlights the fact that there are other unknown AhR agonists present in these samples. Preliminary results from the GC-HR-ToF-MS showed a large amount of aromatic unresolved complex mixture (UCM) in the particle F2 fraction. Ongoing work utilising comprehensive gas chromatography coupled to mass spectrometry (GCxGC ToF MS) is focused on teasing out AhR agonists in this aromatic UCM. Further work will focus on more advanced broad-spectrum analytical techniques.

EC07-5

Effects - directed identification of novel antiandrogenic contaminants in fish exposed to wastewater effluents

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The widespread occurrence of feminized male fish downstream of some wastewater treatment works has led to substantial interest from ecologists and public health professionals. A survey of UK waste water treatment works (WwTWs) has revealed that the majority of the effluents sampled contain antiandrogenic as well as estrogenic activity[1]. Another study has demonstrated that feminization of fish was correlated with their predicted exposure to both antiandrogens and estrogens or to antiandrogens alone[2]. Identification of key active compounds in wastewaters is difficult as they can often be present at subnanogram per litre concentrations. However, a wide range of xenobiotics can bioconcentrate in fish bile at concentrations tens of thousands greater than in the effluent itself, facilitating the structural identification of bioavailable contaminants present in the ambient environment [3]. In this study, bile from fish exposed to a WwTW effluent was analysed for antiandrogenic contaminants using a bioassay-directed analytical approach. Fractionated bile was interrogated for anti-androgen activity using yeast and a mammalian-based androgen receptor transcription assay (Anti-YAS) and (AR-CALUX). Fractions containing

antiandrogen activity were analysed by gas chromatography- mass spectrometry (GC-MS) after trimethysilylation and the identities of key structures present in wastewater effluent have been determined. Key compounds identified included triclosan (a biocide- thought to act as a weak estrogen [4]) and chlorophene (a germicide not previously recognized as and antiandrogen). Other novel antiandrogenic compounds identified included: chloroxylenol (an antiseptic), resin acids, metabolites of pyrene and biphenyl, and compounds known as xenoestrogens: nonylphenol and bisphenol A. Together, triclosan and chlorophene accounted for >50% of the total antiandrogenic activity in fish bile and these compounds are currently being tested for antiandrogenic activity in vivo in fish.

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EC07-6

Identification of thyroid hormone-like compounds in polar bear plasma by Effect-Directed Analysis

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Endocrine disrupting compounds (EDCs) released into the environment may bioaccumulate and biomagnify in the foodchain. Significant concentrations of thyroid hormone-like (TH-like) compounds were found in biological tissue (e.g. in fat and blood) of top predators such as humans and polar bears (Ursus maritimus). This study aims to identify TH-like compounds in plasma of polar bear cubs-of-the-year by Effect-Directed Analysis (EDA) using a dedicated plasma sample treatment method.

Ion exchange solid-phase extraction (SPE) followed by liquid-liquid extraction (LLE) was evaluated by known TH-like compounds (hydroxylated polychlorinated biphenyls (OH-PCBs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs), other halogenated phenols (OHPs) and perfluorinated compounds (PFCs)) as well as the less potent non-hydroxylated parent compounds of the PCBs and PBDEs spiked to cow plasma. Good chemically determined recoveries were obtained for OHPs, OH-PBDEs, OH-PCBs extracted from spiked plasma (>90%) and lower recoveries for PFCs (~60%) and for PCBs and PBDEs (~30%). In the radioligand T4*-TTR binding assay the spiked extracts showed TTR binding potencies which were in good agreement with the calculated theoretical spiking levels.

Then the SPE-LLE method was successfully applied to polar bear plasma samples in collaboration with the Norwegian University of Science and Technology (NTNU) and the BearHealth-project and the extracted samples were screened in the T4*-TTR binding assay. Measured TH-like activities were generally higher than the estimated activity based on the measured concentrations of target TH-like compounds. Three samples have been selected for further analysis, where the analyzed target compounds could only explain part of the measured TH-like activity, to identify the compounds causing the remaining activity. Identification studies will be carried out to explain the remaining TH-like activity.

EC08 - Tracking community consumption of illicit drugs and other substances by measuring human metabolic residues in urban wastewater

EC08A-1

Sewage epidemiology: potential of a novel approach

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Sewage epidemiology is a new approach to population studies, which use wastewater analysis for assessing collective voluntary and involuntary exposure of the members of a community to a wide range of chemicals. The rationale of this approach is that traces of almost everything we eat, smoke, drink, ingest, or absorb, are excreted with our urine or stool and end up in wastewater, where they can be measured.

Illicit drugs were the first application: residues of the drugs consumed by a collectivity are excreted in wastewater and their levels, knowing kinetic, metabolism, and behaviour in wastewater, and characteristics of the sewage system, can be used to back-calculate for the type and amount of the substances collectively consumed by the population.

However, this approach has a much wider range of potential applications. This include for instance pharmaceuticals. We know the amount of a given drug which is prescribed or sold in a given area, but we don't know the amount really taken by patients. Wastewater analysis for the metabolites of the pharmaceuticals can help in elucidating the compliance to the treatment, thus contributing to evaluate its efficacy. Other examples include pesticides, PCBs and food contaminants. Monitoring of the metabolites of these substances in wastewater is a new approach to estimate the real exposure of the population to these chemicals.

Sewage epidemiology needs a multidisciplinary approach, with the interaction of experts of several different fields, such as pharmacokinetic, medicine, and analytical chemistry. Monitoring wastewater has the potential to extract useful epidemiological information from qualitative and quantitative profiling of biological indicators entering the sewage system, and to become a useful tool to be used in population studies .

EC08A-2

The overlooked importance of sampling to advance wastewater analysis from a promising method to a useful and reliable tool for the estimation of illicit drug abuse

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Sewers were not designed to assess illicit drug consumption. However, immense advances in analytical chemistry have propagated wastewater analysis as a promising tool to tackle this difficult task. It has been shown for legal drugs that traditionally applied sampling methods are prone to systematic biases and random errors resulting in sampling artefacts ranging from "not significant"to "100% or more". For the first time we present illicit drug concentrations and loads at high temporal resolution in sewers to emphasise the importance of sampling. A unique time series at two-minute time intervals for 11 legal and illicit drugs and selected metabolites reveals high short-term fluctuations. Based on this and previous studies, we provide general recommendations for sampling in any catchment to make the wastewater epidemiology method a reliable and acknowledged tool in the future. Sampling is the first and most crucial step to maximise data quality. Sophisticated chemical and statistical analyses simply cannot make up for any deficiencies in sampling. If traditional sampling methods are not adapted to sample for illicit drugs, the wastewater epidemiology method may not be more accurate and reliable than traditional surveys and interviews.

EC08A-3

The significance of chirality of illicit drugs for the estimation of drugs abuse using the sewage epidemiology approach

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Most illicit drugs are chiral compounds. Their enantiomers reveal different potency and are often characterised by stereoselective disposition in the body. Amphetamines for example are characterised by one asymmetric carbon centre and exist in the form of two enantiomers, which significantly differ in potency, e.g. S(+)-amphetamine has twice as high stimulant activity as R(-)-amphetamine. However, R(-)-amphetamine has been reported to be as effective as the S(+)-enantiomer in the development of the psychotic syndrome. The phenomenon of the chirality of amphetamine is crucial in forensic identification of its illicit use. This is because amphetamine has some limited therapeutic use in narcolepsy and attention deficit hyperactivity disorder. Amphetamine is also formed as a metabolite of methamphetamine and several prescription drugs such as selegiline.

The aim of this presentation is to raise awareness of the importance of the phenomenon of chirality in forensic estimation of drugs abuse via sewage epidemiology approach. The report will present results obtained during a ten month long monitoring programme of several WWTPs in the UK. To the authors' knowledge this is the first report tackling the phenomenon of chirality in the estimation of drugs use using a sewage forensics approach. Among the studied chiral drugs are: amphetamine, methamphetamine, MDMA, MDEA, MDA, ephedrine and pseudoephedrine. The monitoring programme revealed that these chemicals are not released into wastewater in the form of racemic mixtures. It was for example observed that in the case of amphetamine, R(-)-enantiomer was dominant in all analysed wastewater samples. It was also noted that enantiomeric ratios of amphetamine enantiomers differed significantly between sampling points and sampling times and varied from 0.53 to 0.84. Similar patterns were observed in the case of other studied chiral drugs. There are several possible reasons for this behaviour. Among them are: (i) different metabolism patterns of enantiomers of the same drug with preferential metabolism of one enantiomer only, (ii) formation of illicit drugs as a result of metabolism of legally prescribed drugs, or (iii) use of drugs in non-racemic forms of drugs. Although a complex and demanding process, the verification of enantiomeric ratios can provide vital information about patterns of drugs usage and can help in the differentiation between their legal and illicit usage.

EC08A-4

Analysis and interpretation of specific ethanol metabolites in sewage effluent for the quantitative measurement of regional alcohol consumption

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Ethyl sulphate and ethyl glucuronide are excreted in urine following the ingestion of alcohol and are useful biomarkers for the identification of acute alcohol consumption. The present study reports a novel ion-exchange mediated chromatrographic method for the quantitative measurement of ethyl sulphate and ethyl glucuronide in sewage effluent, and presents a novel calculation method for the purposes of relating the resulting sewage concentrations with rates of alcohol consumption in the general population. Sewage samples were collected from a sewage treatment plant servicing 500 000 people over a 25 day period and analysed for levels of ethyl sulphate and ethyl glucuronide. The resulting data were then used to estimate combined alcohol consumption rates for the region, and the results compared with alcohol related sales statistics for the same region.Ethyl glucuronide was found to be unstable in sewage effluent. Ethyl sulphate was stable and measurable in all samples at concentrations ranging from 2 µg/L to 31 µg/L. The highest concentrations of the alcohol biomarker were observed during weekend periods. Sixty one percent of the total mass of ethyl sulphate in sewage effluent corresponds to alcohol consumption on Friday and Saturday. Sales statistics for alcohol show that consumption in the region is approximately 6750 kg/day. The quantity of ethyl sulphate passing through the sewage system is consistent with consumption of 4900 - 7800 kg/day.Sewage epidemiology assessments of ethyl sulphate can provide accurate estimates of community alcohol consumption, and detailed examination of the kinetics of this biomarker in sewage streams can also identify time-dependent trends in alcohol consumption patterns.

EC08A-5

Trace analysis of barbiturates in wastewater by HPLC-LTQ-Orbitrap MS

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In a recent survey, illicit drugs and their degradation products were encountered throughout the water cycle in the Netherland. There was also evidence for the occurrence of at least three barbiturates (barbital, pentobarbital and phenobarbital) in the water cycle. However, the method applied suffered from a lack of sensitivity for these compounds. Earlier research has confirmed that barbiturates are persistent in an aqueous environment and originate probably from leachates of old landfills [1]. There are almost 2500 different kinds of barbiturates known/synthesized from which only a few are ever used for medical purposes. Due their adverse side effects barbiturates are nowadays mostly replaced by other products like benzodiazepines. During the 1930s, barbiturates were used as sedatives but during the 1960s their illegal use became more and more widespread. In the Netherlands barbiturates are nowadays mainly in use as veterinary euthanasia

and antiepileptics. In 2009 the total amount of barbiturates imported in the Netherlands was 2068 kg. The largest part being pentobarbital 1296 kg followed by phenobarbital with 753 kg and barbital with 9.58 kg. Another source of phenobarbital is as the metabolite of primidone. The total prescription of Primidone in The Netherlands was 171 kg in 2007.
The first objective of our study was to set up a method to determine barbiturates in aqueous matrices with an increased sensitivity and accuracy. Secondly, we investigated the presence of barbiturates in wastewater. Finally we back calculated the total consumption of barbiturates and compared it with the import and export figures of the national inspectorate of the Netherlands

EC08A-PS
Poster spotlight
Miscellaneous
Poster spotlight highlighting abstracts WE 060, WE 061, WE 062, WE 063:
- Year-long community level measurement of drug use using passive methods with in situ calibration
- Selective determination of illicit drugs by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry
- Monitoring of drugs of abuse in Dutch sewage water by LC-LTQ FT Orbitrap MS
- Monitoring and uncertainty assessment of cocaine and benzoylecgonine wastewater loads in Switzerland

EC08B-1
Use of legal and illegal drugs in communities: methodological considerations for generating annual estimates of drug excretion based upon municipal wastewater sampling
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Municipal WWTP based testing for medications and drugs of abuse is in its infancy. The ability to document the presence of substances has been shown repeatedly around the world, however the utility of these data is limited until we understand how to reliably gather samples (e.g. for annual estimates) and accurately interpret results accounting for variability.
To address these methodological issues we undertook a year long, weekly sampling campaign in 18 cities in the Northwest region of the United States. Diverse municipalities were chosen for their variable population size, commuting flows, weather and resident characteristics. Data resulting from more than 900 tested samples provide insights into how to design sampling plans that account for day of the week, and longer periods, for stimulant drugs of abuse and opioid pharmaceuticals. Variability in data, and resulting confidence in the results, was found to be impacted by the specific compound of interest, the type of composite sampling utilized by WWTPs and the population characteristics of the municipality.
Determining sewer derived population estimates for drugs excreted appears to be a worthwhile endeavor and has the potential to provide data of sufficient quality to inform practice and policy decisions. However, it does have limitations due to variability that limits the precision of estimates. This variability differs for specific compounds based upon the chemical properties of the compound as well as the consumption pattern(s) for a community. Additionally, community characteristics and the WWTPs' methods for generating 24 hour composite samples influence index loads and variability. Future sewer based community estimates of drug excretion should be explicit about how samples were obtained. Researchers should also be explicit about the variability of estimates and ensure that variability is accounted for when making conclusions about the level or trends in substance use.

EC08B-2
Illicit drugs in prisons: sewage epidemiology to evaluate use and trends
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The prevalence of illicit drug use and drug related problems in prisons has been widely reported. Different strategies for drug detoxification and rehabilitation and to prevent drug entry in prisons are carried out by penitentiary authorities worldwide. Evaluation of success of such programs requires fast and economic tools to monitor illicit drug use by prisoners. In this context, the present work applied the sewage epidemiology approach to assess for the first time drug abuse in a penal complex from the levels of different drug residues measured in the prison sewage waters and evaluated the suitability of this approach to track and control illicit drug usage in such facilities. Drug residues were measured in sewage waters with an analytical method based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Levels of consumption indicators measured in these waters were used to backcalculate drugs usage in the penal complex. Daily use was observed for methadone (13.7 $g.day^{-1}$ on average), ephedrine (4.0 $g.day^{-1}$ on average), cannabis (3.4 $g.day^{-1}$ on average), cocaine (1.1 $g.day^{-1}$ on average) and alprazolam (0.5 $g.day^{-1}$ on average). Sporadic consumption was observed for heroin, amphetamine, methamphetamine, and ecstasy. Additionally, illicit drug use in the prison was compared to illicit drug consumption results obtained with the sewage epidemiology in different cities in Spain, and with extrapolated illicit drug use data at national level. In spite of the fact that the sewage epidemiology approach applied may suffer from bias that still need to be investigated and refined, it provides near 'real-time' information on collective drug use in an anonymous way and constitutes a very useful, economic and fast tool to evaluate the efficiency of measures adopted to control and track drug abuse in this type of facilities (or any other provided that it has an STP associated or an accessible collector system).

EC08B-3
Estimation of illicit drugs consumption by wastewater analysis: a five years-long investigation in Italy
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Wastewater analysis is a novel tool to estimate drug consumption in a population, this method is based on the direct measurement of illicit drugs residues (parent compounds or urine metabolites) in urban wastewater, and the back-calculation of the local drug consumption from the measured levels.
The aim of this study was to investigate the pattern of drug consumption in Italy by wastewater analysis. Drug residues were measured in urban wastewater using a specific multiresidue analytical method based on LC-ESI-MS/MS. Drug consumption was then estimated from the measured levels considering wastewater flow rate, human metabolism and the average dose of each substance. Daily wastewater samples were collected in large and small cities in northern and central Italy, and a five years-long investigation was conducted in Milan. The weekly consumption profiles were similar in all the cities showing an increase in cocaine and amphetamines consumption over the weekend, and a stable consumption of cannabis and heroin through the week. Cannabis was the most used drug (20-33 doses/d/1000inhabitants), followed by cocaine (2-6 doses/d/1000inhabitants), methamphetamine (0.1-4 doses/d/1000inhabitants) and heroin (1-4 doses/d/1000inhabitants). These estimates were generally in line with the national prevalence data from epidemiological studies, furthermore they highlighted local differences in the patterns of consumption. In fact, cocaine and methamphetaminuse were mostly used in large cities, while heroin in smaller cities. The monitoring of drug consumption in Milan from 2006 to 2010 allowed the identification of several changing habits. Cocaine consumption in Milan was 1100 g/d in 2006-2008, but fell to 620 g/d in 2009 and remained stable in 2010. Heroin consumption dropped as well from 80 to 40 g/d in 2008-2009, while methamphetamine consumption rose from 15 to 130 g/d from 2006 to 2010. Daily cannabis consumption was stable (3100-3500 g/d) along all the investigated period. This study confirmed wastewater analysis as a suitable tool to produce objective and updated estimates of drug consumption in a defined population, being therefore able to complement epidemiological studies.

EC08B-4
The consumption of illicit drugs in Brussels (Belgium) through sewage epidemiology
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A one year sampling campaign of influent wastewater samples (235 samples in total) was set up in the largest wastewater treatment plant (WWTP) in Belgium, receiving wastewater from the largest part of Brussels. The consumption of cocaine (COC), amphetamine (AMP), methylenedioxymethamphetamine (MDMA), methamphetamine (METH), methadone (MTD) and heroin (HER) in Brussels was evaluated based on measured concentrations of the parent compound and/or metabolites in daily 24-hour composite influent wastewater samples. The inevitable back-calculations used in the sewage epidemiology approach were adapted to newly available information. When a degradation of the compounds in wastewater was observed, the measured concentrations were corrected for this degradation to overcome underestimations. Further, new insights in excretion patterns of illicit drugs has led to adapted correction factors used in the back-calculations. For COC, three different back-calculation approaches were evaluated; based on benzoylecgonine (BE) or ecgonine methyl ester (EME) alone or on BE and EME together. No significant differences between the different back-calculations were observed. In addition, for the first time, efforts were made to calculate the number of inhabitants living in the catchment area of the WWTP in a real-time and dynamic way, based on concentrations of nitrogen, phosphorus, chemical oxygen demand and biological oxygen demand in the influent wastewater samples. Clear variations in the amount of inhabitants in the catchment area of the WWTP were observed, emphasizing the need for real-time calculations of this parameter instead of using the design capacity of the WWTP. For COC, AMP and MDMA a significant higher use during the weekend and holiday periods was observed ("recreational character") while for HER and MTD no trend in their consumption could be found. METH consumption was negligible. Generally, the sewage epidemiology calculations were in agreement with official statistics, when available. This study shows a further refining of the sewage epidemiology approach which leads to more reliable results. Because of the extensive sampling campaign, a sound evaluation of the results could be made. The approach shows great potential and in the future more efforts should be devoted to further optimize this methodology.

EC08B-5
Illicit drugs in Canadian municipal wastewater and estimates of community drug use
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In this study we evaluated the distribution of selected drugs of abuse (cocaine and its major metabolite, benzoylecgonine (BE), amphetamine, methamphetamine and ecstasy (i.e. MDMA)) in municipal wastewater from three Canadian cities, and we estimated community drug use from the levels of these compounds in untreated wastewater. This is the first study to evaluate community drug use in cities from North America, and the comparison provided on previously published data from European cities provide some valuable insights into differences between North America and Europe in drug usage. Cocaine was the most widely used illicit drug at a median level for the 3 cities of 15.7 doses per day per 1,000 people. For the other drugs, the median doses per day per 1,000 people were 1.8 for amphetamine, 4.5 for methamphetamine and 0.4 for ecstasy. Methamphetamine use was highest in the largest city and cocaine use was lowest in the smallest city. In addition, data obtained on the removals of illicit drugs during wastewater treatment, generally >50% except in a WWTP that uses primary treatment, show interesting comparisons to European data where more advanced technologies are typically used to treat wastewater.

EC08B-6
Estimation of illicit drug consumption via wastewater analysis in South-East Queensland, Australia: uncertainty evaluation
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Illicit drug use is an important public health and social problem. Analysis of wastewater for estimating drug use has become a useful tool to estimate drug consumption at a community level. A key potential application of this tool is the assessment of changes in illicit drug consumption in a given population. However, the successful application relies on understanding the uncertainties associated with all aspects of the measurement. The uncertainties include sampling (U_S), flow measurement (U_F), chemical analysis (U_C), population size (U_P) and excretion fraction (U_E) and biodegradation in sewers (U_B) of a given drug. It is relatively easy to accurately estimate and

thus reduce US and UC compared to UP and UB. Researchers also need to rely on operators at sewage treatment plants (STPs) to obtain best available estimates for U_F. The aims of our study were to (a) reduce the sampling uncertainty through an optimized sampling method, (b) identify and evaluate the total uncertainty associated with our per capita drug consumption estimates, and (c) provide an estimation of illicit drug consumption over 12 days in a urban catchment from South East Queensland, Australia. We predicted our estimates having a remaining uncertainty in a range from 24 - 31% even with the best sampling practice and current chemical analysis. Apparently, the respective uncertainties, particularly U_S and U_F, could be further reduced when there is a platform to normalise loads of DRs with those of other chemicals in wastewater. More effort is needed in the future study to refine the back estimation method so as to improve the confidence of the estimated data.

EH01 - Ecosystem services in natural, agricultural and urban areas

EH01A-1

Going beyond qualitative assessment of ecosystem services

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Understanding ecosystem services and their flows to human populations depends on a reliable assessment of ecological structure and function. The basis for this understanding should be an analysis of food webs and interactions among species because together they provide the mechanisms by which biological communities and associated ecosystems persist. It is necessary to develop both statistical and theoretical relationships between ecosystem structural metrics (e.g., species richness, biomass, population abundance, and habitat connectivity/fragmentation) and functional metrics (e.g., productivity, population persistence, resilience, and nitrification). Because there are few empirical data on basic relationships between ecological structure and function, ecological modelling will continue to play a central role in developing an understanding of ecosystem services in a general as well as site-specific sense. Examples of analytical tools for evaluating ecological structure and function relationships include models of: 1) single-species populations, 2) food webs (biological communities comprised of multiple species), 3) ecosystems (communities plus abiotic components of the environment), and 4) landscapes (including spatial scales encompassing multiple ecosystems). Because empirical assessments of relationships among ecological variables are limited, we maintain that attempts to assess ecosystem services in the absence of modelling populations, food webs, and/or landscapes could fail. We have no shortage of metrics to assess ecological structure, function, and services. The pressing issue, which ecological modelling can help address, is which of these metrics are most useful and cost-effective.

EH01A-2

Mapping soil biodiversity and ecosystem services in the Netherlands

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Soil policy in the Netherlands is in a transition phase - it is changing from a focus on the protection and restoration of soil from threats such as contamination and sealing, towards a focus on sustainable land use. A key aspect of the new focus is that soil quality should be assessed using chemical, biological and physical indicators in a systems approach. Furthermore, soil ecosystem services will be the basic concept in a framework for sustainable land use in order to split up the denominator 'soil quality' in meaningful aspects for society. We used data from the Netherlands Soil Monitoring Network (NMSN), including the Biological Indicator for Soil Quality (BISQ), to produce habitat-response relationships as proxies for soil biodiversity. These proxies were used to map soil biodiversity. Parallel to habitat-response modeling, functions for the performance of ecosystem services were produced based on biological, chemical and physical parameters. These functions will also be used for mapping purposes and their usefulness discussed. Maps were produced showing 1) predicted soil biodiversity (for several proxies of soil biodiversity), 2) predicted performance of ecosystem services (for several ecosystem services) and 3) the difference between predicted and expected performance of ecosystem services when the land use is sustainable. The expected state of a soil with a sustainable management (good ecological status) was characterized by a panel of experts. Maps were generated for both agricultural and nature areas. It is the aim of the maps to raise public awareness and to give support towards transition to sustainable land use. It is given acknowledgement that with current data, knowledge and consensus, the level of uncertainty of these maps is quite high. However, the general trends on a national scale may be helpful to show the potential for ecosystem services in The Netherlands.

EH01A-3

An ecosystem services framework: a case study on citrus production and insecticide use. To what extent are specific ecosystem services affected?

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The findings of this pioneering proof-of-concept study will deliver a case study to inform discussions between scientists and policy makers in pesticide regulation and stakeholder discussions with the European Food Safety Authority (EFSA) where an ecosystem services approach can inform risk management decisions.

In this study, an ecosystem services framework has been developed and is applied to a key insecticide for use in citrus growing. In the southern European Union (E.U.), citrus growing is particularly important both economically and culturally to the communities in these regions and nationally. The study focusses on Southern Spain where generations of farmers have grown citrus fruits with some regional landscapes being dominated by citrus groves. Spain has long been a leading producer and foremost exporter of oranges with nearly 6.5 million tonnes produced by the European Union in 2007/08.

Recent scientific thinking is increasingly focussing on the ecosystems services approach. The concept, advanced by the Millennium Ecosystem Assessment, brings a fresh approach to managing identified ecological risks in a holistic manner. EFSA has recently developed and published a framework for deriving specific protection goals for the environmental risk assessment of pesticides. The EFSA framework is based on the ecosystem services approach.

The ecosystem services approach identifies and values the primary ecosystem services that a habitat or 'property' may provide to humans given different land uses and actions (e.g. food production; recreation; biodiversity). The type, quantity, and quality of ecosystem services provided by an area are influenced by the surrounding landscape and land uses. Human activity can affect the quality and quantity of each ecosystem service provided. Overall, some services may be improved, some services may not be affected, and some services may be harmed. A systematic evaluation of such changes in service flows is required to allow for consistent comparisons across alternatives, as well as to optimise the achievement of environmental objectives while maximising benefits and minimising costs to society.

The framework developed for this study builds on the EFSA specific protection goals and demonstrates how an ecosystem services framework is applied by identifying and valuing the primary environmental services that a habitat may provide given different land uses and actions in citrus growing regions.

EH01A-4

Enhancing multiple ecosystem services in existing grass buffer strips

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Plant and invertebrate diversity plays a key role in terrestrial agro-ecosystems through the provision of multiple ecosystem services. Plants are the primary producers, while bumblebees pollinate crops and wildflowers, and spiders can help control crop pests such as aphids. Butterflies, particularly as larvae, and spiders also represent key food items for higher trophic levels including birds. Despite their importance, agricultural intensification has led to widespread declines in invertebrates and other taxa in the UK and NW Europe. In response to these biodiversity losses Agri-Environment Schemes (AES) were introduced and provide farmers with financial incentives in return for environmentally sensitive farming. A popular option is the establishment of perennial grass buffer strips with over 29,000 ha currently under UK AES agreements. However these strips tend to lack a wildflower component and as such are botanically species-poor. Studies have demonstrated how scarification of the buffer strip surface to create bare ground and application of graminicide to suppress competitive grasses can promote the development of sown wildflowers, benefitting invertebrates. The aim of this study was to investigate how the enhancement of existing grass buffer strips can be used to promote the abundance of bumblebees, butterflies and spiders, thereby supporting multiple ecosystem services.

The study was initiated in spring 2008 on two arable farms in Berkshire, UK on the outer 4 m of existing 6 m grass buffer strips to investigate two management practices: (a) scarification to create bare ground into which wildflower seeds were sown; (b) graminicide (fluazifop-P-butyl) application to reduce the competitive dominance of grasses.

The combination of scarification, sowing and graminicide resulted in the greatest abundance of sown wildflowers, and of bumblebees and butterflies reflecting a higher availability of foraging resource. Abundance of orb-weaving spiders responded to the sown wildflower cover, probably due to the utilisation of vegetation structures, e.g. inflorescences, to construct webs, and also higher prey densities. Incorporating these management tools into existing agri-environment options could benefit key invertebrate groups and support the delivery of multiple ecosystem services in arable landscapes.

EH01A-5

A SETAC Pellston Workshop on the risks from pesticides to pollinators: feedback and basis for the future from a regulatory perspective

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There have been reports, in recent years, of declines in native and managed pollinators in several regions of the world. Modern crop management practices to ensure a proper control of pest populations and diseases have been identified as a factor in these declines. Plant Protection Products (pesticides) are part of these management practices. Regulatory measures have been adopted requiring an assessment of the potential impact of these products on a broad range of taxa; included in the taxa evaluated are terrestrial arthropods especially those which are considered beneficial such as pollinating insects and honey bees (Apis mellifera) in particular.

Concerns which the use of pesticides in crop protection have raised for pollinators varies among countries as a function our understanding of their effect on crop pollination, the nature of the tools that are available for the risk assessment, and observed adverse effects in the field. The regulatory processes aimed at assessing the risks of pesticides to pollinators may differ from one country to another, and may not account for some key potential routes of exposure which were not identified as of concern at a local level.

A global Pellston SETAC workshop on estimating the potential risks of plant protection products to insect pollinators in January 2011 aimed to bring together the best available science regarding exposure and effects assessment methodologies for Apis and non-Apis bee species, and harmonize further the risk assessment approaches among North and South America, Europe, Australia. The workshop reviewed the state of the art science in (1) assessment of the exposure of pollinators to pesticides, (2) assessment effects using laboratory tests, (3) assessment of effects using field studies and (4) risk assessment strategies. A fifth expertise group was charged with evaluating exposure, effects and potential risks of pollinators other than the honey bee (non-Apis bee pollinators). The SETAC Pellston workshop organized around the question of pollinating species exposed to Plant Protection Products is the first experience in the area of sharing science and regulatory expertise at the world scale on a common concern with the aim to further improve the relevance of regulatory recommendations. This presentation will compare and contrast the outcome of the Pellston workshop from the perspective of regulatory agencies in North America and Europe.

EH01A-6

Use of ecosystem services concept to determine need for open soils in urban areas

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The draft Soil Framework Directive contains an obligation to limit the permanent covering of soil with impermeable material as far as possible and, where soil sealing is unavoidable, to mitigate the negative effects of doing so. In order to be prepared for the possible implementation of the Soil Framework Directive, the Dutch Ministry of Environment requested TCB to report on the cases in which sealing needs to be limited and how this can be achieved. As a follow-up the Ministry requested to indicate a minimum percentage of open soil in each type of development area, in order to put a limit to negative effects of sealing on soil functions. This paper addresses the establishment of a minimum percentage of open soils in urban areas. The minimum percentage of open soils in urban areas was estimated by the space needed by soils to provide or support ecosystems services to the inhabitants. The area of open soil necessary for temperature regulation

and contribution to human health and well being is estimated at 5 to 10% of the urban area. Water regulation via soil needs much more space. 'The more, the better' can be concluded for biodiversity in urban areas, provided there is horizontal and vertical diversity in vegetation and good interconnectivity between areas with open soils. Surface water also contributes to certain urban ecosystem services. Based on all findings, it was concluded that 20 to 40% of the urban area should be reserved for open soil and water to provide all necessary ecosystem services. These values should be used at the scale of neighbourhoods. Not only the total area of open soil and water matters, the locations and good connections between these are crucial too.

EH01B-1

Ecosystem services for life cycle assessments

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Integrating ecosystem services considerations into life cycle assessments (LCA) will improve the effectiveness of these assessments by expanding the range of environmental aspects included in assessments and providing more emphasis on the dependence companies have on ecosystem services.
Business and communities both depend and impact ecosystem services, or the benefits derived from ecosystems. For example, forests supply timber and wood, fibre, regulate climate by absorbing carbon dioxide, and yield genetic resources for medicines. Coral reefs attract tourists, serve as nurseries for commercial fish species, and protect properties along coastlines from storm surges. Wetlands absorb waste, help reduce floods, and purify water. However, most life cycle assessments do not consider the full range of ecosystem services related risks and opportunities, possibly leaving firms vulnerable to risks or unaware of opportunities related to their dependence and impact on ecosystem services.
Numerous strategies are emerging to provide life cycle assessment professionals the means to expand LCA efforts. These strategies will be reviewed for the value in different contexts and impediments to advancement will be discussed.

EH01B-2

Indicator based environmental quality and quality of life in urban areas

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AbstractA comprehensive strategy is necessary to protect the ecology and characterise reference conditions in urban heavily modified areas and new towns. The importance of the quality components defined by the "good ecological status", "good chemical status"and "quality of life"will be demonstrated in this study by "on site effects monitoring"tools to promote an environmentally sensitive and sustainable use of the resources in urban areas. The study contributes to the resource management, evaluation of the interaction of ecosystems and urbanisation as well as quantification of risk. The idea behind "Contribution of Sustainability"was to summarize and assess the measures and aims for a sustainable development, while the idea behind "Field of Sustainability"was to assign these aspects to one (or multiple) of the five columns of sustainability (Economy, Ecology, Social, Cultural, Governance).

EH01B-3

Sustainability indicators as decision-support tools for spatial planning

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To ensure sustainability of living, especially in urban areas, spatial planning should be based on reliable evaluation of benefits and cost of each alternative, taking into account also the ecosystem services provided by the natural environment, and their role in support to human living and human societies. It is therefore necessary to include in the evaluation of planning procedure (e.g. within Strategic Environmental Assessment, SEA) some decision support tools that take into account also the private consumption component and its relative effects. The present work is an attempt to perform SEA integrating the more traditional evaluation made through a set of indicators (which results are compared with local limits) with other sustainability assessment methodologies. The idea is to include in the evaluation some issues of global concern, such as resource depletion and climate change and to reinforce the use of the carrying capacity concept within the local planning. The evaluation includes Ecological Footprint (EF) assessment of citizens' consumption and a carbon balance (CB) of the area. The case study presented refers to the implementation of this approach in the Strategic Environmental Assessment of a spatial planning plan of four municipalities in Northern Italy.
The case study presented highlights the importance to support the definition of spatial planning programs with suitable tools, such as sustainability composite indicators, that are able to consider a wider range of aspects, with reference to the carrying capacity concept. The proposed methodology proved to be quite useful, even if there are some limits, such as the fact the EF method doesn't allow for accounting multifunctionality of ecosystems (e.g. carbon storage and wood provision from forests).

EH01B-4

Sustainability assessment of forest biomass supply chain at local scale: carrying capacity of the system for energy valorisation

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Evaluation of the trade-off between the benefits coming from forest resources' use and the conservation of forest ecosystems is needed. Considering the use of biomass for energy purpose, on one hand the use of wood resources should be based on an evaluation of the "carrying capacity"of the forest ecosystem and site-specific characteristics; on the other hand, the role of biomass valorisation has to be assessed considering the socio economic benefit or drawbacks due to the further development of the supply chain.
In the context of a site -specific sustainability assessement of a wood energy supply chain, the research focuses on development of an expeditious methodology to obtain georeferred quantity of biomass at local scale for mountain forest areas, in order to facilitate energy planning that considers the local system carrying capacity and the potential of substitution of fossil fuels.
Methodology developed for the site-specific assessment of the biomass availability, with respect to carrying capacity, consists of quantification and spatial distribution (using Geographic Information System) of forest biomass that considers local features as reported in local territorial plans and it applies Life Cycle Assessment for supporting the overall environmental assessment.
Biomass value calculated has been converted from volume to mass, considering species features and water content. The result is compared with current utilization of wood, and waste products from forestry processing are estimated, in order to quantify the mass available for energy valorisation. Then, the energy potential is estimated, from biomass quantity and from wood features, principally the lower calorific value and water content for each species. Finally, the potential of substitution of fossil fuels is calculated, knowing energy potential from available biomass for energy use.
The methodology is applied to two mountain areas, Comunità Montana Lario Intelvese (CMLI) and Comunità Montana Triangolo Lariano (CMTL), in northen Italy (Como Province).
The proposed methodology evaluates the possibility that forests can provide the supply of raw material for energy production among ecosystem services. In addition, this assessment aims to integrate considerations to protect the other ecosystem services. Moreover, the proposed methodology is useful for a preliminary assessment of the possibility to considering woody biomass in energy planning at local level.

EH01B-5

Is the European honeybee (Apis mellifera mellifera) a good representative for other pollinator species?

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Pollinators are important components of biodiversity and provide a key ecosystem service through pollination. Worldwide pollinator risk assessment is primarily based on European honeybee data. However, the European honeybee is not necessarily the most efficient pollinator for some high value crops (e.g., coffee) and it is not clear if a European honeybee based risk assessment is protective for other relevant pollinators.
In a first attempt to test if *Apis mellifera mellifera* is a good representative for other pollinators a first-tier contact LD_{50} test using the organophosphate dimethoate was performed with several pollinator species originating from The Netherlands, Brazil, and Kenya, respectively. Thus aquired LD_{50} data was used to contruct an Species Sensitivity Distribution curve ranking the different species by their response to direct contact with the toxicant.
Results from identical test set-ups with *Bombus terrestris*, *Apis mellifera mellifera*, *Apis* sp. (africanized), *Apis mellifera scuttelata*, *Scaptotrigona postica*, and *Meliponula ferruginea* show that although the European honeybee s not the most sensitive species tested the difference with the most sensitive species (*Apis mellifera scuttelata*) comprises only a factor 3. This difference does not lead to different outcomes in the risk assessment procedure. Although further testing with solitary bees and compounds with different mode-of-action is needed this first tests seem to indicate that for at least organophosphates the use of European honeybee data is protective for other pollinators.

EH01B-6

Sediment Ecological Risk Assessment (SEcoRA)

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In spite of countless papers invoking the concept, it can be argued that the term sustainable sediment management has little value, as sediments are not managed for their own sake, nor, in many cases, are they managed in a truly sustainable manner. Managing sustainability in ecosystems requires the understanding and management of all aspects, both natural and anthropogenic, of the ecological and biophysical environment in a manner that sustains ecosystem services. Sediments, or the soil/sediment continuum are essential, but just part of that system and their management does not stand alone. At best, we can provide frameworks for understanding and managing the role of sediments in sustaining ecosystem services. This paper will describe an effort to provide a language and conceptual framework in support of that goal. For the most part, soil and sediment themselves do not provide ecosystem services. Rather, soil and sediment status provides a range of functions essential to the viability and sustainability of a variety of ecosystem services. Sediment (or soil) status, defined here as a combination of the attributes quality, quantity, transport and location, is controlled by landscape and watershed biophysical conditions. These attributes are affected by natural and intrinsic conditions and by anthropogenic management of the landscape to optimize preferred ecosystem services. A range of biotic and abiotic endpoints have sediment status requirements; the extent to which sediment status meets those needs affects the role of sediment (positive or negative) in terms of that endpoint, and in terms of how sediment effects ecosystem services provided by or represented by that endpoint. ERA concepts have been adapted to identify the pathways of impact by which sediments link the ultization of ecosystem services on land affects downstream aquatic ecosystem services; these approaches will be descibed. The development of sediment ecological risk assessment (SEcoRA) approaches will allow for a better understanding of the interacting positive and negative roles of sediment in the maintenance of ecosystems and the socioeconomic functioning of rivers, considering various dynamic aspects of the interactions between sediment status and various endpoints in a spatially explicit manner.

EH02 - Monitoring and modeling stressed ecosystems to support ecosystem-based management

EH02-1

Modelling the impact of endocrine disruptions on aquatic ecosystems: an experimental lake study

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Despite growing concern towards endocrine disrupting chemicals (EDCs), impact on wild populations and their consequences on ecosystem services remain unknown. Single-species tests are prefered over experiments in enclosed ecosystems for the experimental ease of work and the higher reproducibility. Ecosystem modelling is not used as a general tool because of the need for extensive calibration for a specific ecosystem. However, the goal of environmental risk assessment and ecosystem-based management is to maintain ecosystem functions and services. Thus, ecosystem modelling is the logical next step in predicting impact of chemicals on aquatic and terrestrial ecosystems. This study aims to develop a simplified ecosystem model that can be used as a tool to predict EDC impacts on aquatic ecosystems. The model development is more focused on predicting ecological effects of EDCs rather than their impact on population dynamics. An object oriented framework for ecosystem modelling was developed in the software package WEST with

equations based on the AQUATOX model (USEPA, 2002). Reproductive endpoints had to be added in the model to incorporate the endocrine disruptions that are commonly measured. The model consists of (i) a food web model (ii) toxic effect sub-models and (iii) a model for nutrient and detritus cycling. Field data are used to help in developping and validating the model. A multi-year whole-ecosystem study is performed at an experimental lake with exposure of well-defined fish and lower-trophic-level populations to environmentally-relevant concentrations of the synthetic hormone 17α-ethinylestradiol (EE2). The experimental lake is located in an undisturbed watershed and contains naturally reproducing populations of fish, benthic invertebrates, zooplankton and algae. The study started in 1999 with baseline data collected until 2000 on aquatic populations in the experimental lake and references lakes. Between 2001 and 2003, EE2 was added continously in the experimental lake. Since 2004, EE2 addition was stopped to measure ecosystem stability and recovery after stressor removal. In contrast with the reference lake, the Fathead minnow population in the experimental lake collapsed after the second year of EE2 addition. This reproductive failure was maintained after the EE2 addition was stopped, although few small individuals indicate some reproduction was occuring.

EH02-2

Response of red fox populations to rodent field controls with bromadiolone: a 6 year study on regional scale

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Anticoagulants rodenticides are mostly used to control rodent populations and may cause severe secondary poisoning of rodent predators. In 90's, hundreds of predators (mainly common buzzard, red fox, and red kite) died after large-scale treatments with bromadiolone bait (60,000 ha) in the Doubs department (France). This poisoning led to changes in the legislation and treatment practices.The present study aims at checking whether Arvicola terrestris controls with bromadiolone have a long term depleting effect on fox populations and whether the evolution of treatment practices decrease undesirable side effects on fox populations.

Treatment intensity was quantified as the quantity of baits used each year on each commune. Red fox populations were monitored by night spotlight counts performed along 472 transects of 1-2 km. Fox counts were carried out in spring and small mammal controls in autumn. Therefore, fox densities obtained per commune for a year n (2004 to 2009) were related to treatments the year n-1 (2003 to 2008). Treatments of the year n-2 were used to investigate possible delays. For each transect a Kilometric Abundance Index (KAI) was calculated and values were interpolated on the centroid of each commune by ordinary kriging.

The model with the lowest AICc selected to explain kriged fox densities a year n includes bromadiolone treatments of the years n-1 and n-2. Intensive treatments led to KAI decrease. Impact was important in 2004, fox counts being lower in the areas where treatments were carried out in 2003. Visual examinations of maps show that KAI generally stayed extremely low in large areas until 2005 and partially recovered the following years. The same areas were treated again from 2006 to 2008 during the next vole outbreak and bait quantities per hectare were decreased by 2 at the minimum. Those treatments were not followed by a decrease of fox KAI.

The present work is the first one to address the issue of the impact of bromadiolone treatments on natural foxes populations on a very large scale (about 5,000 km2) and permitted to evaluate the resilience of populations to treatment. This approach shows an additionnal example of how monitoring wildlife population on the long term using index method may provide valuable informations about adverse effects of pesticide treatment. It also shows that treatments carried out at low density of vole population are likely to have a lesser impact on fox populations.

EH02-3

PCB-induced changes of a benthic community and expected ecosystem recovery following in-situ remediation

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The benthic community was analyzed to evaluate pollution-induced changes for the polychlorinated biphenyl (PCB)-contaminated site at Hunters Point (HP, California) and 30 reference sites in the San Francisco Bay. An analysis based on functional traits of feeding, reproduction, and position in the sediment shows that HP is deprived of deposit feeders, subsurface carnivores, egg laying species, and species with no/weak protective barrier. Sediment chemistry analysis shows that PCBs are the major risk drivers at HP (1570 ppb) and that the reference sites contain much lower levels of PCB contamination (9 ppb). Different feeding traits present a direct pathway of exposure, which can be mechanistically linked to PCB bioaccumulation by biodynamic modeling. The deposit feeder Neanthes arenaceodentata accumulates about 20-times more PCBs in its lipids than the facultative deposit feeder Macoma balthica and up to 180-times more than the filter feeder Mytilus edulis accumulates though the aqueous phase. In situ sorbent amendment can reduce the PCB bioavailability from sediment but long term monitoring data of full-scale field amendments are not existing to date. To overcome the lack of data, the modeling framework was used to evaluate the remedial success regarding ecosystem recovery. The comparison of different exposure scenarios suggests that PCB tissue concentrations at HP are two orders of magnitude higher than at the reference sites. The model further predicts that a full-scale sediment amendment with activated carbon can reduce PCB bioaccumulation at HP by up to 85 to 90% under favorable field and treatment conditions. The modeling demonstrates that such remedial success corresponds to exposure conditions suggested by the sediment quality guidelines and the cleanup goal for HP but concentrations remain slightly higher than at the reference sites. The present study demonstrates how the remedial success of an untested sorbent amendment, which lowers the PCB availability, can be compared to reference conditions and traditional cleanup goals, which are commonly based on total sediment concentrations.

EH02-4

Recovery potential of diatomic biofilms after industrial contamination (Cd, Zn) : field and experimental studies

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This study was conducted in a context of metal impacted hydrosystem remediation (Cadmium and Zinc) on periphytic diatoms biofilms, dominant primary producers in freshwaters. It aims to combine in situ and laboratory studies to assess first biological modifications on biofilms in decontamination conditions in order to understand the remediation procedure effects.

The colonization of biofilms was conducted during 24 days on a site undergoing industrial discharges and on a metal-free site considered as control. After 24 days, a first batch of contaminated biofilms was translocated to the metal-free site to undergo natural decontamination, and a second was brought to laboratory into artificial streams containing metal-free medium, to carry out decontamination experiments under controlled conditions. Biofilms from the metal-free site were also brought in the laboratory as control biofilms.

Different tests were then performed: analyses of metal bioaccumulation, taxonomic investigations, biovolumes and teratological forms measurements, diatom densities numerations, completed by physicochemical measurements in water.

Contaminated biofilms translocated to the metal-free site showed a very fast metallic decontamination potential and after only 24 days most of species were similar to those found into control biofilms : only a few species, like Eolimna minima, considered as metal-resistant, were still found into biofilms, but in very low densities.

Otherwise, metal concentrations into biofilms under laboratory decontamination showed a sharp decrease but both Cd and Zn stay significatively higher than concentrations analyzed into control biofilms after 56 days of decontamination. Moreover, taxonomic inventories do not highlight a complete recovery of diatom communities : metal-resistant species persist with high abundances after 56 days of decontamination.

By comparing results from field and laboratory experiments, it appears that decontamination under natural conditions was faster. This assemblage recovery suggests the involvement of species immigration and emigration from the natural environment which prevail over multiplication rates of pre-established species.

Complementary experiments would be necessary to assess the real importance of diatom import or export into biofilm and their effects on the evolution and then recovery potential of diatom community structures.

EH02-5

Establishing environmental risk based management for industrial operations in (sub-) Arctic marine areas. Linking early warning signs to whole organism effects from individual to population levels.

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Population and community responses to multiple stressors can be assessed by combining fate and effect modelling with information from whole organisms and individual internal processes followed by further assessment by population dynamic modelling on the population and community levels. This presentation gives an example of a foundation for such an approach based on a combination of experimental studies, monitoring and model tools.

Two subsequent projects (BioSea I&II; 2001-10) were focused on establishment of environmental monitoring-, risk-, and model tools for oil based discharges to the marine environment to aid environmental management. Crude oil consists of a large number of chemical constituents representing a combination of different stressors.

An objective of the BioSea II project (2007-10) was to adopt the tools to (sub-) Arctic areas. This was fulfilled through a series of experiments with ecologically relevant (sub-) Arctic fish and invertebrate species (Atlantic cod, halibut/long rough dab, Icelandic scallop and northern shrimp) at different development stages (eggs, larvae, juvenile and adults).

The following achievements were made:

_ Critical harmful effect concentrations of dispersed oil were determined based on organism survival and growth (fitness) of vulnerable early life stages.

_ Two suites of biomarkers for fish and invertebrates were evaluated for detection of responses to oil based exposures. These have been further developed into operative monitoring tools by establishing field background and threshold levels.

_ Field background levels of biomarkers were determined in species inhabiting (sub-) Arctic areas.

_ Biomarker threshold values were determined for juvenile and adults at concentrations where detrimental effects were observed in fitness of individual organisms at early, vulnerable life stages.

_ A model approach was developed to assess adverse effects on population level for one of the test species (northern shrimp).

_ Reactions of the studied species to different test conditions were studied by multivariate analyses to assess similarities/dissimilarities.

_ The use of biomarkers to monitor hydrocarbon discharges from oil and gas activities as 'Environmental Indicators' within an ecosystem based environmental management approach for the Barents Sea region was considered.

EH02-6

Organic pollutants, bacteria, primary producers and global change. Are we tracking the problem adequately?

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The total number of synthetic organic chemicals introduced to the environment by Humans has never been quantified, but it is not lower than thousands. A fraction of these chemicals have toxic effects to coastal organisms and presumably affect ecosystems structure and function. During the last decades, some of the processes affecting the transport, degradation and fate of a limited number of chemicals have been studied, and the rising concern for environmental risk of organic chemical has lead to the regulation of a few of them by national and international organisms. However, the environmental inventory of organic pollutants is far from being quantified, and current methodologies used in most toxicological tests only allow to determine effects of individual chemicals to organisms. There are major limitations on appropriate methodologies to assess the effects of organic pollutants at population and ecosystem levels and the effects induced by complex mixtures of organic pollutants present in natural environments. The modification of the composition of the biosphere by a myriad of organic pollutants at ultra-trace levels is not yet regarded as another vector of environmental change which is irreversible due to the persistent

character of many of these chemicals and due to its global coverage. Here, we claim that the modification of the atmosphere, water, sediments and biota composition is a factor to be taken into account in ecosystems, and that its pressure on the environment has been exponentially increasing during the last 6 decades of the anthropocene.
In addition, we will point out to the interactions between ecosystem functioning, trophic status, fate and transport of pollutants and carbon cycle during the anthropocene

EP01 - Alternative flame retardants: Environmental exposure, fate and trends

EP01-1
Determination of organophosphorous flame retardants (OPFRs) in various matrices - including first result of OPFRs in the food chain of the Western Scheldt
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Halogenated flame retardants like polybrominated diphenyl ethers (PBDEs) are used in high volumes in different kind of products to enhance fire safety. Due their persistency, bioaccumulative properties and toxicity the US have started to phase-out the production of the Penta- and Octa-BDE commercial mixtures voluntary in 2004. In 2009 also the production and application of Deca-BDE commercial product has been limited. The phase-out of both products has led to an increase in the use of alternative flame retardants. Organophosphorous flame retardants (OPFRs) is one of the flame retardant groups that show an increasing production over recent years. OPFRs are also used as plasticizers and anti-foaming agents in many products such as furniture, textiles, cables, building materials, insulation materials, paints, floor polishes, hydraulic fluids and electronic appliances. In most applications OPFRs are used as additive chemical and thus not covalently bound to the polymeric materials. The objectives of the present study were to develop an analytical method to analyze OPFRs in various matrices, and to study the OPFRs in a pelagic and benthic food web in the Western Scheldt, The Netherlands. An analytical method was developed for the analysis of OPFRs in sediment and biota using LC-MS/MS. Samples of a pelagic and benthic food web from the Western Scheldt were analysed for OPFRs. The first results show that OPFRs were found in Western Scheldt suspended particulate matter (SPM) and biota. In SPM TPP, TCP and EHDP were detected, and TPP and EHDP were only detected in the benthic organism's cockle and sole. Low concentrations of TCP were also found in sole. However, higher in the pelagic food web, in herring and common tern egg concentrations of these OPFRS were below the LOD. The OPFR concentrations in sole are in the same range as BDE47, BDE99, BDE209 and α-HBCD. In conclusion, the first results show that TPP, EHDP and TCP may accumulate in the benthic food web but not in the pelagic food web.

EP01-2
Fate of organophosphorus flame retardants - Determination of their metabolites in human urine
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Trialkylphosphates (TAP) are a group of flame retardants that is used increasingly, also as substitute for brominated flame retardants. Besides that TAP are used as pesticides, plasticizers and as additive in many products. Owing to their widespread use TAP are also widely distributed in the environment [1]. Due to the presence in consumer products humans are exposed to TAP, and TAP have been found in biomonitoring studies, in human breast milk as well as in urine.
Depending on their alcohol moiety TAP are more or less stable and hydrolysis may occur. These hydrolysis products, dialkyl phosphates (DAP) and monoalkyl phosphates (MAP), are far less toroughly investigated [2]. We have recently developed a method for the detection 14 metabolites of TAP (DAP and MAP) in human urine. This method is based on liquid-chromatography-mass spectrometry (LC-MS/MS) using ion-pair chromatography [2]. Several analytes first had to be synthesized to allow their quantitation. The method was exemplarily applied to 19 samples of different individuals [3]
In this study five MAP and two DAP were detected for the first time in human urine. Monobutyl, diethyl, diphenyl and diethylhexyl phosphate were determined with median concentrations in the µg/L-range. These first results generated with the new LC-MS/MS method suggest that the body burden for organophosphorus compounds may be much higher than visible from the sole analysis of the TAP. The inclusion of TAP metabolites in future biomonitoring studies should provide a more comprehensive picture of the exposure of humans to organophosphorus compounds.
It needs to be clarified whether the DAP and MAP determined in human urine had been formed from TAP in the human body after uptake or were transformed before uptake and incorporated as DAP and MAP, already. For that purpose also the environmental fate of TAP needs further investigation. This will also help to assess which route of exposure is most relevant for humans.
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[2] Quintana J.B., Rodil R. and Reemtsma T. (2006) Anal. Chem. 78, 1644-1650.
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EP01-3
Bioaccumulation of selected halogenated organic flame retardants in remote lakes and in the Great Lakes
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In this study the bioaccumulation and concentrations in surface waters of a wide range of non-legacy halogenated organic compounds (HOCs) including replacement halogenated and non-halogenated organophosphate flame retardants (OPFRs) was determined in remote lakes within the Canadian shield and in the open Great Lakes. Large volume samples of surface waters (100 L) were collected from two remote lakes Lake Opeongo (Algonquin Provincial Park, ON) and Siskiwit Lake (Isle Royale National Park, MI) as well as from surface waters of the lower Great Lakes over the period 2005-2010. Zooplankton (>100 um), mysids, forage fish and lake trout were obtained from Lakes Erie, Ontario and Opeongo while only lake trout were available from Siskiwit Lake. Extracts were screened for 27 individual BDEs (Br3-Br10) and 20 Br3-Br6 compounds/ PBDE replacements and other HOCs using GC-electron capture negative ion mass spectrometry (GC-EC NIMS) with a HP-5MS and RTX-1614 capillary columns. OPRFs were analysed by GC-EIMS using an HP-5MS column. Br3-Br6 compounds and Dechlorane Plus (DP) were non-detect (<0.1 pg/L) in Siskiwit and near detection limits in Opeongo lake waters. Pentabromo-ethylbenzene (PBEB), 1,3,5-tribromo-2-methoxy-4-methylbenzene (Br3MeBz), allyl 2,4,6-tribromophenyl ether (ATE) and dibromopropyl 2,4,6-tribromophenyl ether (DPTE) were the most prominent non-PBDE HOCs in Opeongo lake waters; present at sub-pg/L concentrations. A larger suite of HOCs were detectable in Lake Ontario waters including DP, Br3MeBz, PBEB, DPTE, 12345-pentabromobenzene (Br5Bz), penta- and hexabromotoluene, and 2-ethyl-1-hexyl 2,3,4,5-tetrabromobenzoate (EHTeBB) although concentrations were near or at MDLs. The tris-chloroalkyl phosphates (TCEP, TCPP, TDCP) were present at 5-50 ng/L in central Lake Ontario waters. A wide range of Br3-Br6 compounds were detected in zooplankton from Lake Ontario including Br3MeBz, 1,3,5-tribromobenzene (TBB), tetrabromoxylene (TBX), bis(tribromophenoxy)ethane (BTBPE), PBEB, and BPTE. Lake Opeongo zooplankton had a more limited suite, with Br3MeBz, ATE and Br5Bz present at low pg/g (wet wt) concentrations. BDE47, BDE153 and Br3MeBz had the highest trophic magnification factors based on the slope of the log (lipid wt) concentrations versus trophic level. TBX, 1,3,5-TBB and BTBPE showed trophic dilution while other compounds e.g. BDE202 (and other hepta- and octaBDEs) were detectable in most samples but showed limited increases with trophic level.

EP01-4
Brominated flame retardants and Dechlorane Plus in air and seawater from the German Bight, North Sea
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Brominated flame retardants (BFRs) have been used for more than 50 years to reduce the flammability of various industrial and commercial products. Polybrominated diphenyl ethers (PBDEs), which are currently the most well studied BFRs, are known to be toxic, bioaccumulative, persistent, are ubiquitous in the environment and undergo atmospheric transport over long-ranges. As a result of the banishment of the industrial Penta-, Octa-, and DecaBDE mixtures, the industry shifts towards alternative, unregulated brominated (BFRs) and non-brominated flame retardants. Even though some alternative BFRs such as 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and, only recently, the chlorinated flame retardant Dechlorane Plus (DP) have been detected in the environment, their environmental fate and transport behavior, especially in the marine environment, is not well understood. Therefore, we investigated the occurrence and air-sea exchange of PBDEs, non-PBDE BFRs and DP in the German North Sea. Paired air and water samples were taken aboard the German research vessel Heincke during three cruises in 2010. The samples were Soxhlet extracted and analyzed by a GC-ECNI-MS system.
PBDEs were detected in concentrations from 1 to 15 pg m^{-3} and from <1 to 10 pg L^{-1} in air and seawater, respectively, while the congener profile was dominated by BDE-47, -99, -100 and BDE-209. Among the non-PBDE BFRs, 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and hexabromobenzene (HBB) were detected in concentrations similar to the PentaBDE congeners and DP showed similar concentrations and spatial trends as BDE-209. This suggests that DP is used as replacement for BDE-209. The highest atmospheric concentrations resulted from land air masses passing Germany and the Netherlands while lower concentrations were observed for oceanic and Scandinavian air masses. The air-seawater exchange was dominated by deposition into the North Sea showing that atmospheric transport is an important source of flame retardants in the North Sea besides dry and wet deposition and riverine discharges from highly industrialized rivers such as the Rivers Elbe and Scheldt.

EP01-5
Emerging flame retardants in the atmosphere of the Great Lakes
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Flame retardants are a large group of chemicals, of which polybrominated diphenyl ethers (PBDEs) represent the vast majority. Flame retardants are added to numerous products to prevent fire, and their usage has increased rapidly since about 1980s, probably as a result of stringent fire safety regulations. In 2004, two commercial PBDEs mixtures were voluntarily retired from the United States market (Penta-BDE and Octa-BDE); the other widely used commercial mixture, Deca-BDE, is still largely unregulated in the United States and Canada, although it was recently banned in the European Union. As a result of their heavy usage, PBDEs are ubiquitous in the environment, having been detected in air, sediments, biota, and people. In addition, the market has been continuously replacing banned or retired products with unregulated compounds. Air samples (vapor and particles) were collected within the Integrated Atmospheric Deposition Network (IADN) at five sites around the Great Lakes. In this study, we focused on "emerging"brominated flame retardants including i.e. α-, β-, γ-, and δ-tetrabromo-ethylcyclohexane (α-, β-, γ-, and δ-TBECH), allyl-2,4,6-tribromophenyl ether (ATE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), 2-bromoallyl-2,4,6-tribromophenyl ether (BATE), octabromotrimethylphenylindane (OBIND), hexachlorocyclopentenyldibromocyclooctane (HCDBCO), bis(2-ethyl-1-hexyl) tetrabromophthalate (TBPH), and 2-ethyl-1-hexyl-2,3,4,5-tetrabromobenzoate (TBB). With the exception of OBIND, all of the compounds listed above were detected in air samples, although for some of them, the frequency of detection was low. Interestingly, TBPH and TBB have not been found in biota, indicating that they may be readily metabolized, but their presence in air suggests that they are subject to long range transport. These two "emerging"flame retardants were found most frequently and at highest levels at the urban sites (Chicago and Cleveland), with some detection at a rural site (Sturgeon Point). Their concentrations were generally higher in the particulate phase as compared to the vapor phase. The levels of TBPH and TBB in the filters were on the order of 5 pg/m^3, which is comparable to those of BDE-47. The majority of the other compounds were found in the vapor phase and at all the sites, from rural to urban with levels around 1 pg/m^3, which are similar to the levels of BDE-47 at the rural sites.

EP01-6
Flame retardants hexabromobenzene, pentabromotoluene and pentabromoethylbenzene: their presence and Norway and environmental partitioning properties
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The brominated flame retardants (BFRs) pentabromotoluene (PBT), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB) have been in use for several decades. HBB is used for fireproofing plastics, textiles and wood, and can be released from BFR polymers. PBT and PBEB are found in unsaturated polyesters and are used for textiles and adhesives. These BFRs have physical chemical properties similar to other persistent organic pollutants (POPs), and have been identified as potential arctic contaminants in screening studies. Studies on these BFRs can be found from the 1980s, especially in Japan regarding HBB. However, not much research was done during the 1990s and only recently are these three compounds being reported again in environmental samples. The reason for this disappearance and reappearance in the environmental

literature is unclear, and could reflect changing production levels or simply changing concern for these compounds. To investigate the presence of HBB, PBT and PBEB, we conducted a screening study of these samples in multi-media environmental samples (diverse biota, air particles, waste water treatment plant samples, etc.) in three areas of Norway that are suspected source zones for BFRs: Drammen, Lillehammer and Tromsø. Additionally, we used various approaches to estimate their partitioning properties and environmental behavior.

EP02 - Antimicrobial resistance in the environment

EP02-1

Exploring the metagenomes of environmental antibiotic hotspots to identify resistance factors that we may face in the clinic tomorrow

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There is accumulating evidence that extensive resistance is promoted within environments contaminated with high levels of antibiotics. Such "hotspots"are unique in the sense that they contain diverse collections of bacteria living under unprecedented long-term antibiotic selection pressures. Thus, there is a possibility that these environments harbour yet undescribed resistance genes, resistance plasmids and chromosomal mutations that we may face in pathogens in the clinic tomorrow. Here, three parallel approaches were taken to identify novel potential resistance factors in DNA isolated from Indian river sediments contaminated with antibiotics from drug manufacturing. First, massively parallel metagenomic sequencing revealed 37 known resistance genes in the sediment, including high levels of several mobile QNR genes that are known to cause moderate resistance to quinolones. A novel resistance plasmid harboring qnrD was assembled from the sequence data and confirmed by PCR. A repeat amino-acid motif, conserved among known QNR proteins, was generated and used to identify other potential QNR genes in the metagenomes. The functionality of these tentative quinolone resistance genes remains to be tested. Second, selected regions of the genes encoding type II topoisomerases, the specific targets for quinolones, were amplified by PCR and sequenced by 454 technology to explore the presence of novel, potential resistance-carrying mutations. As a third approach, river sediment DNA was fragmented, inserted in an expression plasmid and transformed into E. coli to construct metagenomic libraries. As a positive control, we screened the libraries for acquired resistance to sulfamethoxazole, as sul2 was the most common resistance gene found by the metagenomic sequencing. Several thousand colonies of E. coli transformants were able to grow on sulfamethoxazole plates, whereas the control host strain, transfected with a plasmid without insert, was sensitive. Similar results were obtained when screening for acquired fluroquinolone resistance. These result shows that such an assay, indeed, can be used to identify resistance-conferring DNA sequences. We are currently screening the libraries for inserts providing resistance to a variety of antibiotics. Growing clones are isolated and selected inserts will be amplified by PCR and sequenced.

EP02-2

Sources, pathways and mitigation of antibiotic resistance genes at the watershed scale

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This presentation will provide a framework for understanding the behavior of anthropogenic sources of antibiotic resistance in the environment as well as insight into potential strategies for reducing the risk associated with antibiotic resistance gene (ARG) "pollution". The first objective was to identify key sources and processes contributing to elevated ARGs in river environment, which was accomplished through the development and application of a novel ARG molecular signatures approach. The molecular signatures approach was demonstrated on the Cache La Poudre River, indicating that this particular river system was primarily influenced by wastewater treatment plant (WWTP) sources of ARGs. This suggests that appropriate treatment of WWTP waste streams may help reduce levels of ARGs in the aquatic receiving environment. Thus, the second objective was to explore WWTP sludge digestion and UV disinfection as potential mitigation options to reduce the spread of ARGs. Thermal hydrolysis of sludge followed by sequential anaerobic and aerobic digestion steps yielded varied responses for tetracycline and sulfonamide ARGs. It was noted that while thermal hydrolysis resulted in physical destruction of ARGs, the concentrations increased by up to an order of magnitude in the anaerobic digestion step. The subsequent aerobic digestion step resulted in further increase of sulfonamide ARGs, by almost two orders of magnitude in the case of sulI. However, tetracycline ARGs, tetO and tetW, decreased in response to aerobic digestion. In the disinfection studies, a typical inactivation curve was obtained for methicillin resistant Staphylococcus aureus in response to UV exposure, but corresponding mecA ARGs remained largely intact. The results indicate that while WWTPs are a promising node for controlling the spread of anthropogenic sources of antibiotic resistance, ARG attenuation at the WWTP is not likely to be achieved via standard practices and may require more aggressive treatments.

EP02-3

Antibiotic resistance downstream of abandoned mine sites: where organisms and genes tell different stories

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Rapid and increasing development of antibiotic resistant bacteria (AR) is eroding the huge benefits of antibiotics in the treatment of disease. Although this problem partially results from inappropriate antibiotic use in medicine and agriculture, there is growing evidence that untreated wastes that contain organic and heavy metal pollutants also play a role in environmental AR development. One pollution setting where elevated AR might be apparent due to pollution is downstream of historic abandoned zinc (Zn) and lead (Pb) mines. This study compared AR using both culturing and genetic methods to quantify AR at various locations in the River Tyne watershed in Northern England, which is an ideal for assessing the effects of metal pollution on AR because one arm of the river was heavily impacted by mining, whereas the other arm is relatively pristine. Sediment Zn levels in the South Tyne ranged from ~ 4000 to 12500 mg-Zn/Kg sediment, whereas levels in the North Tyne were < 300 mg-Zn/Kg sediment. Further, culturing methods found ~ 70% of the isolated colonies were ampicillin resistant in the South Tyne and only ~ 1.0% were resistant in North Tyne. However, despite this broad difference in resistant cultured species, antibiotic gene data suggested no significant relative difference in resistance gene levels. These data show that studies that solely rely on resistance genes for detecting apparent resistance can provide misleading results, and we suggest that all future studies on environmental antibiotic resistance use culturing methods to detect resistant strains not covered by known resistance genetic markers.

EP02-4

Effects of veterinary medicines introduced via manure into soil on transferable antibiotic resistance in soil bacterial communities

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Due to the use of veterinary antibiotics in animal husbandry, considerable amounts of antibiotics are introduced into soil with manure. Antibiotics entering the environments are assumed to affect abundance of antibiotic resistance genes and their transferability. The approach of capturing transferable plasmids (conjugative or mobilizable) into a selectable recipient strain was used to study transferable antibiotic resistances in manure and manure treated soils. This cultivation-independent method allows to gain insights into plasmid diversity that might play an important role in the dissemination of antibiotic resistance genes in soils. In this study transferable plasmids conferring sulfadiazine (SDZ) resistance were directly captured in *E. coli* from manures and manure treated soils. Transfer frequencies of capturing SDZ resistance plasmids into *E. coli* were determined in soil microcosm, mesocosm and field experiments with soil treated with manured containing SDZ or not. Higher transfer frequencies were observed for soils treated with SDZ containing manure. A total of 204 captured plasmids were analysed. The majority of plasmids captured belonged to the recently discovered group of LowG+C plasmids and 15% to the IncP-1e subgroup. Restriction profiles and the plasmid encoded resistances showed that a high diversity of antibiotic resistance plasmids was captured from soil and rhizosphere samples. Although potential transconjugants were only selected based on the captured SDZ resistance, the plasmids obtained typically conferred multiple antibiotic resistances. Sequencing of these plasmids allowed further insights into the type of antibiotic resistance genes and mobile genetic elements carried on similar plasmid backbones.
Our study showed that spreading manure on agricultural soils promotes spreading of transferable antibiotic resistances and residual veterinary medicines in agricultural soils. Our data indicate that LowG+C and IncP1-_e plasmids play an important role in the dissemination of multiple antibiotic resistance in agroecosystems.
This study was funded by the Deutsche Forschungsgemeinschaft, research group FOR566 "Veterinary Medicines in Soils: Fate and Effects".

EP02-5

Does fertilization with sewage sludge promote antibiotic resistance in bacteria isolated from food crops?

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The recycling of municipal sewage sludge (biosolids) onto agricultural land as a valued source of crop nutrients is a common farming practice. Sewage sludge can contain significant concentrations of several antibiotics and broad spectrum antimicrobial agents, as well as an abundance of bacteria resistant to a variety of antibiotics of human clinical significance. In the present study a field experiment evaluated the impact of sewage sludge use according to normal farming practice on the abundance and the characteristics of viable antibiotic-resistant bacteria detected on tomatoes, potatoes, carrots and sweet corn (maize) at harvest. Municipal sewage sludge was applied at a commercial rate in May 2009 to a 72 m2 plot, with an untreated adjacent plot as a control, both of which were then cropped to barley (Hordeum vulgare). The following spring, in May 2010, both the treated and the control plots were planted with the four vegetable crops in a randomized block design. In late August 2010 the crops were harvested, bacteria washed off the surface of the vegetables, and the abundance of bacteria resistant to each of 27 antibiotics established by viable count on IsoSensitest agar medium containing each antibiotic at the clinical breakpoint concentration. There was no significant effect of sewage sludge utilization on the abundance of bacteria resistant to any antibiotic. Augmentin-, cefoxatime-, and cefoxitin-resistant bacteria were isolated and purified from carrots, tomatoes and sweet corn, foods that can be eaten raw. These were screened for resistance to the full panel of antibiotics to establish patterns of multiple antibiotic resistance (MAR). Preliminary analysis of the results suggests that the abundance of bacteria resistant to multiple (eg. >10) antibiotics is higher on the vegetables that were grown in soil fertilized with sewage sludge than in control soil. Overall, If these results are confirmed they would indicate that further investigation of a relationship between sewage sludge use in agriculture and the abundance of MAR bacteria in human food crops is warranted.

EP02-6

Assessing the environmental hazard of antibiotic resistance. Considerations from a regulatory view.

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The development of antibiotic resistances in microorganisms has been identified as a major global public health problem (WHO 2001 [1], UNEP 2005 [2]). The importance of the topic has been addressed in the current guidance for the authorization of veterinary (CVMP/VICH/644/2001 [3]) and human pharmaceuticals (CPMP/EWP/520/96) [4]. In contrast, the potential adverse impact of the development and spreading of antibiotic resistances on the environment is not reflected in the current guidance for the environmental risk assessment for human as well as veterinary pharmaceuticals. Consequently, the aim of this study was to screen and evaluate available literature of antibiotic resistances in the environment in order to give conclusions how antibiotic resistances could be included in the current guidance for the environmental risk assessment of pharmaceuticals. As a first step, the recent scientific literature on the development and/ or spread of antibiotic resistance was screened for the occurrence of adverse effects of antibiotic resistance in the environment. The aim was to give a comprehensive overview of the full range of observable effects and to identify the key parameters for the selection and dissemination of antibiotic resistance. For each individual antibiotic or class of antibiotic, respectively, the type of resistance developed and the potential for horizontal transfer of the resistance genes was recorded. In a next step, a comparison and evaluation of methods for the determination of threshold concentrations of antibiotics at which resistance is likely to be induced was conducted using available literature studies. Finally, derived thresholds from the literature were linked to consumption data for Germany to assess the probability that these concentrations might be reached in environmental compartments. The results show, that there are indications for adverse effects of the spread of antibiotic resistance in the environment. The literature review supports the requirement of including antibiotic resistance in the environmental risk assessment of pharmaceuticals. The determination of a Minimum Effect Concentration (MEC) for antibiotics can hereby be regarded as

an appropriate method in order to set a threshold concentration above which the development of resistance is likely to occur in the environment and further steps should be taken.

EP04 - Emission of chemicals from consumer goods - from emissions to effects

EP04-1

Conceptualizing emissions of chemicals from products - foundations of a modelling approach

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The research reported here is approaching the task of conceptually linking chemicals in products to their occurrence in environmental samples building on earlier work applying the concepts of substance and material flow analysis and bridging that modelling with chemical diffusive mass-transfer models. The bridging of these concepts is possible using the "combined nomenclature"(CN) and concepts such as total stock, total product area and mean composition of materials in the surface of products.

The process of conceptual modelling used builds on principles used in design of information systems in multidomain user settings. A conceptual model of the information i.e. the nomenclatures, terminologies and categorisations of concepts and terms, has been identified through workshops with researchers, a references group and a literature study.

The aim of the model is to cover the processes that lead to emissions from environmental relevant processes of products along the entire "life-cycle". The core concepts of the technical system are "product"and "use". The "product"concept hence includes any physical product not limited to a specific category. Each product consists of one or several components, which in turn are made of one or several materials, which consists of one or several chemicals. Different types of properties and descriptions are related to each of the levels of physical representation from product category (CN-categories) to chemical level.

The "use"concept is describing an episode during the "life-time"of a given product. The amount of chemical substances emitted from a product depends on the type of use, the length of time it is used in this particular way and the ambient conditions in the direct vicinity where use takes place. The use concept is thus very wide and encompasses the different stages of a product life-cycle which require a thorough definition of "use types"and ambient conditions.

A further specification of concepts has been done, covering around 20 concepts and their further relations and connections to data and data sources useful for calculations will be presented.

EP04-2

Human exposure to PBDEs in Europe and North America

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Polybrominated diphenylethers (PBDEs) are flame retardants used to reduce the ignition and spread of fire in a broad range of products such as computer screens, mattresses, or upholstery in transport vehicles. Lower-brominated PBDEs (penta and octa mixtures) have been banned in Europe and voluntarily phased out in North America. Deca-BDE is still in use in most states of the US, but will be phased out by the end of 2013. In Europe deca-BDE is no longer used in electronics and electrical applications.

The aim of this study is to show with a consistent methodology whether the different legal settings, the different use patterns of PBDEs, as well as the differences in behaviour (e.g. eating habits) lead to different dose levels of PBDEs taken in/up by humans. The exposure was modelled probabilistically for five regions (North America, UK, Northern, Central and Southern Europe), seven consumer age groups, eight most important PBDE congeners and eight pathways (e.g. intake via food, dust, soil, and organic films).

The following results were derived: (1) Americans experience higher doses of PBDEs than Europeans. (2) Consumption of food and inadvertent ingestion of dust as well as dermal contact to dust contribute most to the exposure to PBDEs. In most cases food represents the dominant pathway for median dose estimates, whereas in the higher dose percentiles the contribution of dust becomes more important. (3) Infants experience the highest doses, followed by toddlers and children; and then teenagers and adults with about 5 times the dose of adults. This hockey-stick like dose pattern is also visible in biomonitoring data, confirming our model results.

Most likely, the reason for Americans to experience higher doses of PBDEs is that more consumer products are treated with flame retardants in North America compared to Europe. Oral uptake of food and dust and dermal uptake of dust are the most important pathways due to the persistent and bioaccumulative nature of PBDEs and their application in products that are used mainly indoors. Younger consumers take up higher doses mainly due to their higher ingestion of food and dust compared to body weight.

Using a consistent methodology we show that Americans take up higher doses than Europeans. We also show that food and dust are important sources of exposure and infants take up the highest doses. There are, however, large uncertainties associated with the oral dust intake rates and dermal uptake rates for PBDEs.

EP04-3

Ecotoxicological assessment of UV filter substances

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Chemical UV-filters are used in sun protection products and personal care products in order to protect consumers from ultraviolet (UV) radiation. Therefore, the production volume of UV-filters has increased continuously in the last decade. Prior studies have shown that UV-filters may be introduced to surface water directly via release from skin during swimming and indirectly via wastewater treatment plants from household use as well as further washing off during rainfall. Due to their physiochemical properties, UV-filters are regarded to be persistent in the environment. The occurrence of UV filters in soil and sediment has attracted much less attention compared to water bodies, and the existing studies are limited to a few filters. For this reason an analytical assessment of widely used UV filters in sediments via GC-MS was done. We found, that some of these substances tend to accumulate in sediments and are present in high concentrations during summer.

Considering this environmental exposure, there is only a limited knowledge on ecotoxicological and toxicological effects of UV-filters on aquatic invertebrates. Some in-vivo-tests with vertebrates (fish, rats, mice) and in-vitro-tests with isolated receptors and human cell lines elucidated the endocrine disrupting and toxic potency of these substances. Consequently, exposure to UV-filters may result in serious health problems for aquatic wildlife.

The study aims to evaluate the presence of the selected substances in the environment and the effects of most common UV-filters (Ethylhexyl-methoxycinnamate, Octocrylene and Butyl-methoxydibenzoylmethane) on aquatic organisms, focussing particularly on infaunal and epibenthic invertebrates (*Chironomus riparius*; *Lumbriculus variegatus* and *Potamopyrgus antipodarum*). Due to their life habits, these organisms are especially affected by lipophilic substances.

Results of the analytic and effect studies will be presented and discussed

EP04-4

From sources to urban fate: a contrast of PCBS, PBDES, PAHs and synthetic musks

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Diamond and Harrad (2009) proposed a conceptual model for human exposure to chemicals contained within a stock or inventory of materials and products. The inventory is held mainly indoors, which is the first environment into which the chemicals migrate. From indoors chemicals migrate outdoors into the urban environment. Persistent chemicals will be exported from the urban area enabling transfer through terrestrial and aquatic food webs from which the chemical may return to the urban area in foods that we eat.

To evaluate the model we quantified and contrasted the emissions, movement and inventory of 4 compounds in Canada's largest urban area, Toronto (2.5 million people). Through material flow analysis, multimedia measurements and mass balance modelling, we examined PCBs (e.g., in interior and exterior building sealants), PBDEs (in the pre-2008 electrical and electronic products, textiles, etc.), polycyclic musks or PCMs (synthetic fragrances in "down the drain"consumer products), and PAH (combustion by-products and pavement coatings).

Indoor air concentrations of PCBs, PBDEs and PCMs all exceeded those outdoors and the magnitude of outdoor air concentrations of PCBs and PBDEs corresponded to their respective spatial inventories. Modelling results showed that 85-98% of emissions are advected via air from the urban area allowing for persistent PCBs and PBDEs to enter the terrestrial food web. Measurements corroborated that a fraction of PCB, PBDE and PAH emissions enter urban soils and surface waters where the later, we postulate, originate from wash off of atmospherically deposited surface films on impervious surfaces and soil entrainment during storm events. In contrast, the fate of more volatile PCMs is controlled by waste water treatment plant discharges and atmospheric processes. Thus, PBDEs are released from indoors to the outdoor environment, from which air and surface waters deliver these emissions to the surrounding environment. PBDEs are also released from treated waste water effluent. The fate of PCBs, which have the greatest emissions in the downtown area where the largest inventory was located, is mostly controlled by atmospheric movement. PAH emissions are dominated by transfer to surface waters whereas "down-the- drain"PCMs are mostly emitted to the environment via treated waste water treatment plant discharges.

EP04-5

Predictive emission model for organic compounds added to materials in consumer goods

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Additives are added to materials in consumer products to give different functions as plasticizers, flame retardants, stabilizers, and biocides and such additives may be emitted and reach the environment. Phthalates and other organic compounds are, for example, released from indoor materials and building products and found in water and sediment. Building products have long residence time and large amounts are stored in the society, which may lead to long-term release. Substances that are added in large quantities to products today and are migrating slowly might therefore become problems in the future.

The objective of this study was to develop a general model that can predict emissions of organic compounds from articles based on material properties and the chemical structure of the organic compounds.

The calculation of emissions was made with predictions of partition coefficient, diffusion coefficent and convective mass transfer coefficient and with infromation on material thickness, content and life time from The Swedish Flooring Trade Association and Swedish manufacturers of plastic flooring. Sewage sludge data originating from the Swedish annual environmental montoring program was used for modeled validation.

The model overestimated diffusion coefficient due to the conservatively set material constants. A comparison of model and experimental data for di-(2-ethyl hexyl) phthalate (DEHP) emissions from vinyl flooring showed an overestimation of 20-50%.

The substitution of DEHP with di-iso-nonyl phthalate (DINP) in the beginning of 2003 are expected to result in lower overall emissions. It is also reflected of DEHP measured in sewage sludge for the past few years (2007 to 2009). However, the predicted annual emitted mass was 4-fold less than the mass found in Swedish sewage sludge. This may indicate that other plasticizer containing materials are also emitting DEHP. The model was also used to compare future scenarios in which DINP was replaced by alternative plasticizers such as isosorbate diethylhexyl (isDEH) and di-iso-nonyl cyclohexane (DINCH) and it was shown that use of DINCH would yield lower, and use of isDEH higher, future emissions as compared to DINP.

The method developed seems to give a fairly good estimation of the emitted amounts. However, it does not take into condsideration processes such as particle-aided releases and emissions of wear particles, and cannot accurately deal with multilayer-materials. Work in these directions are in progress.

EP04-PS

Poster spotlight: Perspectives on emissions of new and old substances

Miscellaneous

Poster spotlight highlighting abstracts MO 123, MO 124, MO 125, MO 126:

- Origin, source, exposure and toxicology of Non-Aroclor PCB11
- PAH release from rubber granulates of artificial turf fields: preliminary hazard assessment for the athletes
- Intense sweeteners in the environment- is there a reason for concern for wildlife effects?
- Alcylphenols and Alcylphenol ethoxylates in consumer goods and their contribution to a Wastewater Treatment Plant in Stockholm, Sweden

EP05 - Plastic pollution - polluted plastics: Fate, effects and life cycle assessment

EP05-1

On the lookout for microplastics in sediments and biota: exploring new techniques

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Marine litter has been an issue of concern for decades. However, recently international attention has increased due to the fact that the annual global production of plastics is increasing and the buildup of these materials in the environment may become problematic.
Under influence of UV-light and mechanical forces, plastics also tend to break down into smaller particles in the size range of 1 mm and smaller. While no clear adverse effects of these so called microplastics on marine organisms have been reported, they have been detected in the water column and sediments at high concentrations. The most widely used method to extract microplastics from sediments is based on flotation of the plastics in a saturated salt solution. This method is not 100% efficient, as PVC for example has a density higher than that of a saturated salt solution. High density chemicals like sodium iodide (NaI) are expensive to use on a large scale. Here, a new method using a fluidized sand-bath and the use of NaI on a small scale is proposed. This technique allowed to achieve an extraction efficiency of 100% for PVC particles and 98% of fibres after one sand-bath extraction and maximally 3 subsequent NaI extractions.
Uptake of microplastics has already been observed in mussels, sea cucumbers, barnacles and other organisms during laboratory trials. For mussels for example, it has also been shown that particles smaller than 20 µm have the potential to translocate to the circulatory system. However, in field collected organisms, no evidence exists yet that microplastics are taken up inside the tissue. A method to extract microplastics - based on tissue digestion - from such organisms is proposed.

EP05-2

Biodegradation in soil of an experimental polyester

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The resistance to all form of degradation of traditional plastics allows them to be used in many applications, but their disposal contributes to increase the amount of municipal wastes. Biodegradable polymers can be used in specific applications (i.e. compostable waste bags, carrier bags, single-use tableware, mulch films) allowing to avoid landfill disposal , thus reducing the cost for waste management and the accumulation in the environment. Products used in agriculture (in particular mulch films) are applied to soil and can be left there. A biodegradable polymer is completely converted by microorganisms (under aerobic condition) to carbon dioxide, water and new biomass. Standard test methods are based on the measure of the amount of CO2 evolved in a closed or a ventilated respirometer. However, CO2 is only one component in carbon balance. The determination of biomass development and of the polymer metabolites and their fate, for example in soil, is very important in order to obtain a complete description of the process. Most commercial biodegradable plastics are made of biodegradable polyesters. In this work the mineralization of a model polyester in soil was evaluated and compared with cellulose and polycaprolactone (PCL). The tests were carried out according to ASTM 5988-96. The tested materials were added to agricultural soil enriched with compost and mineral salts and put in hermetic jars. PCL and cellulose were quickly mineralized and at the end of the test (after about five months of incubation) they reached a not well defined plateau phase. Their final mineralization was about 60% and 50% respectively. The model polyester mineralization shows a different pattern: after a lag phase around 15 days mineralization proceeded regularly till the end of the test reaching about 53%. Only half of its carbon content is converted into CO2, butanediol and sebacic acid (used for its synthesis) resulted to be completely biodegradable, with about 50% of CO2 production and 50% converted into biomass, so that it is likely that no carbon remains in soil as material residue. If normalized to cellulose (used as control because considered a fully biodegradable polymer) the polyester mineralization can be considered as 100%. Results suggest that the experimental polymer was completely biodegraded, but to complete the study a simple and reliable method to measure the biomass production should be developed and applied.

EP05-3

Toxic metals derived from plastic litter in a beach

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In this study we established a novel and reliable method to estimate the total mass of beach litter and toxic metals in Ookushi beach, Goto Island, Japan. Using a balloon with an attached digital camera we measured the area of beach covered by litter, and then randomly assigned ten boxes of 2x2 m across the beach to obtain *in-situ* measurements of the litter mass per unit area. The mass (kg) of beach litter is estimated by multiplying the area (m^2) covered by litter with the average litter-weight per unit area (kg/m^2) and found as 716±259 kg from a t-test with 95% confidence limit. Among randomly collected beach litter within the square areas, plastics occupy 74% of the total mass, of which light plastics such as polyethylene (PE; 234±96 kg) are more prevalent than heavier materials. Second, the concentrations (mg/kg) of toxic metals in plastic litter are estimated using handheld X-ray fluorescence analyzer (XRF, Innov-X Systems Inc., a-6500). Toxic metals in polymers are widely used as plasticizers, catalysts, stabilizing additives, and pigments. Among various toxic metals, lead (Pb) and total chromium (Cr) are detected in PE plastic litter and their concentrations are estimated at 44.7±13.7 mg/kg and 13.7±5.5 mg/kg respectively by analyzing 432 pieces of plastic samples. We also estimate the total mass (g) of Pb and total Cr carried by PE litter by multiplying the concentration (mg/kg) with the estimate PE plastic mass (kg) over the beach and calculate as 10.4±5.2 g and 3.2±1.9 g, respectively. However, Pb and total Cr contained in PE litter occasionally exceed 100 mg/kg, that is the EU regulations on packaging and packaging waste. These toxic metals in polymers are often used as pigments and are potentially released into the beach environment during the degradation of plastics. The results of other polymers will be shown in our presentation.

EP05-4

Association of TiO2-nanoparticles with small plastic fragments in beach sand at the Island Norderney (North Sea)

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Titanium dioxide (TiO_2)-nanoparticles are added to plastics during manufacture as white pigments or UV blockers. In this study the occurrence of TiO_2-nanoparticles associated with small plastic fragments in the intertidal sediments at Norderney, an island situated in the German Bight in the north-west of Germany, was studied.
At two locations, N1 (N53°43.187′ E007°15.946′) and N2 (N53°43.106′ E007°17.768′), 1.9 kg of sand from each sampling site were collected directly from the surface. A method based on the separation of different dense components via floating in a saline solution was optimised and applied. The total sample volume was divided into portions of 175 g. Each aliquot was poured into a 2-L separatory funnel and filled up with 600 mL of a NaCl solution (26 % w/w). The funnel was plugged and shaken. After a settling time of 1 h the sediments and 300 mL of the solution were drained down. This procedure was repeated twice by adding 300 mL or 100 mL of the NaCl solution and draining down sediments and 200 mL or 50 mL of the solution. Finally the funnel was rinsed with 1 L of distilled water and the content of the funnel containing all less dense particles was filtered by a vacuum filtration unit with a 0.45 µm cellulose acetate filter. The filter was dried at room temperature, sealed in petry dishes and optically analysed under a stereomicroscope at 6.5-40-fold magnification.
Overall there were 24 plastic objects per 3.8 kg sand. Among the separated objects were green, blue and red fibres and blue, green or white shards. The smallest identifiable objects were a tenth of a millimetre, the biggest were 2 mm.
First results of the analyses of the microscopic plastic fragments using an environmental scanning electron microscope (ESEM) demonstrated the association of TiO_2-nanoparticles with some of the fragments. Based on our field data first estimates of TiO2 materials with plastic particles result in a range from 4 to 7 g ng L^{-1} of sea surface .
Our results point out that plastic debris in rivers and oceans must be considered as a source of TiO_2-nanoparticles in the aquatic environment. Because of an enhanced density of microplastics containing TiO_2 an accumulation of such fragments in sedimentary habitats is expected. Our findings highlight for the first time a potential risk for marine ecosystems from TiO_2-nanoparticles associated with microplastics.

EP05-5

Understanding leaching from plastics into the environment: lessons learned from food contact plastics use

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Since the start of plastics mass production around 60 years ago the prevalence of environmental plastics debris has increased, and the extent of this contamination is gradually becoming apparent now. Due to its material properties plastic are not biodegraded. Weathering slowly leads to mechanical break-down, resulting in small plastic fragments being distributed worldwide. This observed widespread distribution of plastic fragments implies chemical interactions with the biotic and abiotic environment. While the adsorption of persistent environmental organic pollutants (POPs) to plastic particles is being studied, the release and migration of chemicals from the plastic into the environment is far less well characterized. For environmental research the rich body of literature concerning migration from food contact material (FCM) plastics into food and food simulants can be a very useful source. This paper reviews FCM plastics migration studies and highlights the presence of endocrine disrupting chemicals (EDCs) in FCM. A good understanding of plastics' qualitative and quantitative characteristics will be imperative to address environmental pollution and understand toxicity of plastic leacheate. Furthermore, plastics used to package foods and beverages constitute between 10 to 20% of all plastics currently manufactured. As single-use items the highly abundant types of plastic packaging are thought to constitute a significant fraction of marine plastics debris. A closer look at their chemical composition is useful for assessing and characterizing the nature of plastics chemical pollution.

EP05-6

What do plastic additives add to life cycle assessment results?

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Plastics are produced in countless varieties of polymer mixtures and additives such as plasticizers, flame retardants and stabilizers. In life cycle assessment (LCA) studies, due to a lack of specific composition information it is common to reduce this complexity to the assumption that plastics are composed of pure single polymers.
The current study investigates how the calculated environmental impact of plastics would change when additives are included in LCA calculations. Several examples will be presented to illustrate the contribution of additives to the total environmental impact of plastics. The first example considers flame retardants used in plastics for electronic equipment (e.g. bisphenol-A derivatives in polystyrene blends, often present at ~20% weight). Other examples to be discussed include plasticizers and fillers, which can constitute up to 40% of the weight of the plastic. While the use of phthalates in plastics increases the potential ecotoxicological effects, the use of the filler material calcium carbonate leads to a reduction of ecotoxicological and other impacts.
The consequences of the uncertainties and variability in the detailed composition of plastics will be discussed in relation to other model uncertainties such as exposure routes of these additives to organisms in the environment (leaching from landfills, volatilization during use phase).

ET01 - Alternatives to animal testing in ecotoxicology

ET01A-1

Comparison of cartilage and bone malformations in the head of zebrafish embryos after exposure with dithiocarbamates and hydrazides

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Dithiocarbamates such as disulfiram are well-known teratogens causing wave-like deformation of the notochord and cartilage malformation in fish embryos. Although different in chemical class, molecular weight and log KOW, hydrazides, especially acetic hydrazide, generate the same morphological effects. Similar results are also found for benzhydrazide, formic acid hydrazide and isoniazide. Fertilized zebrafish eggs were exposed for 6 days, fixed in paraformaldehyde and stained as whole mounts. Disulfiram induced strong cartilage malformations after exposure to ≥ 80 µg/L, whereas acetic hydrazide caused cartilage alterations from 1.5 g/L. Wavy notochords occurred after exposure to disulfiram even at the lowest test concentration of 20 µg/L, whereas at the two lowest concentrations of acetic hydrazide (0.375 and 0.75 g/L) mainly fractures of the notochord were observed. Concentrations of acetic hydrazide ≥ 1.5 g/L resulted in undulated notochords, similar to disulfiram. Cartilages and ossifications of the cranium, including the cleithrum, were

analyzed individually assessing the severity of malformation and the degree of ossification of bones in a semi-quantitative approach. Cartilages of the neurocranium, such as the ethmoid plate, proved to be more stable than cartilages of the pharyngeal skeleton such as, e.g., Meckel's cartilage. Hence, ossification is much more susceptible to the test compounds than cartilage, showing alterations at lower concentrations, mostly as a reduction of bone mass.

ET01A-2

Further development of a gene expression fish embryo test as a potential alternative to the fish early life stage test

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To date, there are no alternatives to chronic fish toxicity tests such as the fish early life stage test. In an earlier project, a gene expression *Danio rerio* embryo test (Gene-*Dar*T) was developed based on the fish embryo toxicity test with the zebrafish by including an additional endpoint, the analysis of differential gene expression. For most tested substances, lowest observed effect concentrations (LOECs) derived with the Gene-*Dar*T agreed reasonably well with LOECs of fish early life stage tests. However, for some substances, larger differences were observed indicating the need to improve the test protocol. Therefore, the Gene-*Dar*T protocol was further developed in the current project by identifying additional marker genes (i.e. genes that are sensitive to toxicants) and optimising the test protocol to allow an effective analysis of the larger gene set. The improved test protocol is used for testing of a range of substances.

A list of 10 model substances and 30 test substances was compiled covering different modes of action and a wide range of toxicities in the fish early life stage test. Marker genes were identified by microarray analyses following exposure of zebrafish embryos to the ten model substances and subsequent verification of sensitive genes with quantitative real time polymerase chain reaction (qRT-PCR). Eighteen genes exhibiting robust expression patterns were selected as potential marker genes. A semi-quantitative multiplex RT-PCR protocol was developed to investigate the effects of the test substances on the expression of these marker genes. This protocol has been used to study the effects of approx. 20 test substances on expression of the marker genes following exposure of zebrafish from 1 to 49 hours post fertilisation.

Range finding tests were performed with 19 test substances, definitive tests with three substances. Preliminary results indicate that substance concentrations eliciting differential gene expression deviate by factor of not more than ± 6 from the effect concentrations in fish early life stage test. Currently, the effects of further substances on differential gene expression are evaluated in definitive tests. Based on the results, the suitability of the marker genes and the correlation of the results of the Gene-*Dar*T with fish early life stage toxicity data will be evaluated. First conclusions on the application range and the limitations of the Gene-*Dar*T will be drawn.

ET01A-3

Danio rerio embryos as a convenient animal alternative model for neurotoxicity assessment

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The developing nervous system is far more sensitive to injury caused by neurotoxic substances than is the adult one. This conflicts with the growing number of chemicals released into the environment deemed to be neuroactive. Despite the relevance of neurotoxic effects in the hazard evaluation of chemicals, testing for neurotoxicity is currently only based on expensive, time-consuming and ethically disputed mammalian experiments, which are also unsuitable for large scale screening applications. Moreover, the present testing approaches endorsed by the OECD or the US-EPA, focus on the assessment of the human health risks only, while the environmental relevance of the neurotoxic compounds remains disregarded. In ecotoxicology, the zebrafish embryo test (zFET) has long been used as an alternative to acute and developmental fish toxicity, and there is good evidence suggesting that the embryos may also qualify as promising animal alternatives in neurotoxicity testing. The UNIFISH project tests the applicability of the zebrafish embryo assay as a screening tool for neurotoxic chemicals by pursuing an approach, which combines morphometric with immunobiochemical endpoints. Fish embryos were exposed to thiocyclam, cartap, and disulfiram for 48 h and subsequently, whole-mount immunostaining with the neuron-specific antibodies znp1 (primary motor neurons) and zn8 (secondary motor neurons) was applied. It was possible to link neuronal defects to a characteristic morphological phenotype of the embryos after the exposure. The quantification of the neurotoxic defects was based on a specified classification system, whereby the severity of neurotoxic damage to individual motor neurons can be assessed. The comparison of the EC50 values determined for neurotoxicity effects and by standard zFET displayed a good correlation between the two test systems. Therefore, the zFET for neurotoxicity could be a sensitive, convenient and reliable alternative approach to assess the neurodevelopmental hazard of environmental chemicals.

ET01A-4

Transgenic fluorescent zebrafish- a promising tool to refine the zebrafish embryo toxicity test zFET

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The fish embryo toxicity test zFET is highly attractive as an animal alternative testing approach in ecotoxicology. To date, the application of the zFET has focused on acute toxicity assessment, but there is plenty of evidence for its high potential to also evaluate specific and even chronic toxicity like teratogenicity or endocrine disruption. This, however, requires a refined endpoint evaluation using detailed morphological and submorphological analysis methods, which are so far neither established nor standardised. For screening, the zFET procedures will also need adjustments to high throughput applications. The use of gene expression markers can be instrumented for a better understanding of adverse effect mechanisms. They can also help to increase the sensitivity and specificity of the zFET in comparison to the solely on macroscopic morphological endpoints based original test assessment.

This project is aimed to prove the suitability of a transgenic zebrafish line for the zFET to test chemical toxicity on vasculogenesis. The transgenic line Tg(fli1:EGFP)y1 expresses enhanced green fluorescent protein (EGFP) in the vasculature under the control of the fli1 promoter. Fli1 belongs to the ETS transcription family and is crucial for the development of the vascular system. Thus, fli1 driven EGFP-expression may serve as a relevant marker for the detection of vascular defects.

For this proposal zebrafish embryos of the Tg(fli1:EGFP)y1 line and the wild type strain were exposed to triclosan, genistein, fenazaquin and cartap for 48 h. By using a fluorescent signal based assessment method, adverse effects on vasculogenesis could be detected earlier or even at all in the Tg(fli1:EGFP)y1 embryos than in the wild type embryos. Fli1 is one of the first ETS transcription factors, and the onset of Fli1- expression in the developing endothelial vessels is as early as 14 hpf. Hence, the fluorescent assessment enables an earlier and more sensitive detection of vasotoxicity than in wild type zebrafish embryos. The transgenic zebrafish line Tg(fli1:EGFP)y1 is a promising tool to refine the zFET for vasotoxic substances.

ET01A-5

Evaluation of the toxic potential of 34 organic chemicals towards a rainbow trout gill cell line

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The EU regulation REACH requires that all chemicals produced or imported into the EU need to be tested with respect to their impact on human health and the environment. At a production volume of 10 tonnes per year the test for acute fish toxicity (OECD 203) is obligatory. This test uses death as endpoint and requires a substantial number of fish. It is expected that in the first 10 years of REACH alone, 4.4 million fish will be needed. Therefore the development of appropriate alternative methods is timely. One promising approach is the use of fish cell lines; however, several studies indicated that fish cell lines appear less sensitive than fish. During the project CEllSens (funded by CEFIC-LRI/UK-DEFRA), we optimized the fish cell line approach; we used several steps to increase the sensitivity of the *in vitro* assay. These included the selection of the fish cell line, modification of the exposure medium, selection of toxic endpoints and the determination of the chemical bioavailability. We further showed that the chemical toxicity is also dependent on the solvent and dosing procedure. Based on these findings, we designed dosing and exposure protocols that account for factors otherwise compromising the *in vivo-in vitro* correlation. The optimized cell line approach was now used to screen the toxic potential of 34 industrial organic chemicals towards the rainbow trout gill cell line, RTgill-W1. The tested chemicals are part of the systematically derived CEllSens chemical list. The selected chemicals have a wide range of mode of action, physico-chemical properties and *in vivo* fish toxicity values. Results indeed reveal a good agreement of the acute fish toxicity data and the gill cell cytotoxicity values. Outliers from the *in vivo-in vitro* correlation can be explained by the mode of toxic action. The compounds that give the greatest deviation of *in vivo* and *in vitro* values are either neurotoxicants or need to be biotransformed into more toxic metabolites. Therefore it can be concluded that these mode of actions are not mimicked by the gill cells. By combining our results with our methods we aim to propose a strategy to use fish cell lines as surrogates for acute fish toxicity studies.

ET01A-6

Combined toxicity of estrogen receptor agonists and antagonists in a fish in vitro assay

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Primary hepatocytes from fish such as rainbow trout (*Oncorhynchus mykiss*) represent a well-characterised high-throughput screening tool for single chemicals and mixtures. These native cells provide assessment of multiple mode of action (MoA) and has successfully characterised the potency of both estrogen receptor (ER) agonists and ER antagonists *in vitro* through use of the estrogenic biomarker vitellogenin (Vtg). Fish in the environment are continually exposed to a mixture of chemicals and the growing concern about the combined effect of mixtures has resulted in development of prediction models for the combined effect of mixtures. Two of these prediction models, the concentration addition (CA) and the independent action (IA) prediction models are widely applied for direct endpoints, like *in vivo* induction of cellular reponses such as the estrogenic biomarker Vtg and *in vitro* activation of the ER. The present work assess the CA and IA prediction models's ability to predict the combined effect of mixtures of ER agonists and antagonists in a primary culture of rainbow trout hepatocytes. The ER agonists showed a concentration dependent increase in the ER-mediated Vtg production, whereas the ER antagonists showed a concentration-dependent inhibition of the estrogen-induced production of Vtg. Mixtures of ER agonists were designed based on the CA prediction model, and experimental results were compared to the predicted respons. Except for deviations from the prediction models at the higher relative mixture concentrations of the ER agonist mixtures, our results are in agreement with other *in vitro* and *in vivo* studies where CA has shown to accurately predict the combined effect of estrogen agonists. Our results show that the use of *in vitro* native cells such as primary rainbow trout hepatocytes in combination with mixture toxicity prediction models may become useful tools in assessing the combined effects of ER agonists and ongoing studies with ER antagonists will determine whether the prediction models are able to successfully predict more complex MoA such as that caused by ER antagonists.

ET01B-1

Alternatives to in vivo tests to detect endocrine disrupting chemicals (EDCs) in fish and amphibians

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A significant amount of current research in risk assessment of chemicals is targeted to evaluate alternative test methods that may replace, reduce, or refine (3Rs) the use of animals, while ensuring human and environmental health and safety. In 2009, the US EPA began implementation of the Endocrine Disruptor Screening Program which includes Tier 1 screening assays in fish and frog species which are closely aligned with the OECD test guideline series 229 and 231. How-

ever, these assays use a large number of animals and are quite long in duration relative to an ideal screening assay. As the Tier 1 assays screen and prioritize a large number of chemicals for possible endocrine activity shorter-term and alternative to animal tests would be advantageous. In order to identify potential alternatives, a literature search was conducted and a database with alternatives to fish and frog tests testing methodologies assembled. Data from 1995 to present were collected related to the detection/testing of estrogen-, androgen-, and thyroid-active chemicals in the following test systems: cell lines, primary cells, fish/frog embryos, yeast, bacteria, cell free systems, and "omics"technologies. A critical analysis was performed to (1) determine the strengths and limitations of each alternative assay identified and (2) present conclusions regarding chemical specificity, sensitivity, and correlation with in vivo data. A summary of the most promising alternative assays will be presented.

ET01B-2

Exploring the potential of the ZFET beyond acute toxicity

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In terms of fish toxicity test alternatives, the fish embryo toxicity test FET can certainly be considered the most promising approach, and in the context of the OECD Test Guideline Program, the FET with zebrafish (zFET) is being validated as a replacement for the fish acute toxicity test. Meanwhile, the applicability of the FET to also evaluate specific, non-acute toxicity and teratogenicity or to predict chronic adverse outcome pathways, is widely acknowledged. A prerequisite to take full advantage of the potential of the FET, however, is to agree on common standard protocols for specific applications and to address sources of uncertainty of the test procedures. In our studies, we address basic FET related methodological issues like the selection of test vessels or the test duration, to facilitate standardisation and prevalidation of the assay, but also seek to refine the FET through the integration of additional toxicological endpoints. One focus is on trancriptomics as an endpoint and the evaluation of its value to inform on underlying mechanism of toxicity. Several fish embryo studies with zebrafish were conducted either following the DIN/ISO test guidlines or following a prolonged test protocol, which includes post-hatch stages until 120 hpf. The objectives of these studies were e.g. to investigate i) the differences between the two different well-plate types and the influence of the oxygen saturation, ii) the influence of organic solvent use on the toxicity of a test compound and on the transcriptome, iii) how genistein affects gene expression and whether the estrogenic mode of action can be discerned from the transcriptome response. The results demonstrate, among other things, that oxygen can become a limiting factor in an extended FET if the test protocol is not adjusted and that the use of the organic solvent dimethylformamide (DMF) can modulate gene expression of thousands of genes. Genistein enhanced the expression of mainly estrogenic genes, which also respond in medaka and adult zebrafish. Our studies show that the ZFET has excellent potentials for application beyond acute toxicity, but for regulatory acceptance effective measures to minimise the level of uncertainty of in particular, integrated molecular omics-based methodologies are still lacking.

ET01B-3

Using a toxicological model for hypothesis testing of aquatic toxicity data

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<p style='margin-bottom: 0in;'>The validity, utility, and applicability of toxicity test results from standard testing protocols is contingent on whether the employed toxicological model and assumptions are appropriate and sufficient. As an initial attempt to determine the possible consequences of the lack of toxicological model specification and validation the standard 96 h acute toxicity test protocol was subjected to hypothesis testing of the enabling assumptions. A one-compartment, first-order kinetics model was specified as the toxicological model. The first assumption was that a steady-state LC50 must be either be reached or estimated from the observed data. The second assumption is that a constant time to achieving the LC50 is most appropriate. A subset of the U.S. EPA fathead minnow 96 h acute toxicity database was employed. Approximately 8% of the over 350 tests examined failed the first validation step as steady-state was not reached and could not be estimated. The remainder of the test results failed the second validation step as the time to achieve LC50 was not constant at 96h but varied widely, by a factor of over 2000 times. Using exposure-based dose data from unvalidated and uncorrected toxicity test results can introduce substantial variability/uncertainty in estimates of relative toxic potency, even with a single test protocol with a single species from the same laboratory. Formal adoption of explicit specification of toxicological model and associated assumptions, combined with routine validation, as is currently done for the statistical component of toxicity testing, is clearly required to improve the interpretation of toxicity testing data and enhance its use in estimation of both hazard and risk.

ET01B-4

Characterization of test conditions of the OECD 210 fish early life stage test

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A long term need in animal alternatives research is to address chronic fish toxicity. The complexity of chronic endpoints, such as growth, survival and reproduction, make this especially challenging because the "endpoint"of interest integrates a wide variety of biochemical, physiological, behavioural and ecological factors. To better define the target for 3R activities, we initiated a systematic assessment of current fish chronic toxicity tests. The Fish Early Life Stage Test (OECD 210, FELS) is the most commonly used ecotoxicity test to develop estimations of effects from long term exposure of fish to chemicals. As part of a larger effort to understand the statistical power of the OECD 210 FELS test, we summarized >100 FELS studies. In addition to power analyses, the data set was probed for the general ability of laboratories to meet control performance criteria (minimum levels of viable eggs, control survival). The data set provides an opportunity to assess the breadth of known test conditions and probe relationships with control performance, test outcomes, and species-specific tendencies in conducting OECD 210 tests. The database includes relevant information such as test durations, temperature, conductivity, pH, hardness, analytical verification of exposure, type of exposure system, use of solvents, and effect concentrations. This paper provides an overview of the characteristics of test conditions commonly encountered in the OECD 210 FELS Test. A total of 89 chemicals in 109 FELS from 15 laboratories studies were reviewed. Fathead minnow studies were most common (72%), followed by zebrafish (12%) and rainbow trout (12%). Test temperatures and dissolved oxygen recorded during tests span a fairly significant range. All studies met the minimum DO saturation requirement (60%) although studies at the low end of percent saturation were associated with the lowest length and weight and had acceptable, but marginal, percent survival. Approximately 50% of studies did not define a LOEC while 65% of the time an EC10 was obtained. Low statistical power associated with low replication in the FELS has been noted. Test conditions were most variable for the fathead minnow whereas Zebrafish and rainbow trout studies appeared to be more tightly controlled. The OECD 210 Guideline does not provide detailed guidance on several aspects of the tests which provides opportunities for diverse interpretations which could be rectified in a careful updating of the guideline.

ET01B-5

Evaluation of the OECD 210 Fish Early Life Stage Chronic Toxicity Test - Setting the Target for Future Animal Alternative Efforts

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Several promising animal alternatives approaches now exist for acute fish toxicity and bioaccumulation. In the context of international chemical management programs a larger need now looms to address chronic fish toxicity alternatives. Many obstacles need to be overcome to make chronic ecotoxicity alternatives a reality; however, a fundamental tenet of any approach should be that the alternative should not increase uncertainty in hazard and risk assessment. Therefore, understanding the present level of uncertainty in identification of hazards based on traditional assays sets the bar for future alternative developments. The chronic fish toxicity assay most commonly used to establish chronic effects is the OECD 210, Fish Early Life Stage Test. We have collated a database (>100 studies and compounds from 15 different laboratories) to probe the data characteristics of the OECD 210. Studies were constrained to fathead minnow (72%), rainbow trout (12%) and zebrafish (16%), which form the majority of studies in the past three decades. Studies were summarized with respect to experimental design, water quality, quantifying chemical exposure, and measured test endpoints (hatchability, post-hatch survival, wet and dry weight, length, developmental abnormalities). Information was collected at the level of individual replicates with the goal of determining the statistical power of the reviewed studies to detect biologically meaningful effects. Results of the analysis indicated that improvements in the sensitivity of the test could be made by maximizing the number of replicate chambers per treatment concentration rather than by maximizing the number of organisms per chamber, by increasing the acceptable level of control hatching success and larval survival compared to current levels, by using wet weight measurements rather than dry weight, and by focusing test effort on species that demonstrate less variability in outcome measures. Effects endpoints were represented primarily as NOECs and less often as EC10 or EC20 values. From these analyses we provide evidence to support the level of uncertainty and power to expect from traditional OECD 210 studies as a target for future animal alternative methods for chronic toxicity testing in fish.

ET01B-6

The OECD fish testing framework project: summary of workshop recommendations

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An integrated Fish Testing Framework project was initiated in 2009 as OECD Project 2.30 with the US as the lead country. The objectives were to review regulatory needs and data requirements for fish testing and review the currency of existing OECD Test Guidelines. In addition, the project aimed to support animal welfare concerns by identifying unnecessary test methods, minimizing the number of *in vivo* tests, and ensuring the optimal use of data derived from *in vivo* studies. A fall 2010 workshop with participation from ~40 experts was organized with the goal of producing a guidance document that provides a detailed discussion of issues, relevant endpoints, and considerations for a harmonized testing framework for fish. In addition to reviews of OECD fish test guidelines, topic areas included general test methods, regulatory needs & data requirements for fish testing, statistical considerations, animal welfare considerations and alternative approaches to testing. General guidance on strategies for approaching hazard testing with fish was developed by building broad principles to guide testing which can be adapted for specific circumstances and types of chemicals.

Preliminary recommendations focused largely on revisions to existing TGs and/or the need for expert group discussions, workshops, or reviews to address critical issues. Existing TGs that warrant revision and updating include TG210 and TG203. Several other TGs (212, 215) may require updating upon completion of the Fish Embryo Test validation. TG204 was recommended for deletion. Several issues necessitate expert working groups, workshops, or review papers before any revisions can be made including: evaluation of solvent effects; choice of test concentrations for fish endocrine screening assays; test acceptance & validity criteria; water & nutrition quality; review & analysis of existing fish data to further inform TG229 development; development of high-quality fish chronic data sets; review of practical applications of mode of action; assessment of the appropriateness of the various recommended or optional fish species in TGs; and harmonization of definitions across various TGs. This presentation will highlight the conclusions and recommendations and discuss the developed framework document.

ET02 - Assessing the exposure, effects and risks of Pharmaceuticals and Personal Care Products (PPCPs) in the environment

ET02A-1

Comparing prioritisation schemes for environmental risk assessment of human pharmaceuticals

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The presence of pharmaceuticals in the aquatic environment, and the concerns that these pharmaceuticals might negatively affect aquatic organisms, has gained increasing attention over the last years. Ecotoxicity data are lacking for most pharmaceuticals and it is therefore important to prioritise pharmaceutical substances for ecotoxicity testing and environmental monitoring. An ideal prioritisation procedure would be systematic, transparent, based on relevant data and applicable to every API on the market.

In the present study, we have ranked aproximately 600 active pharmaceutical ingredients (APIs) currently on the Swedish market according to chemical, environmental effect, sales, and pharmacological data, and combinations thereof, with the aim to discuss the consequences and implications of using different parameters for ranking pharmaceuticals. Particular focus is put on the fish plasma model, originally proposed by Huggett et al (Hum Ecol Risk Ass 2003;9:1789-1799), and its application by the MistraPharma research programme in which we participate. The fish plasma model estimates fish plasma concentrations of pharmaceuticals based on PEC and lipophilicity (logP) values and compares the predicted fish blood plasma levels to human therapeutic plasma levels, thereby creating a concentration ratio (CR).

The fish plasma model takes into account both the estimated internal exposure at the drug target(s) and an estimate of potency (based on read-across from humans, i.e. data that are available for most pharmaceutical substances). This makes it a theoretically attractive concept for predicting risks for pharmacological effects in fish, whether adverse or not. We would like to stress, however, that any prioritisation scheme should be evaluated empirically for accuracy prior to implementation. A comparison of predicted or measured fish plasma levels and human therapeutic levels correctly identifies ethinylestradiol and levonorgestrel as pharmaceuticals of high environmental risks. This adds confidence to the overall concept of the fish plasma model.

ET02A-2

Perspectives on prioritization approaches for studying pharmaceuticals in the environment

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Human and veterinary pharmaceutical residues in the environment have received unprecedented attention from citizens, environmental scientists, engineers and managers, and regulatory authorities over the past decade. An increasing body of literature is reporting the accumulation of therapeutics in wildlife, though an understanding of relationships among internal exposure, therapeutic mechanisms/modes of action and adverse effect thresholds is lacking. Further, some of the unique properties of pharmaceuticals present unique challenges to the assessment of environmental risk and hazard. Fortunately, pharmacological safety data is often more available than many other classes of industrial chemicals, which allows for physical-chemical and biological read-across applications among target and non-target organisms. Various efforts have attempted to identify pharmaceutical properties associated with greater risk to aquatic and terrestrial organisms. In this presentation we critically review such approaches for developing prioritization hazard and risk frameworks in prospective and retrospective environmental assessments. Because secondary poisoning of diclofenac represents a definitive example of wildlife impacts by a pharmaceutical, we specifically examined the potential for secondary poisoning to terrestrial wildlife using a trickling filter case study. Herein we accounted for the influence of pH on partitioning of weak acids and weak bases to predict relationships between oral wildlife exposure and recommended daily dose in humans. We then assessed these high priority pharmaceuticals for side effects and common contraindications, and further examined their potential hazards to aquatic life. This study thus provides a novel approach for considering aquatic and terrestrial receptors for prioritizing environmental risk and hazards of human and veterinary medicines.

ET02A-3

Human health risk assessment of pharmaceuticals in Chinese drinking water

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Pharmaceuticals are a class of emerging chemicals that are of environmental concern due to their inherent bioactivity. Their ubiquitous occurrence in various environmental compartments and their effects on non-target organisms are well documented. Pharmaceutical residues and metabolites may contaminate potable water sources after being released into surface waters or groundwater, passing unchanged through drinking water treatments and ending up in tap water, where they may pose a chronic hazard to human health. However, regulatory levels for pharmaceuticals in potable water have not been established anywhere in the world. In China, existences of pharmaceuticals in sewage, surface water and sediment were previously reported but related studies in drinking water are scarce.

In this investigation, tap water was sampled from ten cities in China"'Hong Kong, Macau, Wuhan, Shanghai, Xiamen, Shenzhen, Yahcheng, Guangzhou, Nanjing, and Changsha"'during September and November, 2010. Tap water samples were collected from different individual residences spread throughout a city (n=5 per city). A total of 38 pharmaceuticals, including antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, lipid regulators, psychiatric drugs, cardiac drugs, diuretic drugs and anti-hypertension drugs, were analyzed by solid phase extraction (SPE) and high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Preliminary results from six cities (n = 30) showed that 16 compounds were present at detectable concentrations in at least one drinking water sample among the six cities. Of all of the detected pharmaceuticals, caffeine and salicylic acid were predominant, occurring in over 90% of the analyzed samples, followed by carbamazepine and erythromycin-H2O. To assess possible risks on human health, acceptable daily intake levels obtained from the available literature or derived from animal toxicity data, ingestion rate and exposure period will be applied to the pharmaceutical levels measured in the drinking water samples.

ET02A-4

Developing environmental assessment regulations for pharmaceuticals: a drug's character is its fate

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While timely access to pharmaceuticals and health care products is of utmost concern to the government of Canada, there is growing recognition and concern that substances in these types of products are being found in the environment in concentrations that may pose a risk. The Canadian Environmental Protection Act (CEPA) 1999 requires that all new substances for use in Canada be evaluated for their potential risks to the Canadian environment and human health. Currently substances in products regulated by the Food and Drugs Act (F&DA) (including therapeutic drug ingredients) are legally obliged to undergo an environmental assessment. However the current environmental assessment regulations were developed primarily for industrial chemicals and are not appropriate for assessing the potential environmental risk of the types of substances and release scenarios associated with drug products. As such, Health Canada, in consultation with representatives from industry, non-governmental organizations and consumer groups, initiated a project to develop Environmental Assessment Regulations (EARs) with specific information requirements for these types of substances. A framework has been developed which seeks to align with the drug development process while leveraging a testing strategy that considers the potential fate and exposure profile of the drug substance to direct the type of ecotoxicological testing to be required.

The starting premise in the development of a regulatory framework for drug substances is that the regulatory requirements must be science-based and in proportion to potential for risk and allow for the continually expanding knowledgebase of the behaviour of active ingredients in the environment to refine requirements for environmental assessments. Action limits and data requirements should be commensurate with the characteristics of the compound and its expected release into and fate in the environment. Fate/exposure directed toxicity testing ensures that all compartments of the environment are considered and will be protected, allowing the science to establish testing requirements, rather than being driven by prescriptive measures. This talk will explore some of these concepts.

ET02A-5

Multi-biomarker approach to assess sub-lethal effects induced by a mixture of three common non-steroidal anti-inflammatory drugs (NSAIDs) on the zebra mussel (Dreissena polymorpha)

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Pharmaceutical compounds are an emergent class of environmental pollutants included in the broad category of PPCPs (pharmaceutical and personal care products). Among these, non-steroidal anti-inflammatory drugs (NSAIDs), the sixth most sold drugs worldwide with an estimated annual production of several kilotons, are commonly revealed in aquatic ecosystems in the high ng L-1 to low µg L-1 range. Some studies investigated the acute toxicity of the most common NSAIDs on non-target organisms, but the information on their capability to induce chronic effects is completely inadequate. Few investigations have only evaluated the potential toxicity of single molecules, excluding the major environmental problem due to drug mixtures. This work deals with the assessment of sub-lethal effects induced by three concentrations of a mixture of three common NSAIDs, namely diclofenac, ibuprofen and paracetamol, on the freshwater bivalve zebra mussel (*Dreissena polymorpha*). Our goal was reached by a multi-biomarker approach in order to highlight cyto-genotoxic effects, as well as the unbalance of the oxidative status of treated-specimens. The single cell gel electrophoresis (SCGE) assay, the DNA Diffusion assay, the micronucleus test (MN test) and the Neutral Red Retention Assay (NRRA) were applied on mussel haemocytes as cyto-genotoxic biomarkers. In addition, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) was measured in the cytosolic fraction extracted from a pool of bivalves to reveal a possible oxidative status unbalance.

Each mixture concentration was able to induce a significant increase of primary genetic damage, which preluded fixed genetic injuries, as highlighted by the increase of both apoptotic and micronucleated cells. Notwithstanding, an unexpected reduction of DNA damage was found at the end of exposure at each concentration. In addition, the NRRA showed that exposure to mixture was able to cause a noteworthy increase of cellular stress in bivalves, above all at the highest concentration, probably due to the rise of oxidative stress, as pointed out by the unbalance of oxidative status of treated specimens. Considering the high potential toxicity of NSAIDs mixture on *D. polymorpha* further in-depth analyses should be necessary in order to investigate the involved mechanism of action and enlarge the knowledge on this fundamental ecotoxicological topic.

ET02A-6

Benthic invertebrate exposure and chronic toxicity analysis for cVMS materials - a probabilistic approach and comparison to the target lipid model

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Lipophilic chemicals, such as the cyclic volatile methylsiloxane materials octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), adsorb extensively to particles and surfaces in aqueous systems, making sediments a key sink when performing risk assessment evaluations. A widely accepted step for estimating the possible risk posed by such chemicals to sediment-dwelling species is to compare the observed sediment concentration with either published ecotoxicity guidelines or to chronic no-observed effect concentrations (NOECs) from toxicity testing with benthic invertebrates. The comparison of field concentrations with chronic NOEC levels can be done with simple worst-case simulations or using a probabilistic distribution approach. In this work, a comparison was made using probabilistic methods of D4 and D5 residues from sediments and organisms collected in the United States (Lake Pepin, Minnesota) and Norway (Inner Oslofjord) to chronic NOEC values determined using EPA/OECD test species such as Chironomus tentans, Chironomus riparius, Hyalella azteca, and Lumbriculus variegates; both sites are downstream from municipal wastewater treatment plants, the likely sources of the D4 and D5 residues. The risk assessment was extended using ecotoxicity data on more than 50 species using the target lipid model (TLM), which assumes a narcotic mode of action (MOA) for ecotoxicity. Comparisons were made using sediment levels on a dry weight and organic carbon basis, and with organism residues on a lipid-adjusted basis. Probabilistic endpoints of 95% exposure and 5% chronic NOEC were extrapolated from the data, which were fit using log-normal assumed distributions. The cVMS acute-to-chronic ratios (ACRs) were consistent with TLM data and with a narcosis MOA (average 2-5). Using either probabilistic techniques or the TLM database, field D4 and D5 concentrations were far below chronic threshold NOEC values with benthic invertebrates, therefore very limited risk appears to exist for benthic invertebrate species with these materials; this evaluation is based on both sediment- and tissue-based analyses and also leveraging the TLM database and narcotic MOA for cVMS materials.

ET02B-1

Bioaccumulation and molecular effects of the contraceptive hormone levonorgestrel in the non-target organism Dreissena polymorpha

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There is increasing concern about the adverse effects of contraceptive steroids found in wastewaters worldwide. Levonorgestrel (LNG), a synthetic steroid used as a contraceptive, as well as a post-coital contraception modality, has been detected in the effluent of STP, in surface waters, in ground water and in sediment. The effects of LNG on invertebrates, in particular on mussels are as yet unexplored. The species Dreissena polymorpha was depicted as a model organism to study adverse effects of LNG on mussels. It was the aim to explore to which extent LNG bioaccumulates in D. polymorpha, thus the bioconcentration factor (BCF) was assessed and to examine if LNG has a direct effect on cell functions of biotransformation, elimination, prevention from oxidative stress and protein damage.

Mussels were exposed for seven days in the flow-through system to increasing concentrations of LNG: 0, 0.3, 3 and 6 µg L^{-1}. Gill and digestive gland tissue were sampled after 1, 4 and 7 days of exposure. Real-time PCR assays were run to study changes in mRNA levels. Whole tissue was sampled at each time point for analysis of LNG tissue content. LNG water and tissue concentration were determined by LC-MS/MS.

Within four days mussels exposed to 0.3 µg L^{-1} LNG bioaccumulated the substance 95-fold. Mussels exposed to the higher concentrations displayed lower BCFs (30 and 56, respectively). After one week, amounts of LNG in mussels exposed to the two lower concentrations were even increased. Only for the highest concentration a decrease of the BCF within one week could be observed.

After only one day we found an immediate up-regulation of pi class glutathione S-transferase (piGST) in both examined tissues in all treatment groups, indicating phase II biotransformation processes. Also superoxide dismutase (SOD) and metallothionein (MT) mRNA was significantly up-regulated after only one day in the digestive gland hinting on oxidative stress. After four days we found an up-regulation of P-glycoprotein (P gp), as well as increased levels of SOD and MT mRNA in mussels exposed to the highest concentration, suggesting enhanced elimination processes and ongoing oxidative stress. After one week exposure to LNG enhanced elimination processes were indicated for mussels exposed to the highest concentration also by the up-regulation of P gp in gills. An enhanced requirement for protein repair, transport or protective processes was evidenced by hsp70 induction in gills.

ET02B-2

Do nonprescription pain relievers have endocrine disrupting potential in zebrafish (Danio rerio)?

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Nonprescription pain relievers such as ibuprofen (ipren, ibumetin), acetylsalicylic acid (aspirin) and paracetamol (panodil, pinex, pamol) exert their effect by inhibting enzymes involved in prostaglandin synthesis. Prostaglandins are signalling molecules involved in induction of pain, inflammation and fever but they are also involved in various housekeeping functions like platelet aggregation and renal blood flow. Nonprescription pain relievers (mild analgesics) are used to treat pain, fever and inflammation and therefore found in most homes. Due to high consumption of mild analgesics in many industrialised countries several analgesics have been detected in waste water and surface water. In mammalian studies inhibition of prostaglandin synthesis have been found to affect steroid levels and sexual behaviour. Recently, also maternal consumption of paracetamol during pregnancy has been correlated with increased risk of cryptorchidism in newborn boys, and administration of paracetamol to pregnant dams reduced the anogenital distance of male rats.

In a set of studies we investigated the effects of prostaglandin inhibitors on sexual differentiation and reproductive parameters in zebrafish, which is a commonly used test organism in endocrine research. Sex ratio and vitellogenin levels in sexually mature zebrafish remained unchanged after exposure to acetylsalicylic acid (0.2-8.2 mg/L) and paracetamol (0.22-29.55 mg/L) during sexual differentiation. Both acetylsalicylic acid (3.8 mg/L) and ibuprofen (21-506 µg/L) reduced PGE2 levels in male and female zebrafish after short term adult exposure but steroid levels remained unchanged. Ibuprofen did not affect other reproductive endpoints except for vitellogenin levels in males. In conclusion, commonly used reproductive parameters in zebrafish do not seem to be sensitive towards prostaglandin inhibitors, but further investigations are required because the reduction of prostaglandins could influence various physiological processes.

ET02B-3

Early life progestin exposure causes sterility in adult female Xenopus tropicalis frogs

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Levonorgestrel (LNG) is a pharmaceutical progestin commonly used in contraceptives. It has been found in the aquatic environment and shown to bioconcentrate effectively and impair reproduction in adult fish at environmentally realistic concentrations. As yet, however, there is a lack of data on toxicity of progestins in amphibians. Moreover, information on the long-term toxicity of progestins following early life exposure is scant. The aim was to characterize effects of developmental LNG exposure on sex differentiation, reproductive organ development and fertility using the model frog Xenopus tropicalis. Tadpoles were exposed to 19 or 156 ng/L LNG (0.5 or 0.06 nM, measured concentrations) via the water from shortly after hatching until metamorphosis. At metamorphosis, exposure was discontinued and effects on gonadal differentiation were assessed using a subsample of the animals. Remaining metamorphosed frogs were held unexposed for nine months, at which time reproductive organ structure, function and fertility were analysed. LNG exposure severely impaired oviduct development and fertility. All adult females (10) in the 0.5 nM group completely lacked oviducts. Upon mating with unexposed males, only one of the 11 LNG-exposed females laid a few eggs (none of which was fertilized), i.e. all LNG exposed females were sterile. All (8) control females laid fertile eggs. No effects on sperm count, sexual behaviour or fertility were observed in the males. No effects on sex ratio or gonadal histology were detected at metamorphosis but devastating effects on reproductive function were detected in adult females, emphasising the importance of investigating the long-term consequences of developmental exposure. To our knowledge this is the first study of developmental reproductive effects of progestin using an aquatic vertebrate. Considering that several progestins are found in the aquatic environment further investigation into the sensitivity of frogs to these compounds is warranted to understand the potential risk progestins pose to wild frog populations.

ET02B-4

Effects of the antidepressant venlafaxine (Effexor®) on fish brain chemistry and predation behavior

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Antidepressants found in wastewater effluent and receiving streams have been shown to be relatively non toxic using traditional fish aquatic toxicity testing. But the psychotropic mode of action of these compounds warrants examination of the behavioral effects these chemicals may have on aquatic organisms. Previous results indicate the antidepressant fluoxetine (Prozac®), causes decreased brain serotonin levels in fish, resulting in a decreased ability to capture prey. Though concentrations required to cause this effect (low µg/L) are approximately two orders of magnitude higher than environmentally measured concentrations (low ng/L).

The antidepressant venlafaxine (Effexor®) has been found at low µg/L concentrations in wastewater effluent. It has a slightly different mode of action than fluoxetine, in that it is designed to alter brain serotonin as well as norepinephrine. The objective of this study was to determine the effects of venlafaxine on fish brain chemistry and predation behavior. A predator prey bioassay, designed in our lab, using hybrid striped bass (Morone saxatilis x Morone chrysops) as the predator and fathead minnows (Pimephales promelas) as prey, was used to test effects. Bass were exposed to venlafaxine for a period of 6 days and then allowed to recover for 6 days. During both exposure and recovery bass were fed four minnows every third day. The time to capture the minnows was quantified and compared among treatments. After each feeding event a subset of bass was removed and their brains analyzed for monoamine concentrations. Behavioral data was then correlated with brain chemistry data to determine if there was a mechanistic link between brain chemistry alterations and predatory behavior.

ET02B-5

Effects of caffeine and ibuprofen in the health status of the European green crab

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The constant input of pharmaceuticals to the aquatic environment has the consequence of exposing biota to unknown chronic effects, thus affecting the heath of organisms even at low concentrations (µg/L to ng/L). The aim of this study was to evaluate heath status of the European green crab C. maenas analyzing sublethal responses after exposure to different concentrations of caffeine and ibuprofen (including environmental concentrations) under laboratory conditions during one month. Sea water was spiked every two days with caffeine (0; 0.1; 5; 15; 50 µg/L) and ibuprofen (0; 0.1; 5; 10; 50 µg/L). Stock solutions of pharmaceuticals were diluted in 0.001% DMSO. Sublethal effects were first evaluated in vivo applying the lysosomal membrane stability test (LMS). Together with LMS, Phase I and Phase II detoxification enzyme activities, lipid peroxidation and DNA damage were determined in gill, hepatopancreas, muscle and gonad tissues. Results showed a dose-dependent and time-dependent effect of pharmaceuticals. Lysosomal membrane stability in crabs exposed to environmental concentrations of caffeine and ibuprofen was significantly destabilized compared to controls ($p < 0.05$) indicating general stress syndrome. EROD (ethoxyresorufin O-deethylase) and DBF (dibenzylfluorescein) enzymatic activities were significantly induced by caffeine (5; 15; 50 µg/L) and ibuprofen (5, 10, 50 µg/L) in gill and hepatopancreas tissues ($p < 0.05$) compared to control crabs. GST (glutation-S-transferase) enzymatic activity was significantly activated ($p < 0.05$) in all tissues by ibuprofen (5, 10, 50 µg/L) and by caffeine at higher concentration. Oxidative stress and DNA strand breaks showed and induction compared to controls ($p < 0.05$) in gills and hepatopancreas tissues exposed to the highest concentration of caffeine and ibuprofen. Results revealed that C. maenas exposed to environmental concentrations of caffeine and ibuprofen can develop general stress syndrome. Results indicated that LMS, Phase I, Phase II detoxification enzymes and antioxidant enzyme activities; lipid peroxidation and DNA strand breaks selected as sublethal responses, constituted suitable biomarkers in the assessment of pharmaceuticals pollution in marine ecosystems using the crab Carcinus maenas as bioindicator species.

ET02B-6

Microbial degradation of gemfibrozil and naproxen drugs in a river water ecosystem

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The presence of pharmaceuticals as widespread ecosystem contaminants has increased attention among researchers, lawmakers, regulators, and the public in the last years. They continuously reach the environment owing to the lack of degradation in the wastewater treatment plant, and for this reason they are detected in aquatic environments. Their concentrations range from µg/L in the effluents from wastewater treatment plants to ng/L in surface waters. Pharmaceuticals are specifically designed to be biologically active, but there is limited understanding of their ecological effects. Consequently, there is the need to investigate on their possible interactions with non-target organisms in the environment, included microorganisms.

Microorganisms have a key role in the cycles of elements and in ecosystem energy flows and owing to their adaptability and metabolic potentiality, they are able to degrade most of xenobiotic compounds. Consequently they are essential in the overall processes that contribute to the quality state of natural ecosystems.

The present work aims to evaluate the environmental fate of two pharmaceuticals, such as naproxen (anti-inflammatory) and gemfibrozil (lipid regulator) commonly found as contaminants in the river Tiber (Italy). For this purpose, different water microcosms were set up (presence/absence of microbial community) and treated with naproxen or gemfibrozil at a concentration of 100 µg/l. Pharmaceutical degradations were assessed and bacterial community structure and function were investigated. At different times, bacterial abundance, cell viability and phylogenetic composition assessed by fluorescence in situ hybridization were analysed in water samples treated versus microbiological control (no treated water samples). Moreover, the degradations of naproxen and gemfibrozil were also evaluated in microcosms simultaneously treated with both pharmaceuticals

ET02C-1

Drugs of abuse as new aquatic contaminants: cocaine effects on zebra mussel (Dreissena polymorpha)

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Cocaine is one of the most common illicitly used drugs and it has been recently found in several aquatic ecosystems worldwide. This drug was chosen for this research since a considerable amount of data are available for this substance concerning consumption and concentrations in

waste and surface waters, which allow us to validate the proposed model. Present study is the first work to evaluate the cocaine cyto-genotoxic effects on aquatic non-target organisms, such as the freshwater bivalve *Dreissena polymorpha*. We chose three different cocaine concentrations very similar to those found in environment, in order to give information useful in the real world: 40 ng/L, 220 ng/L and 10000 ng/L. We evaluated the cytogenotoxicity in mussel hemocytes in *in vivo* experiments by the lysosomal membrane stability (Neutral Red Retention Assay), the single cell gel electrophoresis (SCGE) assay, the micronucleus test (MN test) and the assessment of the apoptotic frequency (DNA diffusion assay). The biomarker battery demonstrated a slight or moderate cyto-genotoxicity in zebra mussel hemocytes at the two lowest concentrations, but significant effects at the highest dose. This can lead to the hypothesis that, with the increasing of the exposure time, a significant increase in both the primary and irreversible DNA damage would be detected, as well as a general increase of cellular stress.

ET02C-2

The effects of furaltadone and chloramphenicol on Ulva lactuca

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The increase in human population carries with it a higher demand for food supplies which translate in higher production of farmed animals. The use of pharmaceuticals both prophylatic and therapeutically is necessary to promot animal health, but may have significant consequences to natural ecosystems. The extent of the effects of antibiotics released to the environment in non-target organisms is already under the scope of researchers but little attention has been given to primary producers such as macroalgae, which are in the bottom of the trophic webs. The present study tested the effects of two antibacterial agents, furaltadone and chloramphenicol, on the growth of Ulva lactuca. Results showed differences in growth rates when submitted to prophylactic and therapeutic concentrations (25 and 50 ug/ml, respectively) for both drugs. Therapeutic concentration showed a higher interference in growth than the prophylactic, causing its decline in the presence of furaltadone and an increased growth with chloramphenicol.The exposure of macroalgae to pharmaceuticals in the environment will have significant effects on growth that will depend on the characteristics and also on the concentrations of the chemical present.

ET02C-3

In vivo experiments for the evaluation of different biomarkers and alterations in proteins expression profile of Triclosan in Zebra mussel (D. polymorpha)

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Among the emerging class of environmental pollutants of PPCPs (Pharmaceuticals and Personal Care Products), one of the most widely used groups is that of antibacterial agents. While these products at low concentrations are probably not pharmaceutically active in humans, they may still be potential pollutants in aquatic environments. Triclosan (TCS, 2,4,4'-trichloro-2'-hydroxy-diphenyl ether) is one of the main known antibacterial agents and its increasing environmental levels is causing growing concern about its presence in freshwaters. In this work, we investigated the effects and the mechanism of action of the antibacterial agent Triclosan in hemocytes of the freshwater bivalve Zebra mussel (*Dreissena polymorpha*). For this purpose we used several biomarkers for in vivo experiments (96 h of exposure) carried out at three possible environmental Triclosan concentrations (1, 2, 3 nM). We used the single cell gel electrophoresis (SCGE) assay, the micronucleus test (MN test) and the measure of the apoptotic frequency (Halo assay) to measure the genotoxic potential of Triclosan, and the neutral red retention assay (NRRA) as a measure of lysosomal membrane stability to identify general cellular stress. We also evaluated the activity of enzymes related both to oxidative stress (superoxide dismutase -SOD-, catalase -CAT- and glutathione peroxidase -GPX-) and phase II metabolism (glutathione S-transferase -GST-) on the cytosolic fraction of the whole body mussels. Finally, we applied a proteomic technique in order to identify changes in protein expression profiles in the mussel gills.
We observed significant increases in all of the genotoxic biomarkers examined as early as 24 h after initial exposure, as well as a clear destabilization of lysosomal membranes, indicating that this chemical is potentially dangerous for the entire aquatic biocoenosis. A comparison of these in vivo data with existing data from in vitro experiments allowed us to suggest possible mechanisms of action for Triclosan in this bivalve. The role of the identified proteins in this mechanism of action is discussed.
Although further studies are needed to confirm the possible modes of action, our study is the first to report on the effects of this widespread antibiotic on freshwater invertebrates.

ET02C-4

The impact of triclosan on ecosystem services of freshwater biofilms

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a broad-spectrum antibacterial that received much attention during recent years since considerable loads accumulate in the aquatic habitats with toxic effects on bacteria and on non-target organisms. Consequently, research has been conducted on the mode of TCS action as well as on TCS impact on the growth, physiology and species composition of bacteria, microalgae and multicellular organisms such as shrimps. Since biofilms are ubiquitous members of freshwaters that provide important ecosystem services (e.g. self-purification), it is valuable to look beyond the criteria of descriptive and functional parameters.
In this paper, we investigated the impairment of the stabilisation capacity of freshwater biofilms by TCS exposure to different concentrations (2 µg/L - 400 µg/L) over time (2 weeks). Biostabilisation was determined using a new developed device (MagPI), the EPS (extracellular polymeric substances) quantity and quality was assessed and the biomass / cell numbers (microalgae, bacteria) of the microbial community determined along with its composition (microscopic analysis for microalgae, DGGE / FISH for bacteria).
The stabilisation capacity of the biofilm was impressive (up to 4 times higher than the control) but significantly impaired by the TCS exposure, showing the lowest sediment stability in the treatment with the highest TCS concentration (decrease of stability to 65% of the initial value). While the bacterial cell numbers showed first a continuous increase despite TCS spiking, bacterial growth was soon significantly hampered in the treatments with the higher TCS concentrations (85 - 400 µg/L). In the treatments with the lower TCS concentrations (2 - 25 µg/L), the bacterial growth showed a similar trend when compared to the control biofilm without TCS. These data suggest a different mode of TCS action from suppressing metabolism to bactericidal effects depending on the TCS concentration. TCS exposure clearly induced shifts within the bacterial and microalgal community that explained, along with significant changes in EPS quantity and quality over time, the observed changes in biostabilisation capacity.
In summary, triclosan exposure affects more than structural and functional parameters of freshwater biofilm; in impairing the stabilisation capacity, it has a great influence on the dynamics of sediments and associated pollutants with wider implications for the aquatic ecosystems and beyond.

ET02C-5

Triclosan persistence through waste water treatment plants and its potential effects on fluvial systems

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Numerous chemicals are continuously released into the environment as a result of their use in industry, agriculture or household activities. The so-called emerging compounds are thought to be potential threats to environmental ecosystems. Among them, triclosan is a commonly used bactericide that survives several degradation steps in wastewater treatment plants (WWTP) and potentially reaches fluvial ecosystems. Moreover, in Mediterranean areas where water scarcity results in low dilution capacity, the potential environmental risk of triclosan increases.
A set of experimental channels was used to examine the short-term effects of triclosan (from 0.05 to 500 µg/L) on biofilm algae and bacteria. Environmental concentrations of triclosan caused an increase of bacterial mortality (EC10= 0.6µg/L). The photosynthetic efficiency of algae was inhibited (EC10= 3.4µg/L), as well as the non-photochemical quenching mechanisms (EC10= 1.3µg/L). Diatom cell viability was also affected with increasing concentrations of triclosan (EC10= 3.7µg/L).
Triclosan toxicity was also evaluated when applied in binary mixtures with i) the phenylurea herbicide diuron and ii) the pharmaceutical product propranolol (β-blocker) on the green algae Scenedesmus obliquus. The toxicity of the first mixture was accurately predicted with the current available models. However, these models underestimated the joint toxicity when triclosan was mixed with propranolol. This mixture had a greater negative impact that predicted by models, showing a synergistic effect.
Although triclosan is present at low concentrations in fluvial systems, the capacity of triclosan to survive through WWTP processes, the toxicity detected on the co-occurring non-target components (algae) of the biofilm community and the increase in toxicity when mixed with other substances demonstrates that triclosan may be toxic to fluvial communities. The results obtained highlight the need of studies covering both single exposures and mixtures in order to assess the environmental risk of emerging pollutants.

ET02C-6

Tertiary treatment methods reduce the ecotoxicity of wastewater for Gammars fossarum (Crustacea, Amphipoda)

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Among other chemicals, pharmaceuticals and personal care products enter the aquatic environment via treated wastewater and are thus frequently detected in surface waters. Tertiary treatment methods, like ozonation, pulverized activated carbon (PAC) and titanium dioxide in combination with ultraviolet (UV) irradiation, are considered to be suitable to reduce the load of those contaminants (=micropollutants) in wastewaters. However, these treatment methods were hardly investigated regarding their potential to alter the ecotoxicity of wastewater. Therefore, we sampled secondary treated wastewater from two wastewater treatment pants (WWTPs) and treated each of the samples with these tertiary methods at the lab-scale. Subsequently, the amphipod shredder *Gammarus fossarum* was exposed to secondary treated wastewater, wastewater additionally treated with ozone, PAC or TiO_2 & UV, and river water (control) for seven days under laboratory conditions (n=20). Gammarids exposed to secondary treated wastewater from both WWTPs showed a significantly reduced (<50%) feeding rate compared to river water. Ozonation as well as the combination of TiO_2 with UV-irradiation reduced the ecotoxicity compared to secondary treated wastewater for *G. fossarum* displayed by a significantly (twofold) increased feeding rate. In contrast, the application of pulverised activated carbon in secondary treated wastewater, did not cause a significantly reduction of the adverse effects related to secondary treated wastewater. However, following the amendment of nutrients to PAC-treated wastewater the feeding rate was at the control level suggesting that activated carbon also removes trace elements from wastewater overriding the positive effect of the micropollutant removal. Finally, all three tertiary treatment methods assessed in the present study seem to be suitable to reduce the ecotoxicity of secondary treated wastewater and hence may have the potential to improve both the chemical and ecological status of surface waters as required by the Water Framework Directive.

ET04 - Ecologically relevant endpoints

ET04-1

In situ feeding assay with Gammarus fossarum: move forward to an ecologically relevant biomonitoring of water chemical quality

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As shown by many laboratory studies since 1990s, a large range of chemical stressors can inhibit gammarids feeding rate. Feeding inhibition also constitutes in most case one of the first traits observed in responses to environmental pollutions. Studying the effects of pollutants on the feeding behaviour is of ecological concern; as it can be related to life history traits like growth, survival or fertility thus allowing assessing the effects of toxicants on higher biological organization levels, such as population. A mechanistic modelling approach is proposed as a perspective to perform this extrapolation between biological scales.
Our work was focused on the crustacean Gammarus fossarum widely present in European rivers. In a first part, we illustrate how taking into account of the influence of biotic and abiotic factors

(body size, temperature, water hardness) on feeding activity allows one to improve the interpretation of in situ feeding rate assays for the evaluation of water quality. For this, we performed a three-steps approach: (i) we characterized the influence of these important confounding factors in laboratory conditions, (ii) we validated the robustness of feeding activity reference values; these latter were established through in situ caging experiments with transplanted standard organisms in reference streams with contrasted abiotic profiles at different seasons (iii) finally, by considering in situ caging in contaminated streams, we underlined the importance of taking into account the influence of such factors for a better toxicological bio-monitoring of freshwater ecosystems.
In a second part, we show that feeding activity can be linked to life history traits such as fecundity and we propose a modelling methodology to link impact on feeding activity to potential effects at the population level, taking into account the influence of environmental conditions. For this purpose, we developed an environmentally realistic Leslie population model, which allowed us to link individual-level demographic parameters to population dynamics. Ultimately, we illustrate how couple fitness related endpoints measured in bioassays procedures to population modelling.

ET04-2
Effects at a daily resolution of imidacloprid on the individual feeding activity of Gammarus pulex (L.)
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Ecosystems are usually exposed to low concentrations of toxicants. Environmentally-relevant concentrations of toxicants rarely cause mortality. Consequently, observing behavioural changes of organisms evoked by relatively short, low concentration stress in ex-situ studies is a useful tool. Differences between individuals in the endpoint of interest can be equal or even greater than the effect caused by the toxicant, making it necessary to reduce the variability by, for example, focusing on a sub-group of the test species.
A method to measure the feeding activity of Gammarus pulex on a daily basis was developed, taking into account the intraspecific variability caused by food source and infection with acanthocephalan parasites (exp.1) and body mass (exp.2). The method was used to observe the influence of the insecticide imidacloprid on feeding activity in a chronic exposure test (exp.3).
In the present study, the impacts of parasite infection as well as the influence of food quality separately were significant. Parasite infection and lower food quality together yield an even stronger reduction of the feeding rate of Gammarus pulex in relation to uninfected and optimally-fed organisms. In terms of size dependence of the feeding rate, the results of this experiment agreed with those in the literature. Furthermore it could be demonstrated that daily measurement of feeding activity on an individual level was feasible, but there were some unidentified factors which reduced the feeding rate over time.
The observed effect concentration for imidacloprid for an exposure duration of four days was 20 to 380 times lower than the lethal concentration. Furthermore, the effect concentrations were within the range of measured and estimated environmental concentrations.
Intra-specific variability makes it difficult to study sub-lethal effects on an individual level and experimental design should minimise variability wherever possible. It is known that lethal and sub-lethal impacts can still be seen after the toxicant is removed from the exposure medium. In this experiment, a secondary effect (higher feeding rate in previously impacted individuals) also persisted beyond the time when the toxicant was eliminated from the water phase. It is essential that experimental duration should be sufficient to study such compensatory effects.

ET04-3
Non-guideline studies refine and improve the aquatic risk assessment of forest insecticides imidacloprid and neem
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Most pesticide risk assessment frameworks include a problem formulation phase in which relevant measurement and assessment endpoints are identified. Functional attributes or measurement endpoints are not typically used in risk assessments under FIFRA (USA) or the PCPA (Canada) because it is generally accepted that protection of structural endpoints will protect ecosystem function. However, we provide two examples where non-guideline laboratory and outdoor mesocosm studies detected significant alterations to population and community functional attributes at realistic concentrations that would not likely have been predicted from standard or tiered toxicity testing. These studies were conducted in the context of synthetic (imidacloprid) and natural (neem) forest insecticides. Realistic aqueous concentrations of neem (azadirachtin) in outdoor mesocosms selectively reduced a key group of macro-crustaceans and the resulting zooplankton community alterations were sufficient to induce measurable changes in whole ecosystem metabolism. Realistic foliar concentrations from systemic applications of imidacloprid in laboratory mesocosm studies significantly reduced organic matter decomposition rates through sub-lethal feeding inhibition effects on obligate leaf-shredding aquatic insects. No such effects were detected for realistic foliar concentrations of neem (azadirachtin). We show how hypotheses generated by combining known ecological, use pattern, and exposure scenario information were used to direct adaptive, non-guideline studies for assessing risk to aquatic communities. These non-guideline studies can provide ecologically-relevant ancillary data for regulatory guideline data submissions to increase environmental realism, reduce uncertainties, and improve an overall risk assessment for forest insecticides.

ET04-4
Effects and environmental consequences of U exposure on the fish Danio rerio
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Exposure to metals can cause a decrease of reproductive capacity in fish. These endpoints may have serious implications for normal population dynamics and community structures. Uranium's environmental prominence is currently increasing because of new mining and milling activities ([U] in water: 200-3000 µg.L-1). Hence, the objectives were to measure the effects of U in egg (survival rate) and adult stages (reproduction output, fertilization, fecundity) of the fish Danio rerio after short and long exposure duration and to quantify the impact on live-history traits of fish. They could allow to develop/valid toxicity tests more accurate for the environmental risk assessment.
No mortality in eggs and no disruption of the main embryo development stages were observed after direct 96h-U exposure. However, eggs were significantly affected by the U exposure; effects of U on hatching success starting at the concentration of 300 µg.L-1 causing a delay in hatching time.
Effects on the adults, 20d of exposure reduced the number of egg spawned (x5) although no egg was spawned after the 200d of exposure. For the 20 µg.L-1 exposure, the number of eggs was reduced by 3-2.5. Moreover, the viability of eggs was reduced (21%) after 200d. In the case of whole life cycle experiment, sex ratio, fecundity, viability and the ability to spawn were widely modified by U exposure. The sensibility of adult U response was widely influenced by the age at the beginning of the exposure modalities.
A simple population model included these endpoints were used to evaluate the U effects. Dynamic of population was altered by uranium exposure. As expected, the most representative test (long term of exposure/whole live cycle) will provide better information on the environmental risk of U exposure.

ET04-5
Impact of pesticides in small streams in central Germany - Effect of pesticides on macroinvertebrate drift, emergence and feeding rates of Gammarus pulex
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The influence of pesticides on aquatic stream communities was investigated in an intensively used agricultural landscape in central Germany. Pesticide exposure was examined at 10 sampling sites at a total of seven streams using event controlled runoff sampling systems and suspended particle samplers (SP samplers). Samples were taken following heavy rainfall events and analyzed for 14 pesticides. In order to identify the risk to aquatic communities, the macroinvertebrate drift (drift net; n=1), insect emergence (emergence tents; n=4) and feeding rates of *Gammarus pulex* in in situ bioassays (n=20) were assessed at each sampling site.
The water samples of two streams as well as most SP samples contained insecticides at ecotoxicological relevant concentrations. The macroinvertebrate drift of *Gammarus pulex* and *Limnephilus lunatus* was high following a rainfall-induced input of the insecticide lambda-cyhalothrin (0.29 µg/L) at one sampling site. The feeding rate of *Gammarus pulex* was also reduced following this rainfall event. Moreover at a further study site, the insecticide lambda-cyhalothrin (SP: 53.2 µg/kg) was found to be responsible for an increase of the macroinvertebrate drift rate.
Significantly reduced feeding rates in in situ bioassays were identified at two sites ($p = 0.01$ and $p = 0.006$, respectively), which correlated with an input of insecticides to both streams. The influence of physico-chemical parameters (pH, conductivity, water temperature and oxygen content) on the feeding rate was excluded through the use of correlation analysis. No effect of the pesticides on the insect emergence rates was visible since other factors had greater impact (short examination period, difference in appearance of species during the season, temperature).
The observed reduced feeding rates of *Gammarus pulex* in in situ bioassays and the increased drift rates show the impact of pesticides on small streams. The amphipod *Gammarus pulex* plays an important role within stream ecosystems as a detrivore, and the disturbance of this species can have significant effects upon both the invertebrate communities and the stream ecosystem as a whole. Appropriate risk management measures can minimize the observed effects of pesticides. Furthermore positive effects to the aquatic communities are expected from improvements of in-stream structures.

ET04-6
Cause-effect relations of key pollutants on the European rivers biodiversity: keybioeffects
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KEYBIOEFFECTS was a Marie Curie Research Training Network (RTN) aiming to gain a deeper understanding of the anthropogenic toxic compounds released in European water bodies. While environmental risk assessment is commonly based on Priority Pollutants (PPs) data and toxicity results obtained from standardized toxicity tests, Keybioeffects research was focused on several knowledge gaps: identification, toxicity, fate and monitoring of emerging compounds and transformation products; bioavailability, trophic transfer, direct and indirect effects of chemicals on natural populations and communities and the evaluation of the environmental effects of chemical pollution in several European river ecosystems.
In this presentation, the main research findings will be summarized and a few examples presented in detail to illustrate our research output and open questions. Overall, the results obtained demonstrate that chemical pollution, including priority and emerging compounds as well as non-identified toxic compounds, is posing a risk to the ecological integrity of European rivers. Furthermore, sampling strategies, experimental approaches, analytical and bio-analytical methods applied or developed within the network are recommended as innovative and sensitive tools to better address the environmental risk of pollutants and identify the causes of ecological deterioration as the European Water Framework Directive requires.
Acknowledgement - EU project: KEYBIOEFFECTS (MRTN-CT-2006-035695).

ET05 - Ecosystem exposure, toxicity pathways and adverse health outcomes

ET05-1
Effects of ingestion of fungicide- and insecticide-coated seeds on red-legged partridge (Alectoris rufa) health
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Pesticide application is suspected to be a major cause of population decline in farmland birds. Seeds coated wit fungicides or insecticides constitute a main part of the diet of species such as the red-legged partridge (*Alectoris rufa*) during sowing seasons. The aim of this study was to test the effects of coated seed ingestion on *A. rufa* physiology and general fitness. We tested an insecticide (imidacloprid) and two fungicides (difenoconazole and thiram). Each experiment consisted in two groups of six pairs of partridges each: the low dose consisted in seeds treated with the recommended dose and the high dose in seeds treated with a concentration twice the recommended one. A third group was fed with untreated seeds and used as control. Seeds administration was prolonged for a 10-day period, after which all animals were transferred to an untreated diet and monitored for an additional 12 days. Right after the exposure and after the monitoring periods we took body mass, ventilation rate, beak and eye ring pigmentation, hematocrit, plasma testosterone and estradiol levels, vitamin and carotenoid profiles and oxidative stress parameters (TBARs, GPx, SOD and GSSG/GSH ratio). Immunocompetence was analysed at the end of the experiment by testing cellular (PHA test) and humoral (SRBC-agglutination test) responses. High concentrations of imidacloprid and thiram caused 58% and 42% mortality, respectively, whereas >80% of controls survived. Sex-dependent lethal effects were detected for thiram, being males more affected than females. Low concentrations induced significant weight loss, although survivors recover quickly once the exposure was terminated. These low concentrations also induced oxidative stress. SOD was significantly stimulated by thiram ($F_{2,28}$=7.539; p=0.002), whereas imidacloprid induced the activity of GPx ($F_{2,22}$=6.651; p=0.006). Imidacloprid was the only immunosuppressive pesticide, impairing the cellular immune response ($F_{2,25}$=4.809; p=0.017). Beak redness was reduced by the low imidacloprid concentration, although this effect was statistically significant only for females ($F_{2,14}$=4.036; p=0.041). The two fungicides also affected ornamentation by reducing the percentage of eye ring pigmentation (thiram: $F_{2,32}$=6.158; p=0.005. difenoconazole: $F_{2,32}$=4.245; p=0.023). Coated seed consumption may compromise health of farmland birds, especially during seasons when little alternative food sources are available in the field

ET05-2

Ecosystem exposure and adverse human health effects of POPs: a data analysis approach by a discrete mathematical method

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It has been evident for decades that environmental chemicals pose an enormous risk to the environment as well as to humans. However, this important topic is still underestimated in every part of society. The effects of environmental contaminants on health are a major concern because exposure is associated with the alteration of human sex ratio, as well as with a number of diseases, including cancer, diabetes, congenital malformations and infertility, etc.
It is clear that there is increasing pressure to intensify the research and to more efficiently evaluate the data on persistent and bioaccumulative chemicals in the environment as well as in human bodies.
In our data-analysis approach we investigated data sets of breast milk samples of women in Denmark and Finland which contained measurable levels of 32 Persistent Organic Pollutants (POPs). In each country the breast milk of 65 women was analysed. Therefore we have to evaluate a data matrix with 32 rows (the chemicals) and 65 columns (the breast milk samples of 65 anonymous women). Out of the 65 samples (complete data matrix) in each country we selected two different subsets according to healthy boys and boys with cryptorchidism, a malformation of the testis. Furthermore, we investigate if and how the physical-chemical properties logPow and BCF are associated with the contamination of human breast milk samples in 32 selected POPs.
The data-analysis method is based on the theory partially ordered sets. The theory of partial order is a discipline of Discrete Mathematics. An overview can be found in a book edited by the second author. The graphical representation of partial orders is laid down in so-called Hasse diagrams. The software package applied is named PyHasse, written by the second author. This software package is still under development. However, it can be obtained by the second author.
In the Hasse diagram technique the ordering of the chemicals, their different positions in the Hasse diagrams and the quantification using partial order theoretical method are, however, new and important amendments in the findings of chemicals influencing the state of health of human beings. The comparisons (similarity analysis) of data sets of Danish and Finnish breast milk samples (healthy, cryptorchidism) with logPow/BCF of the 32 chemicals reveals similar structures of the diagrams. It shows that the physical chemical properties logPow and BCF are good indicators for the ranking of the used POPs.

ET05-3

Transcriptomic and enzymatic approaches on the freshwater fish Gobio gobio exposed to two PCBs

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The production of polychlorinated biphenyls (PCBs) is forbidden since the 1980's but, due to their high stability in the environment, these molecules are nowadays ubiquitously distributed in freshwater and more particularly in sediments. Moreover, PCBs have the capacity to biomagnify in the aquatic trophic webs. The use of transcriptomic in ecotoxicology allows to observe the effects of PCBs at cellular and molecular levels. This can provide some early predictive indicators of these pollutant effects. Some fish species are commonly used in ecotoxicological studies at transcriptomic level but few are fishes living close to the sediments. The gudgeon Gobio gobio is a benthopelagic freshwater fish, common in European rivers, which is exposed to the pollutants contained in rivers water and sediments.
The purpose of this study was to evaluate the effect of two PCBs on the mRNA expression and the enzymatic activities of gudgeon detoxification systems.
In first step, the gudgeon coding sequences of cytochromes P450 (CYP) 1A, pi-class glutathione S-transferase (pi-GST), selenium-dependent glutathione peroxidase (Se-GPx) and catalase (CAT) have been identified.
In a second step, gudgeons were exposed to a non-dioxin PCB (PCB 153), to a dioxin-like PCB (PCB 77) and to a mixture of both PCBs. Exposures were performed using food containing four PCB doses (10 ng/g, 100 ng/g, 1 µg/g and 10 µg/g) for 1, 3, 7 and 14 days. The expressions of the four detoxification genes have been studied by real-time PCR in the liver of gudgeons.
The PCB 153 had no effect on CYP1A, pi-GST, Se-GPx and CAT expressions whereas the PCB 77 or the mixture of the two PCBs led to expression variations. For example, an induction of CYP1A expression was observed from the first day of exposure with 1 µg/g of PCB 77.
The measurement of the activities of these detoxification enzymes is ongoing in order to compare the responses at the transcriptional and the enzymatic levels.
This study provides the first data about detoxification responses at both transcriptional and enzymatic levels in the liver of Gobio gobio. These parameters could be used as potential biomarkers for pollutants monitoring.

ET05-4

Specific gene responses of exposure to acetylcholineesterase inhibitors are mediated via nicotinergic acetylchline receptors and elevated intracellular calcium levels

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The identification of chemical-specific gene expression patterns can be used to assign mode of actions to non-characterised or unknown chemicals. In order to exemplarily demonstrate that specific expression patterns can also be obtained for compounds that do not directly interfere with transcriptional regulation, we analysed the effects of the neurotoxic acetylcholinesterase inhibitor azinphos-methyl (APM). Zebrafish embryos exposed for 24 and 48 hours were used as a model. Hierarchical cluster analysis of microarray data and comparison with two non-AChE inhibiting control compounds (1,4-dimethoxybenzene, 2,4-dinitrophenol) revealed a highly specific cluster of APM induced genes. Subsequent detailed analysis of one of the most strongly induced genes, hspb11, indicated that changes in expression were mediated via the nicotinergic acetylcholine receptor (nAChR) and increased Ca2+ levels. For instance, the expression patterns were compliant to those observed for various mutant strains. Furthermore, modulators of intracellular calcium levels such as thapsigargin and caffeine induced hspb11. The data demonstrated the strength of combining transcriptome and functional analyses in the zebrafish embryos to assign gene expression changes to a specific mode of action.

ET05-5

Proteome profiling reveals potential toxicity and detoxification pathways following exposure of BEAS-2B cells to engineered nanoparticle titanium dioxide

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Identification of toxicity pathways linked to chemical exposure is critical for a better understanding of biological effects of the exposure, toxic mechanisms, and for enhancement of the prediction of chemical toxicity and adverse health outcomes. To identify toxicity pathways and networks that are associated with exposure to engineered nanomaterials, an integrated proteomic study was conducted using human bronchial epithelial cells, BEAS-2B and nanoscale titanium dioxide. Utilizing two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS), we identified 46 proteins that were altered at protein expression levels. The protein changes detected by 2-DE/MS were verified by functional protein assays. The identified proteins include some key proteins involved in cellular stress response, metabolism, adhesion, cytoskeletal dynamics, cell growth, cell death, and cell signaling. These differentially expressed proteins were also mapped to identify potential toxicity pathways of titanium dioxide using Ingenuity Pathways AnalysisTM (IPA). Twenty protein canonical pathways and tox lists were generated from the analysis, and these pathways were compared to signaling pathways generated from genomic analyses of BEAS-2B cells treated with titanium dioxide. There was a significant overlap in the specific pathways and lists generated from the proteomic and the genomic data. In addition, we also analyzed the phosphorylation profiles of protein kinases in titanium dioxide-treated BEAS-2B cells for a better understanding of upstream signaling pathways in response to the titanium dioxide treatment and the induced oxidative stress. In summary, the present study provides the first protein interacting network maps and novel insights into the biological responses and potential toxicity and detoxification pathways of titanium dioxide.

ET05-6

Modulation of immune parameters by chemical environmental pressures in wild populations of European bullhead, Cottus sp. from Vesle Basin (Champagne, France)

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In freshwater ecosystems, a large number of chemical substances are able to disturb homeostasis and adaptative responses of fish organisms by modulating different physiological functions including immune system. In order to characterize the early biological effects of environmental contaminants on fish health status, some immune-related cellular and biochemical responses were proposed as immunotoxicological biomarkers. The aim of the present study was to evaluate immune status of European bullhead, Cottus sp., natural populations, living in different field contexts characterized by various chemical environmental pressures. In Champagne region (France), five sites of the Vesle watershed in the North East of France were selected with urban (Muizon), intensive agricultural (Bouy and Prunay) or viticultural (Serzy and Prunay) influences. For each location, adult fish were caught by electrofishing in spring, summer and autumn and splenic leucocyte parameters (leucocyte sub populations, cell mortality and phagocytosis) were carried out by flow cytometry. Fish biometric characteristics, such as length and weight, have no impact on immune parameters. Seasonal variations in immune responses depended on the tested immune function without discrepancy between studied sites. For example, the lymphocyte percentages were higher in summer whereas leucocyte mortalities were strongly increased in spring and phagocytosis percentages in autumn. Nevertheless, independently of seasonal variations, environmental characteristics of each site seem to disturb selected immune markers. The highest immune modulations were observed in sites situated in areas with intensive agriculture or viticulture, Bouy, Prunay and Serzy respectively. These first results may indicate possible immunotoxicological impacts of agri-viticultural practices on bullhead natural populations living in Vesle watershed. It is sure that further observations, on many years, have to be done to verify observed immunomodulations. Moreover, data are needed to better characterize the effects of biotic and abiotic confounding factors on these biomarker base levels and to define their natural variability ranges for assessment of wild fish health.

ET06 - Ecotoxicology of amphibians and reptiles

ET06-1

Multi-endpoint assessment of the effects of chlorpyrifos ingestion in a lacertid lizard (Podarcis bocagei)

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Lizards are among the least studied groups in ecotoxicology, and despite a recent increase in the number of studies, there is still a lack of knowledge regarding their response to environmental contamination. In Europe, lacertid lizards have been identified as potential model species for reptile ecotoxicology. Chlorpyrifos is one of the main organophosphorus insecticides used in agricultural areas and has been reported as moderately toxic and a cholinesterase inhibitor. Adult male *Podarcis bocagei* (n=36) were captured in a non-contaminated site and exposed under controlled conditions to a commercial formulation of chlorpyrifos (CICLONE 48 EC - 480 g/L, SAPEC AGRO). Individuals were assigned to three treatments: control (dH2O), CPF1 (96 ng cpf/meal) and CPF2 (960 ng cpf/meal). The CPF was injected into live mealworms, which were provided as meals every second day over a 20-day period. After 20 days feeding, 18 individuals (6 per treatment) were fasted and subjected to individual performance tests and the remaining were sacrificed and dissected for biochemical assays, histopathological analysis in liver and testes and hemoparasite prevalence/intensity. There were no significant differences regarding running speed between treatments. In the predatory behavior experiment there was a tendency for exposed animals to take more time to subdue and swallow the prey. There were no statistical differences in the proportion of lizards with hemoparasites. Exposition to chlorpyrifos did not produce any variation in stress oxidative enzymes regarding the control group in any of the studied tissues. Esterase activity was significantly inhibited by chlorpyrifos with CbEs having a higher sensitivity than AChE. In general, no histopathological changes were observed in livers of treated animals, although CPF2 exposed individuals presented higher levels of congestion. Seminiferous tubule diameter was not different and all spermatogenic stages were present in control and treated individuals. A tendency for larger lumen was observed in treated individuals. When exposed to environmentally relevant doses of chlorpyrifos *P. bocagei* displayed carboxylesterase and acetylcholinesterase inhibition in all tissues examined. These biomarkers of exposure were accompanied by behavioral symptoms of neurotoxicosis, including reduced capacity to respond to prey cues. The implication of these studies is that environmental exposure to chlorpyrifos may affect individual fitness.

ET06-2

Effects of field-exposure to agricultural pesticides on thyroid and on testis in the lizard, Podarcis bocagei: A histopathological study

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The use of pesticides in agriculture is a source of environmental pollution in these environments. Many wildlife species use agriculture fields as habitat and are routinely exposed to these chemicals. Lacertid lizards are an important group of reptiles occupying agricultural landscapes in Europe that have been identified as potential model species for reptile ecotoxicology; however, little is known about the effects of pesticides on these animals. Many pesticides can have endocrine effects observable within organs associated with reproduction, growth and development (e.g. gonads, thyroid). The aim of this study was to evaluate the effects of field-exposure to pesticides on the thyroid gland and on testis function. Adult male lizards were captured in north-western of Portugal, within corn fields treated with pesticides and from agriculture fields designated for biological agriculture and which therefore did not use pesticides. All animals were collected during autumn of 2009. Animals were sacrificed and thyroids and testis were removed for histological analysis. Thyroid histology indicated that exposed animals displayed follicular lumens with more reabsorption vacuoles and a significant difference in follicular area (when compared to thyroids taken from reference animals) On the other hand testis histology indicated that testicular diameters among exposed animals were significantly larger in exposed animals compared to testes taken from reference animals. These results suggest that the complex mixture of pesticides that animals were being exposed in the field had an effect on thyroid homeostasis and on spermatogenesis and may indicate that one or a combination of pesticides are acting as endocrine disruptors among these populations of P. bocagei.

ET06-3

Effects of Roundup® on the protein expression patterns in the liver and brain of metamorphosing common frog Rana temporaria

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The worldwide decline of amphibian populations, reported in a large variety of habitats, is often linked to destruction, disturbance, and fragmentation of the habitat. But the environmental presence of chemical compounds such as pesticides has been considered as a possible factor contributing to the reported decline. One of the widely used herbicides is glyphosate which is, among other things, formulated as Roundup®. Up to now, little information is known about the toxicological effects of Roundup® on amphibians. Since the understanding of the mechanisms by which this herbicide may interact with the normal gene expression is of special interest, we evaluated the potential effects of this relevant environmental pollutant on the protein expression in the brain and liver of metamorphosing *Rana temporaria*. To achieve this goal, tadpoles at stage 28 (Gosner index) were exposed to either 1mg or 10µg a.e./L of Roundup® until they reached metamorphosis at stage 44 (Gosner index). For the proteomic analysis, a 2D-DIGE minimal labeling approach coupled to nano flow liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) was applied. When considering the liver, proteins identification suggests that Roundup® may impair mechanisms such as the energetical metabolism (glycerol-3-phosphate dehydrogenase, transaldolase), cellular cycle regulation (60S acidic ribosomal protein P0), hepatic proteolysis (monomeric alpha-macroglobulin), mitochondrial function (carbamoyl-phosphate synthase), and hepatic haem biosynthesis (coproporphyrinogen oxidase). When considering the effects of Roundup® on the brain, results showed that calcium signalling (Annexin A5), neuronal differentiation (Dihydropyrimidase - like3, Dihydropyrimidinase-related protein 5), cellular cycle regulation (Membrane protein-palmitoylated, ndrg2 protein), cytoskeleton (Microtubule-associated protein RP/EB family member 1, Alpha-tropomyosin, Putative actin-capping protein Z beta subunit variant 1, vinculin[3DOTS]), ions transport (ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A 5), and proteins splicing (Splicing factor, arginine/serine-rich 9) were targeted by Roundup®. In conclusion, we can say that our data demonstrate that environmentally relevant exposure to Roundup® can deeply modify amphibian proteome and argue that these changes have to be taken into account while estimating the toxicological hazard of wild amphibian populations exposed to those chemicals.

ET06-4

The role of metabolization processes in micropollutants bioaccumulation by adult green frogs: comparison of benzo[a]pyrene and fipronil toxicokinetics

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The implication of micropollutants (MP) in the global amphibian biodiversity decline is nowadays well accepted. However, there is a paucity of data concerning the biophysico-chemical interactions of MP with adult frogs. The aim of this study is to compare the toxicokinetics of benzo[a] pyrene (BaP) and fipronil (fip), two MP deferring by their origin (combustion vs agriculture) and by their chemical properties ($K_{ow,BaP}$=6 vs $K_{ow,fip}$=2.8). Frogs were exposed directly in water for 8 days to low concentration of BaP or fip (10 ppb), containing low quantities of respective radiolabelled molecules. The short-term uptake rates we obtained were equivalent for the two MP. In addition, over the 8 days of exposure, the bioaccumulation factors (BAFs) obtained for fipronil were 2 to 3-time higher than those measured for BaP. These results contradicted with the well accepted consensus for which an increase in K_{ow} led to both an increase in uptake rate and an increase in BAF, and could be the consequence of a better metabolization capability of the frog enterohepathic system for BaP if compared to fip. The results we obtained for MP distribution within the organs, the polarity of the metabolites formed, assessed by thin layer chromatography, as well as the induction of the detoxification enzymes (P450 and GST) in liver (and kidneys) after exposure to BaP or fip comforted this hypothesis. In the light of the findings, potential reproductive dysfunction and embryos development impairment leading to decreased-fitness of amphibian populations are discussed.

ET06-5

Consequences of biofuel crop expansion for amphibian diversity in tropical agroindustrial landscapes

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The dawn of a new paradigm in energy supply - biofuels - points to the continued expansion of industrial agriculture in the near future. This expansion could have excellent short term economic benefits for tropical countries, but would have to be carefully planned and monitored as industrial agriculture is one of the most important vectors of habitat destruction and degradation worldwide. This study proposes to test the hypothesis that the expansion of soybean and sugarcane, the two most important biofuel crops in Brazil, is associated with significant changes in amphibian community composition, diversity and structure. This hypothesis was tested by means of field surveys in water bodies distributed across a gradient of environmental degradation, i.e., that comprised of native habitats, pastures and plantations, in a soybean-dominated landscape in Southern Amazon and in a sugarcane dominated landscape in southeastern Brazil. Overall, we found a strong signal of land use on amphibian communities both in sugarcane and soybean landscapes, with amphibian community composition in plantations being a subset of that in pastures. Considering that both plantations and pastures are structurally degraded, but that plantations are in addition exposed to a diverse array of agrochemicals, this observation is consistent with a role for environmental contamination in influencing species distributions. The observation of larval amphibian dieoffs in ponds in sugarcane fields, and of strong community level effects in ponds in soybean plantations, both consistent with the timing of application of pesticides, renders further support to this hypothesis. The hypothesis of a link between pesticide contamination and amphibian performance at the individual, population and community level is now being tested by a research program combining further field sampling, and experimentation in laboratory, mesocosm and field.

ET06-6

Atrazine and glyphosate in the environment and its implications for amphibians

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Herbicides are the top selling pesticides on a global basis. Glyphosate and atrazine are the highest selling herbicides today. Initially registered by Ciba-Geigy in 1958, the triazine herbicide atrazine which inhibits photosynthesis. Monsanto Corp. began to commercially produce glyphosate in 1974. Its mode of action is to disrupt amino acid production in plants. These compounds were registered and their toxicities to organisms have been extensively reviewed without regard for the potential impacts on amphibians or ecosystems in general until recently. The effects of atrazine on amphibians have received wide attention in recent years to the extent that the use of atrazine has been reviewed within the USA based solely on its potential to affect gonadal development in amphibians. The ecosystems and food webs inhabited by herpetofauna may also be altered by atrazine and glyphosate. Our field studies in Canada, indicate that even hatching of amphibian eggs may be affected by atrazine exposure, combined with other pesticides. In the south Okanagan valley, Canada, is a classic location where high biodiversity and human development meet. The intensive use of pesticides combined with loss of habitat and degradation of habitat due to other factors led our team to counter by creating new habitats for amphibians with conservation covenants in the hope that this will preserve the many species at risk that depend on water in this desert region.

ET07 - Endocrine disrupting chemicals in the environment

ET07A-1

Freshwater mudsnail estrogen receptor - identification and regulation

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Molluscs are raising attention as ecotoxicological test organisms due to their high diversity and importance in aquatic ecosystems. The ovoviviparous prosobranch gastropod *Potamopyrgus*

antipodarum (freshwater mudsnail) responds very sensitively to xenobiotics and has therefore been proposed as OECD standard test organism. Endocrine disrupting chemicals influence the reproduction of *P. antipodarum* which can be assessed by embryo numbers in the broodpouch. However, the knowledge about the endocrine system of *P. antipodarum* is rather limited. The aim of this study is to identify an estrogen receptor in the endocrine system of *P. antipodarum* and to investigate if this receptor is differentially expressed under different (xeno-)hormone exposures (i.e. 17α-ethinylestradiol, bisphenol A and 17α-methyltestosterone).
The DNA-binding domain of the identified ER-like transcript has an amino acid identity of 92% compared to the ER of the gastropod *Nucella lapillus* (84% to human ERα) and 83% in the ligand-binding domain (38% to human ERα). Furthermore, the *Potamopyrgus*-ER is transcriptionally regulated as proofed by quantitative Real-Time PCRs of (xeno-) hormone exposed snails.

ET07A-2

Quantification of vitellogenin by mass spectrometry (LC-MS/MS) in the freshwater amphipod, Gammarus fossarum : a potential endocrine disruption biomarker

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Induction of vitellogenin (Vg) in males or juveniles is a well known effect of xenoestrogenic contaminants in fish, and has been extensively used as a specific endocrine disruption biomarker. Despite their obvious ecological importance, relatively few works have been carried out on invertebrates and consequently few tools are available to diagnose an endocrine disruptor exposure in these species. At present, only indirect methods, such as the organic alkali-labile phosphate (APL) assay, have been proposed and applied in invertebrates species. From an analytical point of view, efforts should also be directed toward developing more specific methods to measure Vg levels in invertebrates.
This study focuses on the development of a new Vg quantitative assay in an ecologically relevant freshwater invertebrate, Gammarus fossarum, using liquid chromatography tandem mass spectrometry (LC-MS/MS). For this end, we followed an approach in three steps, i) we developed a quantitative SRM (Single Reaction Monitoring) assay for the detection of proteotypic Vg peptide, ii) the specificity of this proteotypic peptide as indicator of the functional Vg was validated, by assessing its natural variability during the reproductive cycle of female organisms and iii) the use of this approach as biomarker was evaluated, by studying the modulation of the proteotypic peptide in gammarids exposed to known endocrine disruptors. From this study, an analytical method allowing an absolute quantification of vitellogenin in G. fossarum was developed. The use of this assay as a specific endocrine disruption biomarker was validated by assessing the modulation of vitellogenin levels in gammarids females exposed to the 20-hydroxyecdysone and the methyl-farnesoate.

ET07A-3

H295R cells as a steroidogenic model: a broader picture using simultaneous chemical analysis of 7 key steroid hormones exposed to 3 endocrine disruptors

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Much effort has focused on substances interfering with hormone function, and more recently research on substances that interfere with the natural production of hormones has developed. Important work in this field is the validation of the H295R cell line as a steroidogenic model [1].
In the present study prochloraz, ketoconazole and genistein were tested as previous studies have given inputs to understanding where in the steroidogenic pathway they interfere. However, by applying simultaneous chemical analysis of 7 key steroid hormones in the pathway, a better understanding of the rate limiting steps can be achieved. Furthermore this method negates the problems of cross-reactivity encountered in immunoassays.
The H295R steroid hormone synthesis assay (http://www.oecd.org/dataoecd/56/11/44285292.pdf) was performed (with minor modifications) and analyzed according to a simplified version of the method described by Hansen et al [2]. In short 3 pooled wells of cell medium was added internal standards and concentrated on C18 solid phase extraction cartridges. Steroids were then derivatized with TMS and analyzed using GC-MS-MS.
Although all 3 test compounds also leads to decreases in testosterone and 17beta-estradiol as previously described in the literature, effects are observed on all other analyzed steroids (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione and estrone). This study also demonstrate that the steroidogenic pathway is disturbed at different stages by the 3 test compounds. Applying the analytical method used in this study to measure hormone production in the H295R cell line offers significant advantages compared to immunoassays.

- Accurate simultaneous hormone baseline measurements of seven key hormones (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, estrone, testosterone and 17beta-estradiol)
- No cross reactivity
- Differentiation of where the steroidogenic pathway is affected by test compounds

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ET07A-4

Evidence of feminization in wild Elliptio complanata mussels in the receiving waters downstream of a municipal effluent outfall

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The endocrine-disrupting activity of municipal effluents has the potential to induce feminization and alter the reproductive systems of aquatic organisms. The purpose of this study was to examine the sex ratio, vitellogenin (Vtg)-like proteins, serotonin, arachidonate cyclooxygenase (COX) activity and dopamine status in wild mussels living at sites upstream and downstream of two municipal effluent outfalls in the Mille-Iles River (Quebec, Canada). Gonad integrity was also studied by monitoring the gonado-somatic index (GSI), the activity of the rate-limiting enzyme aspartate transcarbamoylase (ATC) for purine synthesis, and changes in lipid peroxidation (LPO). The results showed that the proportion of females was dramatically increased from 25-35% at the upstream sites to 80% at the downstream sites. The levels of Vtg-like proteins were significantly elevated in the male mussels only. Male mussels downstream of the municipal effluent plumes expressed female-specific protein bands (Vtg-like), as determined by high-resolution gel electrophoresis and silver staining. The serotonin/dopamine ratio was significantly decreased in the downstream mussels, indicating that the gonad was in a state of early vitellogenesis. However, this change was not accompanied by changes in ATC, suggesting no significant egg production; this was confirmed by the observation that the downstream mussels displayed significantly low GSIs. Indeed, GSIs were rather dependent on the serotonin/dopamine ratio ($r=0.44$; $p<0.001$), while Vtg-like proteins were dependent on dopamine levels ($r=0.5$; $p<0.001$). The increase in COX activity at the downstream sites and its close relationship with increased serotonin levels suggest a concomitant serotonergic signalling in addition to VTG production. The production of Vtg-like proteins combined with the serotonergic effects of the municipal effluents was associated with oxidative damage (LPO) in the gonad. This study provides the first evidence of feminization in wild mussel populations and the disruption in gonad physiology by exposure to municipal effluents.

ET07A-5

Effect directed analysis of estrogenic effects in sediments of the river Elbe

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The aim of the present study was, to comprehensively identify substances that are responsible for the estrogenic effect of an environmental sediment sample taken from the river Elbe / Germany. The estrogenic effect of the organic sediment extracts was determined using the Yeast-Estrogen-Screen (YES). The sample was fractionated by liquid chromatography for effect directed analysis. The composition of estrogen-active fractions was further investigated by GC-MS and high resolution LC-MS analysis. Identified substances were tested in the YES and additive effects of the mixture were calculated using the concept of concentration addition (CA). The composition of the environmental sample was rebuilt with pure compounds based on the data of the chemical analysis in order to assess the partition of estrogenic activity caused by the identified compounds. The organic sediment extract showed an estrogenic potential of 1,9 ± 0,4 ng/g ethinyl-estradiol-equivalents (EEEQ) in the sediment. We were able to identify 13 substances by effect directed analysis of which eight had xenoestrogenic properties. The most prominent were 17-beta-estradiole and estrone, but alkylphenols, bisphenol-A and stigmasterol could be found as well. Chlorophene (o-benzyl-p-chlorophenol) - a widely used antiseptic that was also identified in the sediment extract - has xenoestrogenic properties in the YES that are in the range of other xenoestrogens like 4-n-Nonylphenol. This is the first report on chlorophene acting as a xenoestrogen. Furthermore, the estrogenic potentials of mixtures comprising of both, xenoestrogens and non-active substances were analyzed. The results indicate a substance-specific modulation of the estrogenic effect by compounds that are present in the analyzed sample and do not stimulate the human estrogen receptor by themselves. As one example the non-active compound tocopherol enhanced in a concentration dependent matter the estrogenic potential of the technical mixture of nonylphenols whereas the potential of bisphenol-A was unaffected. This finding indicates the importance of mixture effects and underlines the need for bio-assays for the integrative analysis of environmental samples.

ET07A-6

In vitro bioassays to monitor estrogenic activity of surface and drinking waters in Brazil

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Estrogenic Endocrine Disrupting Chemicals (EDCs) are compounds able to cause adverse effects to humans and wildlife, and have frequently been detected in the environment. Increasing efforts from governmental sectors and the scientific community are devoted to the development of methods to quantify these compounds and predict the risks they pose to humans. The aim of this work was to evaluate selected EDCs of raw and treated waters in Brazil with different levels of pollution, using chromatography-tandem mass spectrometry (LC-MS-MS) and verify their potential biological effects via in vitro yeast bioassays. Two different bioluminescent yeasts containing the human estrogen receptor were employed. One of the strains contains the *luc* from a firefly and the other the *lux* genes from bacterias (BLYES) as reporters of the estrogenic activity. All raw waters presented endogenous and/or xenoestrogens and showed estrogenic activity in at least one sampling. Estrogenic compounds and estrogenic activity were not detected in treated waters. All samples containing estrogenic compounds presented estrogenic activity in the BLYES method but not in the luc gene based method. In general, the concentrations of target-compounds determined by chemical analysis did not explain the biological responses observed. Because of its simplicity, low cost and good sensitivity, the biological approach showed to be appropriate for monitoring raw and drinking water, indicating hot spots events to be further chemically characterized.

ET07B-1

Triclosan does not alter secondary sexual development or steroidogenesis in juvenile silurana tropicalis

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The impact of the anti-bacterial agent, triclosan (Irgasan® or TCS), on gonadal steroidogenesis during sexual differentiation in Silurana tropicalis was examined. In previous studies, TCS did not alter the normal course of thyroid-mediated metamorphosis in Xenopus laevis, or alter reproductive fecundity or endocrine status in adult fathead minnows (Pimephales promelas) at environmentally relevant concentrations. To evaluate the potential effects of TCS on secondary sexual development and steroidogenesis, a partial lifecycle study initiated with stage 10 embryos and concluding 8 weeks post-metamorphosis was performed using 0.0 (control), 6.3, 12.5, 25, 50 µg TCS/L as target exposure concentrations. Morphological endpoints included visceral development, gonad development, gonad-somatic index (GSI), body weight, and sex ratio. Biochemical endpoints included serum testosterone (T), dihydrotestosterone (DHT), and estradiol (E2); gonadal T; and gonadal CYP 17, CYP 19 (aromatase), and 5α-reductase (5-AR). Exposure to TCS did not alter visceral development, gonad development, GSI, whole body weight, or sex ratios compared to controls. In addition, no significant changes in serum T, E2, and DHT con-

centrations were found in TCS exposed S. tropicalis compared to controls. Significant changes in gonadal T levels; or CYP 17, CYP 19 (aromatase), or 5-AR activities were not observed in the TCS treatments relative to the controls. Overall, these results demonstrate that TCS does not alter secondary sexual differentiation or steroidogenesis in juvenile S. tropicalis.

ET07B-2

Long-term effects of a binary mixture of perfluorooctane sulfonate (PFOS) and bisphenol A (BPA) in zebrafish

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The frequent use of perfluorinated chemicals (PFCs) in industrial applications and domestic products has led on a global basis to a continuous detection of PFCs in a wide range of environmental matrices including aquatic systems. Perfluorooctane sulfonate (PFOS) is the most commonly detected PFC in biotic and abiotic samples. To date, the understanding of the potential effects of PFOS towards biological systems has reached substantial progress. However, the majority of studies have focused on acute effects, leaving long-term effects largely unexplored. Given the persistent properties and the reported membrane altering potential of PFOS a long-term assessment in combination with other pollutants should be a promising strategy to shed more light on the complex toxicology of PFOS. Since PFOS has been shown to act as an endocrine disruptor in fish a combined investigation with another endocrine disrupting chemical (EDC) would represent an approach where specific endpoints such as sex steroid levels could be measured and compared, thus providing a more direct hint of any interactive effects.
In the present study we investigated waterborne PFOS both alone and in a binary mixture with the known EDC bisphenol A (BPA) over two full generations of the zebrafish (Danio rerio). Survival, growth, reproductive success, vitellogenin (VTG) and histological alterations in thyroid, liver and gonads were examined. PFOS (300 µg/L) was found to induce lipid accumulation in liver of F1 generation fish. A parallel finding in PFOS (300 µg/L) exposed fish was the occurrence of granuloma, presumably as a result of bacterial infection. Identical granuloma structures were detected in lower PFOS concentrations in F2 generation fish, indicating a suppressed immune system over generations. BPA tended to increase plasma VTG concentrations whereas the opposite trend was observed for PFOS. Binary mixtures of the two chemicals indicated no synergistic effects. Conclusively our study does not validate the hypothesis that the presence of PFOS increases the endocrine potential of BPA. However, our results indicate an immune suppressing potential of PFOS which seems to be enhanced over generations.

ET07B-3

Contamination of river ecosystem by compounds with specific modes of action assessed in biotic and abiotic matrices using in vitro bioassays

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Organisms living in aquatic environment are continually exposed to various natural and synthetic compounds that can affect their physiological functions, e.g. modulate the endocrine system, disrupt normal reproduction or developmental processes. Monitoring studies are often focused on assessment of contamination by endocrine-disruptive compounds in abiotic matrices, mainly sediments, surface and waste waters. However, presence of environmental contaminants in abiotic compartments does not indicate necessarily uptake and potential effects on organisms. In this context, use of fish tissue samples for biotests should provide more accurate information about contamination and its reflection in organisms. In two year long study we investigated influence of city agglomeration and large wastewater treatment plant (WWTP) on contamination of river ecosystem by compounds with specific modes of action. The studied area, the second largest city in the Czech Republic (400,000 inhabitants), is spread in the basin of two rivers, with variety of industrial activities and large WWTP. In selected locations sediments were collected in spring and autumn seasons. River water and waste waters from influent and effluent of the WWTP were sampled using two types of passive samplers - semipermeable membrane devices (SPMD) for hydrophobic compounds and polar organic chemical integrative samplers (POCIS) for polar pesticides and pharmaceuticals. In addition to abiotic samples and for better characterization of direct effects on aquatic organisms, the chub (*Leuciscus cephalus* L.) was selected as the most suitable indicator species. Organic extracts of sediments, passive samplers (SPMD, POCIS) and fish bile have been tested by battery of *in vitro* bioassays with recombinant yeast and mammalian cell lines. Specific activities, namely dioxin-like, (anti)estrogenic, (anti)androgenic, and also cytotoxic effects have been determined. Combination of biotests with abiotic and biological matrices enabled complex assessment of contamination by endocrine-disruptive compounds in river ecosystem and possible risks for aquatic organisms. The research has been supported by CETOCOEN (CZ.1.05/2.1.00/01.0001) project granted by the European Union and administered by the Ministry of Education, Youth and Sports of the Czech Republic, and by the projects of the MSMT 2B06093 and ENVISCREEN 2B08036.

ET07B-4

A novel type of endocrine disrupting effect: Octylphenol and 17β-oestradiol cause malformations in eelpout embryos

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Monitoring programmes (i.e. in Denmark and Germany) have revealed increasing frequencies of malformations among eelpout embryos during recent years. Malformations can be induced in oviparous fish species by exposure to chemicals in the laboratory and the implicit assumption in the eelpout monitoring programmes is that the observed increase in malformations might be caused by changing environmental conditions - including exposure to chemicals. This study aimed at investigating malformations upon exposure of pregnant eelpout to nominal concentrations of 500 ng l-1 17β-estradiol or 100 µg l-1 octylphenol. In the control group, 77.3% of the embryos developed normally, while only 0.9% and 8,8% did so in the groups exposed to 17β-estradiol and OP, respectively. Early death (with or without malformations) and eye and/or spinal cord malformations were observed. Early death dominated in the group exposed to 17β-estradiol and spinal cord deformations dominated in the group exposed to octylphenol. Exposure of pregnant eelpout females to 17β-estradiol and octylphenol at concentrations in the upper range of concentrations found in the environment thus results in abundant malformations among the embryos. This is a novel type of endocrine disrupting effect, and obviously, we want to establish dose-response relationships and no-effect-levels in further investigations. Likewise, we want to elucidate the mechanisms underlying this effect.

ET07B-5

Progress of the Japanese Program on Endocrine Disruption: from ExTEND2005 to EXTEND2010

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Ministry of the Environment, Japan launched a new research program on endocrine disruption titled "EXTEND2010"(EXTEND: Extended Tasks on Endocrine Disruption) in July 2010, after reviewing the preceding "ExTEND2005"(ExTEND: Enhanced Tack on Endocrine Disruption). Target of the new five-year program is to accelerate hazard assessment on endocrine disruption of chemical substances, prioritizing organisms in the environment, and to consequently conduct risk assessment to see whether any regulatory risk management measures should be introduced.
As well as promoting research on fundamental science and wildlife observation, test protocols of fish, amphibian and invertebrates have been developed through bilateral or multilateral collaborations. Reliability evaluation of existing knowledge that might be relevant to endocrine disruption is being conducted to select possible candidate chemicals subject to testing to assess their endocrine disrupting effects to aquatic organisms. Framework for assessing endocrine disrupting effects to organisms in the environment is being developed and in vitro tests are conducted for some of the candidate chemicals on a trial basis.
Progress and updated situation of the assessment of chemicals in EXTEND2010 will be demonstrated.

ET07B-6

Effects of endocrine disrupting chemicals on global DNA methylation using in vitro models

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Epigenetics is an emerging field that has widespread implications for environmental toxicology. By regulating gene expression patterns, epigenetic mechanisms such as DNA methylation are essential in normal development and differentiation. Environmental factors, including contaminants, may alter epigenetic control of gene expression, with important consequences for development and susceptibility to disease. The implications of early life exposure to environmental chemicals on long-term human health via altered epigenetics is a subject of increasing importance. We have recently started a European Commission-funded project called OBELIX ("OBesogenic Endocrine disrupting chemicals (EDCs): LInking prenatal eXposure to the development of obesity later in life"), which tests the hypothesis that prenatal exposure to EDCs plays a role in the development of obesity later in life (see also www.theobelixproject.org). In this project, possible mechanisms of early programming of obesity by EDCs are researched, including effects on DNA methylation. In the study described here, the effects of ten well-known EDCs on global methylation in in vitro models representing two main obesity-related target tissues (hypothalamus and adipose cells) were examined. In addition, the effects of EDCs on differentiation of murine preadipocyte cells were investigated. Results indicate that selected EDCs promote the differentiation of 3T3-L1 cells and cause reduced global DNA methylation. However, no effects of the EDCs were found on global DNA methylation in murine neuronal cells.

ET08 - Environmental OMICS: a global answer to environmental questions

ET08A-1

A proteomic approach to the development of potential estuarine biomarkers for metal contamination using the Sydney Rock Oyster (Saccostrea glomerata), NSW Australia

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The introduction of waste products into rivers and estuaries in industrial and urbanised areas since the industrial revolution has led to significant increases in chemical contamination. As such, it is now imperative to develop effective monitoring methods to protect biota and the environment. Biomarkers provide information on the cause and effect paradigm of contamination by linking contaminants directly to their effects on biota. However, traditional single parameter biomarker analyses can be insensitive, especially at low contaminant levels. Proteomics provides a new method for identifying potentially hundreds of species specific biomarkers simultaneously at extremely low levels of contamination and over short time periods, allowing early detection of environmental damage.
The current study uses proteomics to assess the effects of metal contamination on Sydney Rock oysters. Saccostrea glomerata were exposed for four days to three environmentally relevant concentrations (100 µg/l, 50 µg/l and 5 µg/l) of copper, lead and zinc. Oyster haemolymph from metal-exposed oysters was compared to haemolymph from non-exposed controls by 2-dimensional electrophoresis to identify differentially expressed proteins. Differential proteins were subsequently characterised using tandem mass spectrometry (LC-MS/MS) so that their putative biological functions could be assigned.
The data suggest that there are unique protein expression profiles not only for each metal, but at each concentration of metal. Exposure to 100 µg/l of copper, lead and zinc yielded a total of 21 differentially expressed proteins. Only one of these proteins was common to all three metals. Differentially expressed proteins were putatively assigned 11 different biological functions, of which cytoskeletal activity accounted for 25%. Ongoing work includes testing the efficacy of these potential protein biomarkers in the natural environment using oyster samples from field studies conducted in Lake Macquarie, NSW, Australia.

ET08A-2

Searching for stable biomarkers of malachite green exposure in Pangasianodon hypophthalmus by a non invasive approach

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This study aims at identifying sensitive multibiomarkers of malachite green (MG) exposure in the Asian catfish, Pangasianodon hypophthalmus, one of the most economically important farmed fish worldwide, by a non invasive sampling of peripheral blood mononuclear cells (PBMCs). Despite the fact that MG is banned in fish produced for consumption, this antiseptic is still commonly used in many fish farms. We tried to obtain a signature of protein expression which could work as an early warning signal of MG application even one month after the end of the exposure. The final objective is to apply this biomarker signature to an in situ monitoring. PBMC proteome has the advantage to be subject to rapid changes in response to external signals and enables regular samplings by a non invasive way. A classic (0.1ppm) dose for therapeutic treatment was applied twice, like Vietnamese farmers application. Directly after the second bath (T1) and after 1 month of decontamination (T2), blood was sampled on 3 fish per tank and PBMC were isolated and finally suspended in DLA buffer for proteomic analysis in Belgium. Two-dimensional differential in gel electrophoresis (2D DIGE) were performed on 24cm, gradient acrylamid 8-13.5 %, pH 4-7, IPG strips followed by analysis with DeCyder software. The number of spots matched in the eight gels was 1195 ± 364 in which 116 showed significant differences in intensity between the treated and the controls and which are common for both periods of sampling with no effect of sampling time (Anova 2, n = 4). Considering single identification per spot, we identified by LC-MS/MS 26 different proteins which are mainly involved in energetic metabolism, protein folding, oxidative stress and DNA/RNA binding. To name just a few, Proline 4-hydroxylase and Pre-mRNA-splicing factor are under-expressed 5.84 and 1.34 times respectively at T2 ($p<0.05$). These identified proteins are suggested to be potential candidates for a Biomaker Protein Expression Signature (BPES) since their expression is still modified one month after the exposure, making them quite stable over a decontamination period. In the opposite, assays of MG residues in the muscle and in the blood of fish show that even if leuco-malachite green is more persistent in the muscle, it has almost totally disappeared after one month of decontamination. These promising results open a new way to the application of fish mononuclear cells to find potential biomarkers of exposure in a non invasive way.

ET08A-3

Analysis of effects of herbicide exposure on Chlamydomonas reinhardtii using a multiple endpoint assay and proteomic profiling

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Herbicides are a heterogenous group of compounds affecting photosynthetic organisms via a wide range of mode of actions such as the inhibition of photosynthetic electron transport or of carotenoid synthesis. For a comprehensive analysis of the effects of three selected herbicides (diuron, norflurazon, paraquat), each representing one specific mode of toxic action, on the single celled green alga Chlamydomonas reinhardtii we developed a multiple endpoint assay. It comprises measuring a combination of physiological endpoints covering a range of cellular parameters in relatively simple setups. These endpoints are, among others, the application of PAM fluorometry, the assessment of cellular esterase activity using the fluorescent reporter dye CFDA-AM, and determination of ATP levels. The presented approach allows discriminating different herbicide modes of action and studying the endpoints' respective sensitivity towards exposure effects assessment and their correlations among each other.
This assay also revealed suitable exposure conditions for sampling for a proteomic profiling analysis applying 2D-LC-MS/MS in combination with the semiquantitative spectral counting approach. The application of proteomics and other OMICS type analyses in ecotoxicology generally aims at linking the physiological effects and modes of action of toxicants to variations in the target organism's proteome. Of special interest are conditions around the lowest observed effect concentration. Exposure of C. reinhardtii to the above-mentioned model herbicides caused a variety of significant positive and negative changes on the proteome level leading to identification of candidate protein biomarkers for the applied conditions. These biomarkers include enzymes involved in antioxidant defense such as L-ascorbate peroxidase and thioredoxins, photosystem I and II components, proteins controlling posttranslational modifications, and general metabolic pathways such as amino acid synthesis and degradation. Functional annotation of the proteomics data was achieved using the MapMan software platform. It enabled us to determine functional groups, like Calvin cycle or protein degradation, responding to the herbicide exposures. This study provided new insights into the effects of the investigated herbicides and shows the possibility of linking variations on the proteome level with their physiological effects and modes of actions.

ET08A-4

Nuclear proteome changes on the social amoeba Dictyostelium discoideum during the molecular adaptations to environmental stress conditions

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The social amoeba *D. discoideum* is a model organism, with a genome fully sequenced, widely used by the scientific community and it is considered as interface between the unicellular and pluricellular organisms.
In the last years we have applied the proteomics approach with 2DE and MS/MS to study the effects induced on the soluble proteins of *D. discoideum* by the exposure to different doses of mercury (Hg^{2+}). Marsano et al. (2010) has demonstrated how 10 µM of Hg^{2+} induces in cells a toxicity response with a generally down regulation of the proteins involved in basic physiological processes.
In the present work, to deepen the mechanisms of stress response induced in *D. discoideum* with Hg^{2+} we have analyzed the nuclear proteins changes.
Nuclear proteins modifications represent a crucial point in the actuation of stress response, as can be seen as a hub between signal transduction events and realization of new gene expression/protein synthesis, phenomena which in turn may lead to the establishment of detoxification and adaptation processes to new environmental conditions or to toxicity.
Histones - between the nuclear proteins - are highly conserved and they do not play simply a structural role, but post-translational modifications at specific aminoacid residues can greatly influence gene expression.
The proteins extracted from nuclei of control's cells and treated with 10 µM Hg^{2+} were labeled using the new technique of Isotope-coded protein/peptide label and then analyzed by LC-ESI MS/MS to highlight the main changes induced by treatment on histones and other nuclear proteins in addition to the main post-translational modifications.
We have identified 170 proteins involved in nuclear processes, such us the DNA and/or RNA processing and we have quantified 150 proteins. Data showed that the exposure of Dictyostelium to 10 µM Hg^{2+} induces a down regulation of the nuclear proteins that are directly involved in major biological events - DNA topoisomerase I & II, ADP-ribosyltransferase, RNA polymerase II and all histones of the nucleosome - and they are indispensable during development and cell division in the organisms.
Also, we have found that the treatment with high dose of Hg^{2+} induces post-translational modifications of histones.
In conclusion, these data represent an important link between genomics and proteomics and provide interesting information on the control of regulation of gene expression in D. discoideum when subjected to environmental stressors.

ET08A-5

Measurement of vitellogenin protein in invertebrates : relevance and usefulness of mass spectrometry (LC-MS/MS) to propose a specific and transferable method across species

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Reproductive success of organisms is related to the quantity and quality of eggs produced by females. Vitellogenin (Vg), precursor molecule of vitellin that is the energy available for embryonic development in oviparous organisms, is proposed as a relevant exposure and effect bio-chemical marker of endocrine disruptors in aquatic vertebrate species (e.g. fish, amphibian). Numerous strategies, such as enzyme-linked immunosorbent assays have been developed to characterise and quantify this protein in vertebrates. On the contrary, in invertebrates few methods are available. This gap mainly results from the low transferability of available antibodies in relation to phylogenetic distance and molecular divergence accumulated by proteins through animal evolution. Recently, our laboratory developed a quantitative assay of Vg in a widespread amphipod, Gammarus fossarum, using liquid chromatography tandem mass spectrometry (LC-MS/MS). This method offers the possibility to identify and quantify Vg, based only on the quantification of proteotypic peptides containing from 5 to 15 amino-acids. Despite a high molecular divergence observed for this type of protein throughout animal evolution, comparative and evolutive genomics revealed that partial conservation between homologous proteins can persist due to high functional constraints acting on residues clustered in the proteic structure forming conserved peptides within phylogenetical groups. In this context, the aim of this study was to assess the possibility to take advantage of peptidic motifs conservation to propose a transferable method across species. For this, our study focused on three model species for which sequence of egg yolk protein is well known, an amphipod, Gammarus fossarum, a cladocerean Daphnia magna and an insect Drosophila melanogaster. In first step, proteotypic peptides of Vg were identified for each species. In the second step, we tried to find these proteotypic peptides in closely related species, such as G. pulex, G. wautierii, D. pulex. This study showed the high relevance of mass spectrometry to propose a specific methodology for Vg measurement. Moreover, methodology proposed to Vg measurement is transferable from a species to another one from the same genus.

ET08A-6

Molecular responses of lymphocytes in European eel Anguilla anguilla exposed to perfluorooctane sulfonate (PFOS). Biomarker discovery using a sub-proteomic approach

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Since the 1980s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population survival. Many factors can be attributed to this decline such as pollution by xenobiotics like PFOS. The aim of this research project was to develop a multi-biomarker approach to set up an evaluation and monitoring tool for assessing the health state of eels. The strategy consists in assessing protein expression profiles (PEPs) in post-nuclear fraction of lymphocytes exposed in vitro to different pollutants, before in vivo validation of biomarkers. The first step of this approach was to find out PEPs of PFOS exposure. For that, we used lymphocyte culture from eel to test the in vitro toxicity of this compound. Exposure time and two sub-lethal concentrations were chosen to avoid cell mortality (48h exposure at 10 µg/l and 1 mg/l). Lymphocytes were isolated from blood by centrifugation over a Ficoll/Hypaque gradient. After in vitro contaminations, the post-nuclear fraction was isolated. Two-dimensional differential in-gel electrophoresis was performed on 24cm, pH 4-7 IPG strips and the results were analysed with DeCyder software. The number of spots detected in the 6 gels was 1665 ± 350, in which 267 ($p<0.05$) and 175 ($p<0.01$) spots showed significant differences between treatments (Anova 1, n=4). The identified differentially expressed proteins can be categorized into diverse functional classes, related to cell structure (e.g. supervillin), protein folding (e.g. protein disulfide isomerase) and signal transduction (e.g. 14-3-3 protein epsilon) for instance with some proteins never found in proteomic studies. These promising results are expected to point out potential biomarkers of exposure to PFOS on fish lymphocytes as well as to highlight unknown mechanisms of action of this pollutant. The second step will be in vivo contaminations of eels in order to validate the PEPs previously defined.

ET08B-1

The Estuarine Sediment Ecology Array (ESEA): a rapid and comprehensive molecular based approach for environmental monitoring and assessment

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Ecological assessments of estuarine environments are restricted to the examination of a small number of macrobenthic taxa, providing a narrow view of a system's true diversity. This is despite strong evidence that many other biotic groups, e.g. meiofauna, may be better indicators of environmental change and condition. However, their inclusion is generally considered too difficult to be routinely practical. The fundamental approaches for assessing and monitoring sedimentary environments has changed little for many decades. Recent advancements in the molecular sciences provide a unique opportunity to measure and understand biological complexity at a previously unattainable level. We have developed a novel approach for sediment ecological assessment, the Estuarine Sediment Ecology Array (ESEA). ESEA is a custom-designed microarray which contains phylogenetically diagnostic gene probes which can be used to identify the presence of an extensive range of organisms ranging from microorganisms to macrobenthic fauna. The underlying approach behind the ESEA is that DNA is extracted from sediments, targeted genes are amplified and hybridized against the array. When the sample's sequences match those on the array, fluorescence signals are produced and the intensities measured. Each probe set is annotated with taxonomic information, enabling rapid identification of a sample's biota. Genes used to create the array were derived from a pyrosequencing study of Sydney Harbour and from

GenBank, an online gene repository. Cross-reaction probe sets were also created for the detection of taxa not specifically represented on the array. The ESEA contains >250,000 probes designed to encapsulate over 53,000 gene sequences. Laboratory trials clearly demonstrated the array's viability. Field trials were performed on eukaryotic meiobiota assemblages sampled from reference and contaminated locations. The results revealed marked differences in the biota between the two treatments, with comparisons involving several thousand taxa. The ESEA has been calibrated and cross checked using pyrosequenced data derived from the same DNA extracts, with extensive field trials currently being performed. The application of ESEA and other genomic technologies now makes it possible to rapidly and comprehensively examine the biological constituents of an environment at a cost similar to that of traditional taxa focussed optical based techniques.

ET08B-2

The DanTox-Project - identification of specific toxicity and molecular modes of action of sediment-bound pollutants in zebrafish

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The aims of the DanTox project are a) to develop a suitable testing strategy for the assessment of bioavailable toxicants in sediments, b) to investigate the molecular and cellular mechanisms of sediment toxicity, and c) to elucidate the causality of biological effects. The long-term objective will be the development of a targeted cDNA-microarray which will be a useful tool for environmental screening.

Sediment samples were collected from the Rhine River (Altrip and Ehrenbreitstein) and from the Vering Canal in Hamburg. For the testing of model chemicals methylmercury(II)-chloride, chlorpyrifos, Aroclor 1254 and bisphenol A were selected. The different methods of the project are categorized into four modules: a) bioassays - selected biomarkers and endpoints; b) gene expression - microarrays and qRT-PCR; c) data assessment - statistical evaluation of the data; d) practical transfer - comparison of the results from sediment contact tests and guideline tests.

At the present stage of the project, results from the fish embryo test, EROD assay, live-imaging of EROD induction and qRT-PCR analysis are available. The highest embryotoxic potential was measured for the sample from Vering Canal after 48 h of exposure (EC50 = 2.6 and 3.6 mg/ml for extract and native sediments, respectively). EC50s for the extracts from Ehrenbreitstein (EC50 = 21.7 mg/ml) and Altrip (EC50 = 18.1 mg/ml) suggest that there is a comparable embryotoxic hazard potential. Only minor EROD induction was measured for TCDD suggesting that the barrier function of the chorion prevents TCDD from harming the embryo. In contrast, sediment extracts showed a clear dose-response-depending EROD induction. Live-imaging of EROD induction with β-naphtoflavone as a positive control documented CYP1 induction at any time of inspection. Due to their molecular structure, methylmercury(II)chloride and chlorpyrifos showed no EROD induction. qRT-PCR revealed clear changes in the transcript abundance of CYP1A1, GST and UGT1A1 genes for the sediment extracts. Up to 600-fold changes in CYP1A1 could be seen for the extract from Vering Canal.

First results from the biotest systems indicate that measurement of specific endpoints is a suitable strategy to identify and detect the bioavailable hazard potential of sediments. In addition, a comparison of the results from the EROD assay and qRT-PCR showed similar tendencies for the sediments indicating that the EROD assay with zebrafish could be a useful tool for routine testing.

ET08B-3

The future role of next-generation DNA sequencing and metagenetics in aquatic biological monitoring programs

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The development of current biological monitoring and bioassessment programs was a drastic improvement over previous programs created for monitoring a limited number of specific chemical pollutants. Although these assessment programs are better designed to address the transient and potentially syngeristic nature of environmental stressors, the reliance on morphological taxonomic identification of samples has several inherent issues limiting the scope of these programs. Biological monitoring programs suffer from high costs, long completion times, quality assurance issues from taxonomic disagreements, and reliance on 'large' animal groups, such as fish and macroinvertebrates, that can be identified by experts. Neglected communities such as microbiota and meiofauna represent a wealth of information that could be applied to assessing ecological condition. Even for those fauna used in bioassessment, many taxa are not readily identified to species, leading to taxonomic impresicion and a potential loss of information. Next generation DNA sequencing (NGS) is a revolutionary technology when considered in the context of biological monitoring programs. This technology allows for bulk DNA extraction of virtually all the specimens in a sample, followed by DNA sequencing of genetic loci used for taxonomic identification. NGS generates large amounts of sequence data that can be applied not only to the identification, with finer taxonomic resolution, of those groups currently used in assessment, but to previously intractable groups such as nematodes, diatoms and other algae, protozoans, and other meiofaunal invertebrate phyla. This presentation will outline the future role that metagenetic data generated by NGS could have in new biological monitoring and assessment programs. The future uses of this metagenetic data would be not only to improve the speed and precision of biological monitoring, but to move programs beyond relatively simple conditions assessments (often 'good,' 'fair,' or 'poor') to the ultimate goal of bioassessment: environmental stressor identification. We will duiscuss the current state of technology, planned research, and future hurdles for the use of metagenetic data in freshwater and marine biological monitoring programs.

ET08B-4

Enchytraeid transcriptome sequencing towards the establishment of a soil ecotoxicogenomics model

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Enchytraeids are an ecologically relevant functional group of the soil mesofauna, distributed globally and present in most soil types. *Enchytraeus crypticus* is a bio-indicator potworm frequently used in soil ecotoxicological tests. The importance of this soil ecotoxicological model is reflected in the establishment of an ISO guideline (ISO16387, 2010) and an OECD guideline (OECD 220, 2004). The drawback is that ecotoxicological testing is time-consuming, labour-intensive and considers endpoints as survival and reproduction thus not informing on the mechanism of toxicity. Alternatively, transcriptional responses are usually fast and provide mechanistic information on the toxic compound. Transcriptomic analyses require sequence information, but this is absolutely lacking for *E. crypticus*. Therefore we present data on transcriptome sequencing of *E. crypticus* using next-generation sequencing technology. The effect concentrations affecting *E. crypticus* survival and reproduction endpoints for five key chemicals were empirically assessed via standard ecotoxicological testing (ISO 16387, 2010; OECD 220, 2004). The EC50 for *E. crypticus* reproduction after 21 days of exposure in LUFA 2.2 soil spiked with carbendazim, cadmium, phenanthrene, 3,5-dichloroaniline and pentachloroaniline were, respectively, <1.0, 35.0, 144, 101 and 278 mg a.i/kg d.w. LUFA soil. Comparing to *F. candida*, the enchytraeid was more sensitive to metals but less susceptible to organic compounds. The different sensitivity of *E. crypticus* emphasizes its relevance as soil ecotoxicological model. In order to obtain most enchytraeid transcriptome information, *E. crypticus* was short-term exposed to the five chemicals at distinct effect concentrations, as well as distinct soil moisture, temperature and pH values. Also, samples from distinct developmental stages and starved animals were taken. The RNA pool, composed of transcripts from above mentioned treatments, was normalized prior to ultra-high throughput 454 Roche GS FLX Titanium pyrosequencing. Approximately 1 million output reads were assembled using distinct programs and the assemblies were combined in order to reach more consistent contigs representing putative transcripts. We performed functional annotation of identified transcripts and linked the obtained biological processes to the applied treatments. This is a crucial first step towards understanding and modelling the mechanistic basis of toxic effects occurring in the enchytraeid.

ET08B-5

Applying next-generation DNA sequencing for Biomonitoring 2.0 assessment in a threatened national park (Wood Buffalo, Canada)

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Biomonitoring seeks to describe and understand biological diversity at different locations, both as a means to learn the typical mix of species that can be found in different habitats, and to establish biological early-warning systems that can indicate when environmental stresses are reaching a critical point. Canada is recognised as a world leader in biomonitoring, but current practices are personnel-intensive, limiting the frequency and intensity of sampling, particularly in remote areas, and focus on a very limited subset of all species that can be found at a given location. Our project introduces 'Biomonitoring 2.0', a DNA-based system utilizing new next-generation sequencing (NGS) technologies and advanced computational analysis, that will simultaneously reduce sample costs while dramatically increasing the knowledge gained from biological samples. Ecosystems are sampled using a multi-habitat approach, and the resulting bulk samples presented for rapid NGS analysis. Using a multi-gene approach, linked to an advanced bioinformatics platform and associated cyberinfrastructure, we can rapidly sort and identify organisms, providing a rich information source for environmental assessment. We are applying this new system in Canada's Wood Buffalo National Park, a World heritage area, containing two internationally-important Ramsar wetlands that are currently threatened by climate change, oil sands extraction, hydro-electric schemes, mining and other human impacts. The project will produce an information source which can be used to develop advanced environmental diagnostics system, to assess the relative contribution of multiple stressors operating on the landscape at different temporal and spatial scales. These stressors include climate-related variables relating to drought (temperature, oxygen, hydroperiod) and contaminants from upstream oil sands activities (including metals and PAHs).

ET08B-6

Relationship between metal bioaccumulation, gene expression and toxic responses in early life stages of Japanese medaka exposed to cadmium-spiked sediments

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Aquatic sediments are well known sinks for numerous persitent pollutants such as POPs and heavy metals. Sediment-accumulated pollutants can be partially bioavailable and may represent a threat for benthic organisms in particular at pollutant-sensitive early life stage. In order to evaluate impact of contaminated sediments on early developmental stage of fish, Japanese medaka embryos were exposed during their whole embryonic development to cadmium-spiked sediments at two environmental concentrations (2 and 20µg/g d.w.). Bioaccumulation, metallothioneins (MTs) content and gene expression levels (rRT-PCR) were assessed at the end of organogenesis (7days post-fertilization) and at hatching. In addition, non invasive endpoints including cardiac activity, developmental abnormalities, body size and survival were examinated during experiment. Results showed significant differences in Cd bioaccumulation levels according to developmental stages and exposure concentrations. Although a dose-dependent accumulation of Cd was reported, no effect on either MTs proteins or mt gene expression was observed. In contrast, morphological deformities mainly on spine column and cardio-vascular system were increased following Cd contamination. Genotoxic potency of tested heavy metal was confirmed by DNA repair, oxydative stress defense system and apoptosis related genes overexpression. Moreover and for the first time, a parallel increase of wnt1 expression was highlighted in Cd-exposed prolarvae.

This study evidenced significant bioaccumulation and effects of cadmium in early developmental stage of medaka following realistic exposure to environmental concentrations of Cd. The same experimental protocol can be used to monitor effect of various sediment-associated pollutants including heavy metals and POPs. Associating early response markers and phentotypical effects observation may improve risk assessment of environmentally persistent chemicals.

ET09 - Laboratory and field measurements and alternative approaches in bioaccumulation

ET09-1

Relating field bioaccumulation metrics for chemicals in a benthic and pelagic food web with existing bioconcentration data

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The current state of bioaccumulation science relies heavily on fish bioconcentration (BCF) testing according to international protocols in order to predict the level of bioaccumulation of chemicals in real organisms in food webs in the field. How similar are BCFs measured in the laboratory to BAF measured in the field? Predictions of bioaccumulation in the field of xenobiotic chemical substances are based on standard bioconcentration (BCF) tests or estimated using regression equations. How easily can we use BCF to predict the behaviour in the field of new chemicals which lack field data so far? How chemicals partition between water and biota, between biota and air are important processes in the understanding of bioaccumulation in food chains and are key to the translation of BCF tests to bioaccumulation in the field. To study bioaccumulation in the field, the Western Scheldt estuary in The Netherlands was selected, as this estuary is characterized by a large land-based pollutant input. We present here examples of how different chemicals, with a wide range of Koa, Kow, and biotransformation rates, bioaccumulate and biomagnify in the pelagic and benthic food chains within this food web, including an air-breathing top predator species (tern). The data illustrates the impact of including bioavailability in the water phase on the calculations of bioaccumulation metrics such as BAF as well as the impact of biotransformation on the biomagnifications (BMF) and trophic magnification factors (TMF). First of all, BCF values are subject to variability (due to protocols followed, but also species-specific differences). When the BAF is examined, we can see that for some compounds, such as PFOA, that the median BAF value is very low (79), however the TMF is >1 in the benthic food web, suggesting biomagnification. The importance of biotransformation as important factor impacting the bioaccumulation of chemicals in a food chain was examined in relation to the BCF, BAF, BMF and TMFs. Biotransformation can have a major impact on bioconcentration and field bioaccumulation as we have seen in the examples of pyrene and BDE209. This also contributes to a low TMF: trophic dilution. Also, the use of dissolved concentrations for these chemicals was demonstrated to have an impact on the BAF calculated in the field. The food web studies demonstrate several key factors for the interpretation of laboratory and field bioaccumulation metrics.

ET09-2

Reducing uncertainty in risk assessment: lessons learned from studying sediment-associated fragrance materials

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Being able to accurately identify PBT substances is key to protecting human health and the environment. Potential hazards and risks of new and existing chemicals are assessed by regulatory agencies based on their P (persistance), B (bioaccumulation) and T (toxicity) potential, and if a compound is characterized as PBT it may be subjected to regulatory control or further characterization to determine its harmfulness. The focus of this presentation will be on the importance of environmental and physiological factors for affecting bioaccumulation, including biotransformation, of organic contaminants in the aquatic environment and thus the importance of these factors for contaminant persistence. Examples will include results from a range of experiments performed in collaboration with the Research Inst. for Fragrance Materials (RIFM) using different sediment-associated fragrance materials (FMs), sediment-dwelling deposit-feeders and different sediment organic contents. Here we focus on: 1. effects of varying organic matter content in the sediment; and 2. impact of species dependent biotransformation differences for the fate (accumulation, biotransformation) of sediment-associated FMs. These studies support the conclusion that the materials presented int he examples are not PBTs.

Due to their feeding strategy, that involves ingesting massive volumes of sediment, and a digestive system optimized to extract organic material from sediment, deposit-feeders may take up large amounts of contaminants from the gut during feeding. However, since at least some deposit feeders also show high biotransformation capacities, which evidently will reduce the body-burden (BB) of organic contaminants, focusing solely on BB will significantly underestimate accumulation and thus increase the uncertainty of e.g., BAF. Since benthic communities play an important role both in the remobilization of sediment-associated organic compounds and in other fate processes (biotransformation) there are implications for the way we evaluate the persistence of organic contaminants in risk assessment. Based on the lessons learned, uncertainty in risk assessment can be reduced by focusing future research on physiological properties that impact accumulation and biotransformation of B and P substances.

ET09-3

Effects of habitat and season on food web accumulation of cadmium to the little owl: a modelling study based on field data

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Little owls (Athene noctua) inhabit different habitats, including floodplains, and have a wide range of potential diet items. However, floodplains are contaminated by legacy contaminants, which may pose a toxicological risk to the little owl. It has been shown that accumulation patterns may differ within floodplains, due to spatial variation in habitat characteristics, soil concentrations and the occurrence of prey items. Field observations showed high seasonality in the occurrence of prey items, so in addition to spatial variation, contaminant accumulation to the little owl is expected to vary temporally, related to this seasonality in diet composition. Modelled exposure of little owls to cadmium indeed showed considerable seasonal and spatial variation. Calculated geometric mean exposure levels were well below threshold levels. However, due to the seasonal variation in diet composition, exposure levels exceeded the threshold values in both spring and fall for a prolonged period of time. This would indicate risks at those moments, which is in contrast with the conclusion based on the geometric mean value. Especially the fact that the threshold is exceeded during the spring period may be of importance, since that is the period of chick rearing. This would have been missed assuming fixed dietary fractions throughout the year, which may underestimate seasonally occurring risks. This presentation illustrates the potential of habitat and season specific food web modelling for more adequate assessments of risks of environmental contaminants to wildlife.

ET09-4

Capacities of phospholipid membrane to accumulate neutral organic chemicals

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Lipids have been considered as the predominant components for accumulation of organic chemicals in biota. It is a common practice to normalize chemical concentrations in the organism to the total lipid content regardless of the classes of chemicals. However, there are at least two types of lipids that are present amply in organisms, storage and membrane lipids. Despite the obvious structural differences between the two types of lipid, their differences in accumulation properties have not systematically been addressed. This study focuses on the equilibrium partition coefficients (K) of neutral organic compounds into phospholipid membrane. We critically evaluated literature liposome-water partition coefficients (K_{lipw}) for neutral compounds and evaluated methods to estimate K_{lipw}.

There was a fairly good correlation between log K_{lipw} and log K_{ow}. Errors were typically up to ± 1 log untis, although there was considerably larger scattering in the region of log K_{ow} > 6, due primarily to the too small K_{lipw} values for PCBs from early studies. However, even recent K_{lipw} values measured by polymer-mediated sampling methods exhibited 1-2 log unit differences between PAHs and PCBs of comparable Kow. Alternatively, polyparameter linear free energy relationships (PP-LFERs) were used for both consistency test and K_{lipw} estimation. The PP-LFERs fit well to the collected K_{lipw} data (R^2 = 0.97; standard devation, 0.3 log units). In contrast to the K_{ow} model, the recent values for both PAHs and PCBs fit well to the regression equation without any indication of the 'hydrophobicity cut-off' proposed previously by others. These results suggest that PP-LFERs can be highly useful for Klipw estimation.

Using the PP-LFER for K_{lipw} obtained, K values representative for membrane lipid-water partitioning were calculated for a number of compounds. Similarly, K values from water to storage lipid were estimated using the PP-LFER for olive oil-water partition coefficients. The comparison suggests that K between both lipids differ by only < 1 log unit for low-polarity compounds (e.g., PAHs) and H-bond-acceptor monopolar compounds (e.g., nitrobenzenes). In contrast, bipolar compounds (e.g., phenols) generally favor membrane lipid over storage lipid and the resulting K values can be > 1 log unit different. Thus, the "total lipid"may not be a suitable normalizer for bipolar compounds, which include a number of compounds such as those with -OH.

ET09-5

Integrated testing strategies (ITS) for bioaccumulation: hierarchical scheme of chemistry-driven modules and definition of applicability domains

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The efficient assessment of the bioaccumulation potential of chemicals under REACH with integrated test strategies (ITS) requires multiple tools. Existing data have to be searched and information from chemical structures and physico-chemical properties need to be evaluated prior to considering to conduct in-vivo experiments with vertebrates. The OSIRIS inventory of chemistry-driven and in-silico BCF modules for ITS compiles:

- Sources of existing data
- Computational methods
- B/nonB classification models
- QSARs
- Physiological models
- Exposure models
- Read across
- in-vitro tools
- 3R (Refine, Reduce, Replace) modules

The ITS components for bioaccumulation listed in the ECHA Guidance on information requirements and chemical safety assessment [1,2] have been extended with new knowledge generated in OSIRIS and complemented with feedback from stakeholders on the actual problems in using ITS for chemical registration. The alternative ITS modules share three major objectives to save time and money by reducing the number of experimental animals required to come to a conclusion about the bioaccumulation potential of chemicals under REACH:

- Classification of non-B/B/vB-compounds
- Omission of BCF studies, that are scientifically unnecessary or technically not feasible
- Waiving of BCF studies, that provide no risk-relevant information

The OSIRIS ITS for bioaccumulation will be publicly available (webtool) after further refinement based on stakeholder feedback. Its concepts and modules, as well as validation results, are presented in detail in a dedicated poster corner.

[1] ECHA. 2008. Guidance on information requirements and chemical safety assessment Chapter R.11: PBT assessment. (http://echa.europa.eu/reach_en.asp). Accessed 6 March 2009.
[2] ECHA. 2008. Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance. (http://echa.europa.eu/reach_en.asp). Accessed 6 March 2009.

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ET09-PS

Poster spotlight: Bioaccumulation measures between lab and field

Miscellaneous

Poster spotlight highlighting abstracts TU 217, TU 218, TU 219, TU 220:
- Antioxidant enzyme activities responses in freshwater biofilm in a metal polluted system
- Mercury accumulation in laboratory-reared Chironomus riparius and in indegenous chironomids' assemblages

- An evaluation of bioaccumulation data for Hexachlorobenze (HCB): can these data be used to convert biota standards into water based risk limits?
- Moving aquatic bioaccumulation assessments to the next level: Progress made and challenges ahead

ET10 - Linking chemical residues with biological responses in wildlife

ET10A-1

Fishing for contaminants in McMurdo Sound, Antarctica: measuring physiological responses of PBDE exposure in Trematomus bernacchii

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Polybrominated diphenyl ethers (PBDEs), which are flame retardants, have been detected at the Antarctic research bases, McMurdo Station and Scott Base. They have also been detected in marine organisms nearby and their presence has been attributed to the sewage sludge released from the research bases. Although there are several published studies showing the toxic effects of both PCBs and PBDEs on temperate fish, nothing is known about the effects of PBDEs on polar fish. Our *main objective* was to determine physiological responses of Antarctic fish, *Trematomus bernacchii*, exposed to two different sub-chronic doses of a PBDE congener mix. Physiological responses were assessed by (a) analysing morphometric data and liver histology and (b) measuring the relative expression of mRNA of cytochrome P450 1A-1 (CYP1A-1) and Cu,Zn superoxide dismutase (Cu,Zn SOD) using quantitative polymerase chain reaction (qPCR).
Live fish were captured at Cape Evans, Antarctica and transported to New Zealand for a ten-week dose-response study. Baseline samples consisted of fish euthanized in Antarctica and fish transported to New Zealand and then euthanized; these fish were not subjected to dose regimes. All remaining fish were divided into three groups and fed gelatine capsules containing either 0-ng (control), 32-ng (low-dose) or 320-ng (high-dose) of a PBDE mix (congeners 28, 47, 99, 100, 153, 154, 183 and 209). Fish were subjected to fortnightly treatments resulting in each group receiving a total of four treatments. Five fish from each group were euthanized at 14-day intervals, biometric data were collected for morphometric analysis (Fulton condition index), fragments of livers were preserved for histology analysis and 60-mg of liver tissue was snap frozen for molecular analysis.
Fulton condition index showed a significant decrease in fish from low- and high- dose groups in comparison to the control group, $F_{2, 42}=3.826$, $p<0.05$. Liver histology indicated a decrease in lipid abundance in the high-dose animals with increasing number of treatments. Furthermore, two-way ANOVA found a significant increase of the normalized mRNA levels of CYP1A-1 in the high-dose group with time ($F_{2, 42}=6.128$, $p<0.05$) but not for Cu,Zn SOD. Future research includes measuring (a) lipid amounts in liver to determine variation in lipid content in different treatment groups and (b) enzymes involved in the defence against oxidative stress such as catalase and glutathione peroxidise using protein assays.

ET10A-2

Exposure to EDCs Disrupts the Expression of cyp19a Isoforms of the Murray River rainbowfish, Melanotaenia fluviatilis

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Cytochrome P450 aromatase is the only steroidogenic enzyme activating the synthesis of estrogens from aromatisable androgens and plays a key role in neural development, sex differentiation, sexual/mating behaviours, reproductive cycles and also in other physiological functions. This study investigated the influence of two endocrine disrupting chemicals (EDCs) exogenous oestrogen 17beta-oestradiol (E2) and the estrogen mimic Bisphenol A (BPA) on the expression of cyp19a isoforms in both sexes of adult Murray River rainbowfish. Reproductively active male and female fish were exposed to either 1, 3, 5 µg/L of E2 or 100, 500 µg/L of BPA for 96 h. The expression analyses of cyp19a isoforms in the brain and gonads were studied using quantitative Real-Time PCR (qPCR). cyp19a1a expression in the ovarian tissues was downregulated and inhibited with E2 exposures. Whereas, it was upregulated until 48 h and reduced at 72 and 96 h with BPA exposure. However, its expression was not detected in the tissues including testes and the brain of both sexes. The expression of cyp19a1b in the female fish brain was upregulated until 48 h and reduced at 72 and 96 h with both EDCs. However, its (cyp19a1b) expression was suppressed with E2 and elevated with BPA exposures in brain of male fish. We also studied the expression of cyp19a1b in the gonads of both sexes where cyp19a1b in the ovarian tissue was downregulated with both EDCs exposure. Meanwhile, its expression in the testes was upregulated with E2 and suppressed with BPA exposure throughout the exposure period. The results showed that E2 and BPA regulate expression of cyp19a isoforms via both positive and negative feedback mechanisms. The results support the hypothesis that the expression of cyp19a isoforms depend on the duration of exposures, tissue and sex of the fish. Collectively the results suggest that, E2 and BPA can have a disruptive effect on the steroidogenic pathways and hence sexual/mating behaviours, sex differentiation and reproductive cycles in this fish.

ET10A-3

A link between environmental contaminants in Southern Alberta Rivers and physiological consequences disrupting reproduction and metabolism in fish

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The objective of the present study was to investigate the presence and adverse developmental effects of environmental contaminants in Southern Alberta rivers by means of chemical analysis, field studies and controlled laboratory experiments. Organic contaminants (natural and synthetic steroids, organic compounds and pharmaceuticals) were detected at all sites sampled along the Oldman river, Bow river and Red Deer river. We used longnose dace to investigate a link between exposures to environmental contaminants with biological response. A significant increase in female to male adult ratio was observed in longnose dace caught down stream of certain municipalities. Significant increases in vitellogenin (VTG) expression were observed in male longnose dace in correlation with female-biased sex ratios, suggesting severe endocrine disruption of gonadal development likely due to presence of compounds with estrogen-like activities. The results are consistent with the hypothesis that exogenous factors resulted in sex changes in longnose dace and caused genotypic males to develop as phenotpypic females. To test this hypothesis, we performed controlled laboratory experiments in which fish in aquaria were exposed to the same concentrations of a selected number of chemicals detected in the river system, individually and as mixtures. The results demonstrate that different chemicals present in the Oldman River disrupt the gene expression profile of the liver, ovary and testis, and the action of these contaminants becomes significantly larger when present in mixtures, compared to the effect of compounds individually. To further investigate the mechanisms of endocrine disruption, we have applied 1H-NMR metabolomics as a tool to measure the concentrations of metabolites in the liver and gonad tissue extracts, and evaluated net metabolic dysregulation due to exposure. The results suggest significant dysregulation of amino acid, lipid, energy, carbohydrate, nucleotide and cofactor/vitamin metabolism. The effect of mixture of contaminants on liver was significantly different from all the individual treatments. The results provide novel information on the effect of contaminants individually and in mixture on global metabolism dysregulation in male goldfish and a framework for better understanding of the metabolic pathways affected by environmental contaminants in fish.

ET10A-4

Oxidative stress and growth in Alligator Gar exposed to environmentally relevant concentrations of the aquatic herbicide, Diquat.

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In Canada Spotted Gar, Lepisosteus oculatus, a species of freshwater fish is known only to three bays of Lake Erie (Long Point, Point Pelee and Rondeau Bay) and is considered a threatened species. The remaining populations are subject to numerous anthropogenic stressors including contaminants, siltation, dredging, and removal of aquatic vegetation through applications of the bipyridal contact aquatic herbicide Diquat (1,1'-ethylene-2,2'-dipyridylium). A potent redox cycler, diquat can increase proliferation of reactive oxygen species leading to cell membrane damage and also potentially impairing growth. During the summer of 2010, water samples collected from a treated area of Rondeau Bay contained 40 _g L-1, 3 hours after treatment, declining to 4 _g L-1 after 24 hours. Diquat was not detected in reference areas. Because experimentation on threatened species is not possible, cultured alligator gar (Atractosteus spatula) were used as a surrogate Lepisosteidian species to examine potential effects of similar diquat concentrations on growth and oxidative stress parameters. Juvenile alligator gar (Xg, n=50/group) were exposed to 0 (reference), 50, 100 or 400 _g L-1 in a static renewal system for a period of 21 days followed by depuration in untreated water. Concentrations of diquat, as well as the metabolites diquat monpyridal and diqaut bipyridal, were quantified (UV/Fluorescence HPLC) in liver immediately after exposure and at 6 weeks post-exposure (n=8/treatment and time). Concentrations of the antioxidant vitamins E (tocopherol) and A (retinoids), as well as peroxidized lipids, were determined in liver as indicators of oxidant damage.

ET10A-5

Reduced host resistance against tuberculosis in fish exposed to elevated levels of POPs

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Extremely high levels of polybrominated diphenyl ethers (PBDEs) have been reported in fish from Lake Mjøsa, and also, high concentrations of PBDEs have been detected in humans that consumed fish from the lake. In addition polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs) are high in fish from Lake Mjøsa while fish from Lake Losna appear to contain background levels of these contaminants in Norway. The high levels of POPs detected in fish in Lake Mjøsa have led to advices from the Norwegian Food Safety Authority, warning children and women in fertile age against eating large trout regularly.
In the present project burbot (*Lota lota*) were sampled from the two lakes. Hepatic levels of POPs and OH metabolites were analysed and the fish were examined for bacterial- and parasite infections and histopathological changes. In accordance with previous studies the levels of PCBs (5310 ng/g lipid weight) and DDTs (3280 ng/g) were 10 times higher, and PBDEs (32900) was 200 times higher, than the corresponding levels in fish from Lake Losna (PCBs 576 ng/g, DDTs 232 ng/g, PBDEs 116 ng/g). To study potential metabolic pathways, OH-metabolites of PCB and PBDEs in blood and bile were analysed. Only 4-OH-CB 146 was found in bile and in blood, and in levels below 2 % of the parent PCB congeners suggesting that the metabolism of PCBs is low in burbot. The level of OH-BDE 49 in blood was approximately 2 % of the corresponding to the level in bile suggesting that bile excretion is the major route of elimination for PBDEs in burbot. The prevalence of pathological lesions (62%) and parasite and *Mycobacterium salmoniphilum* infections (18%) was significant higher in fish from Lake Mjøsa compared to Lake Losna (<1%) suggesting that the high level of contamination in Lake Mjøsa may adversely affect host resistance against opportunistic pathogens.

ET10A-6

EROD activity in peripheral blood lymphocytes: a non-invasive and relevant biomarker of exposure of dairy ruminant to PAHs and other POPs

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The use of biomarkers for evaluating the level of exposure of animals to environmental pollutants should be the first step prior to any exhaustive but often time-consuming and expensive analytical investigations (extractions in complex biological matrices followed by LC or GC-MS analysis).
In this respect, EROD activity has been extensively used over the past decades as a non specific biomarker of exposure of wild fauna to CYP450 1A1/1B1 inducing persistent organic pollutants (POPs) such as PAHs, PCBs, Dioxins and many over pollutants. In fish, EROD activity is measured in the liver or in the gill tissue. In birds and marine mammals, such as seals or penguins, it is often measured in liver. But EROD activity was also measured in duodenum and kidney of laboratory animals such as minipigs or rodents. To date, there is a total lack of information on the possibility to use this activity as a biomarker to evaluate the level of exposure of dairy ruminants to POPs such as PAHs. This could however be useful since POPs are often lipophilic molecules, which can therefore be transferred toward milk in a metabolized or native form with possible issues in terms of food chain. With such big farm animals (cows, goats or sheeps), EROD activity measurements should of course be performed without slaughter. Since it was shown that EROD activity could also be detected in peripheral blood lymphocytes (PBL) of rodent and humans, we decided to check the possibility to use simple blood samplings.
The first objective of this brief presentation is to introduce kinetic and dose/responses results showing that EROD activity in peripheral blood lymphocytes can be used as a relevant and

non-invasive biomarker of chronic (40-day) dairy ruminant oral exposure to PAHs, using goats as a model species. The second objective of this presentation will be to introduce recent results achieved on PBL of rats orally exposed to PAHs over a 28-days period, with simultaneous comparison of the EROD activity in the liver and brain. These results clearly demonstrate that EROD activity in PBL can be linearly correlated to EROD activity in the liver and in brain, thus strengthening the hypothesis according to which EROD activity in PBL could be used to properly evaluate EROD activity in the liver. This last point is interesting since the liver is the main detoxifying organs.

ET10B-1

Predictors of disease susceptibility in quail (Corturnix sp) exposed to immunotoxic environmental contaminants

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We investigated the immune response, physiological markers, plus disease resistance to an infectious agent (E. coli), to characterize the biological costs of exposure to environmental contaminants. Experimental birds (Coturnix c. japonica) were orally exposed to three contaminants of global concern; lead (Pb), malathion (the most widely used organophosphate insecticide in North America), and perfluoroctanoic acid (PFOA). Exposures were environmentally realistic; lead acetate (0, 5, 50ppm), malathion (0, 1, 10ppm) and PFOA (0, 1, 10ppm). Immunity was tested through i) the PHA skin test of T cell response, ii) the antibody mediated B cell response to vaccination with Newcastle Disease Virus, and iii) the innate immune response measured through; iiia)the chemiluminescence assay and iiib) the expression of TLR"ð3 in the bursa of Fabricius. In the PFOA study only, thyroid hormones (T4, T3) were measured in plasma.
No clinical signs of Pb toxicity were observed. Of all immunotoxicity tests, only TLR-3 was different (increased) in the highest exposure group. Mortality after bacterial challenge was lowest in the high exposure group (27.8%) compared to the low dose and control (55.5%) groups, showing a hormetic (immunostimulatory) effect with Pb exposed birds having better survival.
In contrast, malathion was immunosuppressive; mortality after E. coli challenge was higher in the high exposure group (50.0%) compared to the controls (22.2%). Of immunotoxicity tests, only humoral immunity was suppressed ($p<0.05$) in the higher malathion group. Histopathology revealed atrophy of the bursa of Fabricius, explaining the suppressed antibody response.
No clinical signs of PFOA toxicity were seen, morbidity after bacterial challenge was not different among the groups. The T cell response was lower ($p\leq0.02$) in PFOA exposed birds compared to controls. The B cell and innate immune responses were not different, but both T4 and T3 were lower in the PFOA exposed birds.
This work provides evidence that testing integrated immunity using an infectious challenge is a better predictor of immunotoxicity from exposure to environmental contaminants, than individual responses to immunotoxicity tests, and that biological responses are contaminant specific.

ET10B-2

Comparative toxicity and risk evaluation of the anticoagulant rodenticide diphacinone in various species of birds

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Several rodenticides have been identified as hazards to predatory and scavenging birds on a worldwide scale. In 2008, the US EPA imposed sales restrictions on second generation anticoagulant rodenticide active ingredients (brodifacoum, difethialone, bromadiolone, difenacoum), in part due to their persistence in biota. The action may be offset by expanded use of other rodenticides, including diphacinone (DPN). Based upon sublethal responses (prothrombin and Russell's Viper venom times) and lethality, American kestrels (Falco sparverius) were found to be 10 to 30 times more sensitive to an acute oral dose of DPN than Northern bobwhite (Colinus virginianus) and mallards (Anas platyrhynchos). The time course of coagulopathy following DPN administration is delayed, and does not necessarily correspond with maximal tissue residue concentrations. A companion acute toxicity study in Eastern screech owls (Megascops asio) was inconclusive as ~50% of the oral dose was regurgitated. The quantity of DPN retained by owls was estimated to range between 130 to 717 mg/kg, yet signs of toxicity (external bruising, internal hemorrhage, frank bleeding, and lethality) failed to yield a dose-response relationship. A pilot dietary study was undertaken in which DPN mixed into Nebraska Bird of Prey diet (2.5 and 10 ppm) was fed to owls for 7 days. Overt signs of toxicity and prolonged clotting time were observed at remarkably low dietary levels of DPN (estimated daily dose ranging from 50 to 200 µg), and a more definitive feeding trial is being undertaken. A risk evaluation of these and other exposure and effects data (Savarie et al. 1979; Johnston et al. 2005; Eisemann and Swift 2006) suggest that some raptors may be at risk of sublethal DPN exposure. In view of the paucity of threshold effect data following repeated daily exposure for birds of prey, additional DPN feeding trials with captive raptors and a refined risk analysis are warranted to better characterize the risk of secondary poisoning.

ET10B-3

Neuroanatomical and behavioural effects of early exposure to BDE-99 in an integrated avian laboratory and field model system

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Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that have become ubiquitous in the environment. BDE-99 is one of the most abundant congeners, and is consistently found in avian tissue and egg samples throughout the world. Previous avian studies have shown that early developmental conditions, such as nutrition and contaminant exposure, can have long-term neuroanatomical effects on the song control system, which could in turn affect mating behavior and reproductive success. Avian brain structures that underlie song learning and production develop primarily in the first few months post-hatch, which makes their development particularly sensitive to conditions during the nesting period. The objective of this study was to determine neuroanatomical and behavioral effects of early exposure to BDE-99 in an integrated avian laboratory and field model system, using the Zebra Finch (*Taeniopygia guttata*) and the European starling (*Sternus vulgaris*) as model passerine species. The zebra finch is a useful model to monitor effects of contaminants under controlled laboratory conditions, as they reach sexual maturity within 90 days, and readily breed in captivity. The European starling can be used as an ecological equivalent for comparative *in situ* investigations. Starlings are widespread, readily use nest boxes, feed on terrestrial invertebrates, and are easy to sample. In both species, we exposed young for the duration of the nesting cycle to environmentally relevant, sublethal levels of BDE99 (0-250 ng/g bw/day). A preliminary study dosing zebra finch chicks with BDE99 for 21-days during the nestling phase showed that there was a strong dose-dependent relationship for plasma BDE99 levels at 30 days of age. Following dosing, we raised young to sexual maturity. In the zebra finches we assessed male mating behavior and song quality. We then collected the brains from both the zebra finches and the starlings, and measured the song control nuclei to determine whether early developmental stress in the form of exposure to PBDE-99 resulted in impaired development of the song-control system. In the zebra finches, we found no significant effect on the volumes of the song control nuclei. There were negative effects on singing behavior, but not song quality. We also observed negative effects on mating behavior and success. Measurement of starling neuroanatomy is currently underway.

ET10B-4

Changes in retinol and thyroid function of peregrine falcon (Falco peregrinus) nestlings relative to brominated flame retardants and other contaminants

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Many additive brominated flame retardants (BFRs) and historical contaminants (e.g., polychlorinated biphenyls PCBs) are lipophilic, persistent and bioaccumulative, and increasing, including in peregrine falcons. The exposure of wild birds to these chemicals is of concern since such exposure can alter thyroid hormone homeostasis, retinol and thereby potentially development and reproductive success. The objective of this study was to assess potential changes in circulating retinol and thyroid hormones in nestling peregrine falcons relative to contaminant concentrations previously reported. Blood samples were collected from 28-d old nestling peregrine falcons at active nests in urban or remote areas of the Canadian Great Lakes Basin. Urban nestlings were heavier than those from remote regions. Consistent with being heavier, female and male chicks had higher retinol concentrations in urban nests than remote nests and male nestlings had higher circulating triodothyronine (T3) levels. In the male peregrines from the remote nests, there appeared to be a slower conversion of thyroxine to T3. PBDE congeners and metabolites were negatively associated with bone length in these nestlings, and for the male peregrine chicks, there were significantly negative correlations between circulating concentrations of retinol and the major PBDE congeners, ΣPBDEs and ΣPCBs, with ΣPCBs and ΣOH-PCBs negatively correlated with circulating T3 levels.

ET10B-5

CYP1A1 and CYP2B expression in mysticete skin biopsies from the Gulf of California: responses to contaminant or interspecies differences in detoxification ability?

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The main objective of this project was to investigate the inter-species differences in CYP1A1 and CYP2B expression and contaminant levels (OCs, PBDEs and PAHs) in three mysticete species, blue whale (Balaenoptera musculus), fin whale (Balaenoptera physalus) and Bryde's whale (Balaenoptera edeni) of Gulf of California (Mexico) using skin biopsy as diagnostic tools. A suite of sensitive non-lethal biomarkers was applied, for the first time, to the three mysticete species in order to evaluate the toxicological status of this cetacean species in the Gulf of California and also to explore the role of migratory behavior and the feeding habits (zooplankton-eating species fin whale and blue whale, fish-eating species Bryde's whale) in the evolutionary process of the two isoforms of CYP. This "multi-trial diagnostic tool", applied to skin biopsies, underlined differences in POP levels and molecular biomarker responses between the three mysticete species of Gulf of California. Two main factors seem to regulate the expression of different CYP isoforms in the three species studied: the inductive ability of POPs and the different evolution of the two cytochromes related to the different feeding habits of the mysticete species.
With regard to the level of POPs higher levels of DDTs and PCBs were detected in the zooplankton-eating species (fin whale and blue whale) in comparison to the fish-eating species (Bryde's whale). Particular concern can be generate by the high levels of PCBs detected to the migratory specie blue whale, that could bioaccumulate POPs while moving along the Californian coast. This contamination phenomena can have generate induction of both CYP1A1 and CYP2B in this species. On the opposite, extremely high level of both CYP1A1 and CYP2B were detected in the fish-eating species, showing similar level to odontocete species. Lower levels of OCs and high level of the CYP2B were detected in the Bryde's whale specimens, suggesting a higher detoxification ability in the fish-eating species. In conclusion these data indicate that two main factors can regulate the expression of the two CYP proteins in the mysticete species of Gulf of California: a) the inductive phenomenon linked to the presence of both planar (CYP1A1) and globular (CYP2B) POPs of in the blubber of blue whale; b) the role of evolutionary pressures related to the different dietary habits of the species (zooplankton-eating species fin whale and blue whale, fish-eating species Bryde's whale).

ET10B-6

Glutathione-s-transferase protein and activity in epidermal tissue of humpback whales

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Cetaceans (whales dolphins and porpoises) are particularly susceptible to accumulation of toxic burdens of lipophilic organochlorine compounds (OCs) due to their longevity and position at the top of marine food chains. Despite frequently reported elevated levels of blubber OC burdens in these species, the performance of chemical risk assessments is precluded by a lack of anciliary individual and toxicological information. Recently the International Whaling Commission (IWC) steering group on contaminants flagged the importance of driving research towards facilitation of cetacean chemical risk assessment.1

Currently OC toxicology on mysticetes (filter-feeding whales) relies primarily on skin and blubber biopsies due to the rarity of stranding events of these large, often migratory species. As such advancements in mysticete toxicology must be underpinned by utilisation of these tissues for further molecular assessments.
Glutathione-s-tranferase (GST) catalyses the conjugation of glutathione with various xenobitics and therefore plays a major role in preventing oxidative stress2. GST has been detected across the animal kingdom and like many detoxification enzymes is substrate inducible and therefore a potential candidate for biomarker applications. To date no studies have reported the presence of GST in the skin, the most accessible tissue, of cetaceans.
This study verified the presence of GST protein in the skin of humpback whales. Subsequently, GST activity was measured in skin extracts of 33 individual animals. No significant differences in activity were observed between sexes or between northward (post summer feeding) vs. southward (fasted) migration cohorts, although expressed activity was observed to be lower in southward migrating cohorts of both sexes. This is in contrast to the expected increase in lipophilic OC exposure occuring at this time due to remobilisation of contaminant burdens along with lipid reserves. The production of reactive oxygen species is however a consequence of all metabolic processes and it is possible that at this late stage of the migration, following extended fasting, metabolism has been significantly depressed masking the expected increase in lipophilic contaminant exposure. Future work will investigate the relationship between activity and organochlorine (OC) contaminant burdens of the whales to further assess the suitability of the enzyme as a biomarker of OC exposure.

ET11 - Mechanistic modelling for risk assessment: sub-lethal responses and population-level effects

ET11A-1
A matter of trust - stakeholders' perspectives on ecological modelling
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Ecological models are gaining momentum and popularity in ecological risk assessment. While the voices of critics seem to balance those of enthusiasts, we have decided to have a closer look at the role of models in an institutional context. Using the policy arrangements framework we have studied perspectives of three stakeholder groups on ecological modelling. 38 in-depth, semi-structured interviews were conducted so far, with stakeholders from regulatory authorities, pesticide producers and academia all over Europe. Confidential interviews comprised 15 open-ended questions, and were focused on the use of ecological models and recent changes in guidance documents and legislation.
The key informant approach was employed in recruiting our participants. They were first identified as key stakeholders in ecological risk assessment of pesticides and then sampled by means of a purposive (snowball) sampling, where each stakeholder indentified as important by others was interviewed and asked to suggest another key stakeholder for our study.
Results show that participants, although having different institutional backgrounds often presented similar perspectives and concerns about ecological modelling. The analysis of repeating ideas and keywords reveals that all stakeholders expect and require a lot from ecological models. Models are a complex matter for which communication and understanding are necessary.
It seems promising that all three groups see ecological models integrated in future environmental risk assessment, although some of the stakeholders are sceptical. Main hopes associated with models were to reduce the amount of expensive and complex testing and field monitoring, both at the product development stage and as an aid to mitigation measures. The analysis suggests that the needs of different stakeholders often overlap and thus that there is a good chance that consensus on the role and requirements of ecological modelling for risk assessment can be reached.

ET11A-2
Scientific needs of regulators to implement ecological modelling in environmental risk assessment of pesticides
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With higher tier studies, the level of uncertainty in the environmental risk assessment of pesticides is reduced. However, such studies have also their limitations especially because of the limited number of scenarios tested. In this respect, ecological modelling could become a valuable complementary tool for risk assessors. But from the regulatory point of view, the implementation of models into the risk assessment of pesticides depends on a number of scientific considerations: (1) Models should include - or in some cases at least account for - various ecological, ecotoxicological and environmental parameters that influence the effects and processes of recovery (i.e., choice of relevant model species; density dependence processes and population regulation; interspecific competition and predation; sublethal, long-term and delayed effects of toxicants; abiotic parameters (e.g., temperature, pH); spatio- temporal scales and landscape dimension) (2) Models should be useful to assess problems of mixture toxicity and of repeated applications (3) Models should be validated using field information from monitoring data and post-registration studies corresponding to various scenarios of exposure.
Irrespective of the anticipated benefits of ecological models for the risk assessment, outcomes from traditional risk assessment will remain central in the decision making processes, in the case of pesticides which - under their conditions of use - have a potential of causing adverse effects on ecologically vulnerable species.

ET11A-3
Bringing mechanistic modelling down to earth
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Ecologists have been developing mechanistic models of ecological systems ever since the 1960s, when computers capable of numerically solving complex sets of equations first became widely available. Within a few years it became clear that the ability of modelers to write equations and define parameters greatly exceeded the ability of empirical ecologists to measure parameter values and perform experiments needed to validate the models. With the technological tools available to today's ecological modelers, biological processes from the scale of the cell to the scale of an entire landscape can be linked together in a single model. However, the fundamental limitations on ecological modeling that were recognized 40 years ago still remain, and are even more important now than they were at that time. The purpose of this paper is to discuss those limitations, and propose steps that could be taken by today's ecological modelers to ensure that mechanistic models achieve their full potential in ecological risk assessment.
Two types of limitations are involved: process limitations and implementation limitations. The process limitations relate to the fact that the fundamental goals of ecological modeling - realistic representation of ecological processes, precise forecasting of future outcomes, and general applicability to a variety of situations, conflict with each other and cannot be simultaneously achieved. The implementation limitations relate to the difficulty of implementing complex models within regulatory decision-making frameworks.
This paper will illustrate the above limitations through illustrative case studies of past modeling efforts and regulatory applications. The case studies will include theoretical explorations of the tradeoffs between various types of uncertainties affecting ecological models and applications of models to two important environmental problems: assessing impacts of nuclear power plants on fish populations and predicting potential ecological effects of toxic chemicals. Based on these case studies, the paper will describe a generalized approach for ensuring that mechanistic models developed to support ecological risk assessments are not only properly tested and documented, but also suitable for implementation in regulatory applications such as pesticide registration and chemical risk management.

ET11A-4
Using ecological modelling to link ecotoxicological data with protection goals: an industry perspective
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The European Food Safety Authority (EFSA) recently published an opinion about protection goals, which was based on the ecosystems services concept. In the opinion, it was stated that for most ecosystem services the protection goal is at the population (or higher) level of organisation (although for vertebrates the protection goals may remain at the individual level for cultural reasons). The standard ecotoxicity data requirements for pesticide registration are, however, mainly focused at the level of individuals. Ecological modelling can make use of the standard data to assess the potential risks that exposure to pesticides may present to populations or higher levels of organisation and is compatible with current regulation. Here we propose a tiered system for how ecological modelling can bridge the gap between data at the level of individuals and the protection goals. We then we present three examples of how different types of ecological modelling have been used to refine ecological risk assessments for different pesticides. The first example demonstrates how a simple worst case toxicokinetic modelling based on field feeding rates can help refine risk assessments for birds. The second example shows how an individual-based population model was used to assess what impacts a pesticide might have on populations of non-target arthropods. The third example illustrates how ecosystem models can be used to assess the risk of indirect effects on aquatic ecosystems. The three examples presented here demonstrate how such models can help to refine risk assessments and extend the use of standard data to improve risk assessments.

ET11A-5
Population viability analysis for determination of lethal and sublethal effects of pollutants on populations
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Matrix population models are some of the most widely used models by population biologists. They are easy to construct and understand, but are they a good choice for use by ecological risk assessment of chemicals? Here, I discuss the strengths and weaknesses of matrix models as a tool for ecological risk assessment using models developed for a number of organisms exposed to pesticides. Results of this study indicate that matrix models are a good choice for an initial screening of toxicant effects because multiple effects (lethal and sublethal) can be easily incorporated into these models, they are easy to develop, use, and to understand. Therefore, matrix models should be the first choice for an initial risk assessment.

ET11A-6
Gaining insight in the interaction of zinc and population density with a combined Dynamic Energy Budget- and population model
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Laboratory tests are typically conducted under optimal conditions testing the single effect of a toxicant. In the field, due to suboptimal conditions, density dependence can both diminish and enhance effects of toxicants on populations. A review of the literature by Forbes et al. (2001) indicated that general insight on interaction of density and toxicants is lacking, and therefore no predictions on their combined action can be made.
To investigate how density dependence interacts with toxicants one can conduct experiments at the population level and test the joint effect of density and toxicant concentration. Such a study, however, does not give insight in the mechanisms of interaction, and is not feasible for long-lived species. Another strategy is to test the combined effects of toxicants and population density on the demographic rates; survival, growth and reproduction of individuals in a bioassay and extrapolate these effects with population models to assess the impact on population viability. Changes in demographic rates result from changes in the energy budget of an individual. An energy budget model combined with a population model, therefore, is very useful to integrate effects of toxicants and density at the population level. This approach is exemplified by assessing the combined influence of zinc and population density on the population viability of the earthworm Lumbricus rubellus.

ET11B-1
Comparison of bioassays with different exposure patterns: the predictive potential of mechanistic modelling
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Static and flow-through experiments were carried out to test the effects of cadmium on laboratory aquatic microcosms. Our objective was to compare these two experiments characterized by different exposure patterns, time-varying and constant, respectively. Focusing on *Daphnia magna* survival, reproduction and growth, we addressed the questions of organism fitness and sensitivity to cadmium. For this purpose, a classical analysis of data (hypothesis testing, determination of No Observed Effect Concentrations and Effective Concentrations) provided poor information. As a modeling framework, dynamic effect models were built for the three life history traits we

considered (survival, growth and reproduction), accounting for daphnid responses to cadmium exposure through threshold stress functions. These models took the exposure pattern into account, making comparable the results obtained from our two experiments. Contrary to the classical analysis, this modeling framework enabled us to detect an improvement of organism development in flow-through conditions compared to static ones and infer similar sensitivity to cadmium in both exposure patterns.

ET11B-2

Juvenile food limitation: ecotoxicologists, be warned!

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It is in the nature of standard ecotoxicological tests that they are as simple as possible. They are generally conducted under standardised laboratory conditions for the species of interest; conditions which have been proven to maintain the species in a good condition. However, what does good condition mean? Since we do not know exactly what the organisms eat in their natural habitat, and some even change their diet during the life-cycle, it is likely that the chosen food is not optimal for them throughout the whole life-cycle. If we do not know exactly what situations we investigate in the laboratory toxicity tests, how can we trust our extrapolations to populations in the field?

For ecological risk assessment, it is essential to detect only effects that are caused by the chemical of interest, and to exclude any side effects following from experimental conditions. Under food limitation, organisms are stressed, and respond differently to toxicant exposure. Therefore, it is important to closely investigate the organism's food requirements: if the test organisms are stressed due to food limitation, this stress might interact with the effect of the chemical, and lead to an overestimation of the chemical's toxicity. Diagnosing food problems and predicting the interactions between food and toxicants requires a mechanistic modelling framework that explains how food is used to fuel the life-history traits, and how toxicants affect this process. We demonstrate that Dynamic Energy Budget (DEB) theory is very well suited for this purpose.

Lymnaea stagnalis, the great pond snail, has recently been proposed as a good candidate species for developing OECD guidelines devoted to the risk assessment of endocrine disrupting components. Analysing life-cycle data from L. stagnalis, we detected food limitation in the early juvenile stage under controlled laboratory conditions. In a simulation study with the DEB model, we investigated how an initial food limitation in juveniles affects the interpretation of toxicity data, and distorts the extrapolation of toxic effects to the population level.

ET11B-3

The importance of density dependence and intra-specific interactions in population models for use in ecological risk assessment

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The range of endpoints used to assess the effects of test substances in the laboratory cover mortality, growth and development. However, population level processes (e.g. density dependence, behavioural interactions) may mean that statistically significant effects from laboratory based studies may not necessarily reflect their real-world importance for population abundance and biomass. We have formulated an individual-based zebrafish population model using results derived from a survey of wild fish, semi-natural experiments and meta-analysis data. The model, written in Netlogo (CCL, Northwestern University), mimics a 6m square pond. Zebrafish development is divided into four life-stages (eggs, larvae, juveniles and adults) with life history characteristics altered with each time-step. Density dependence is included for growth and survival and parameterised from experimental results. Exposure scenarios causing a 10, 50 and 90% decrease in growth rate and fecundity were investigated. Sex ratio scenarios tested for a 10, 30, 70 and 90% proportion of the population developing into males. Each simulation ran for 3000 days, with each scenario repeated 10 times. Growth depression resulted in significantly reduced abundance of mature females (K-W, DF = 3, P < 0.001). A 50+% decrease in growth rate resulted in population extinction within the 3000 day simulation. Fecundity depression resulted in significantly increased abundance of mature females in the population (ANOVA F3, 36 = 16.93, P < 0.001), likely caused by increased young survival through density dependent processes at lower population densities. Sex ratio changes in the hatched eggs resulted in altered sex ratios in the mature population. However, the abundance of mature adults in the population significantly increased with increasing male sex ratio bias (ANOVA F3, 36 = 9.46, P < 0.001), likely caused by the earlier maturation of male individuals compared with female individuals. In conclusion, firstly, populations are sensitive to changes in the growth rate of individuals, with small reductions in individual growth rates resulting in large reductions in population abundance. Secondly, fecundity reductions often observed via endocrine mediated effects may be compensated for in wild populations due to density dependent processes. Thirdly, although feminisation of fish by endocrine disruptors is commonly found, sex ratio bias is not necessarily a cause for concern at the population level in its own right.

ET11B-4

An agent-based model of woodpigeon populations and its use for risk assessment.

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European regulations concerning pesticide risk assessment focus on the protection of populations [1,2]. However, field experiments to measure this risk are expensive, logistically challenging and involve extensive animal experimentation. Population modelling seems to be one of the best ways of extrapolating to the population scale from individually measured effects and data on the behavioural ecology of the species at risk. Agent-based models (ABMs) that incorporate effects of intraspecies variation and landscape use can minimise the need for animal testing in higher tier risk assessment. In ABMs each individual animal can be modelled separately, and, in contrast to standard population models, we do not need to guesstimate the values of parameters describing population dynamics. Instead the population dynamics emerges from the system in a way that depends on the input parameters describing individuals and their modelled behaviour.

We have developed an ABM of woodpigeon population that can be used for risk assessment of new pesticides. Woodpigeons were chosen because they are an exceptionally vulnerable species, a consequence of their mostly herbivorous diet which is mainly gathered in agricultural terrain. They feed extensively on oilseed rape, sown cereals and leafy vegetables. In the past there have been incidents of woodpigeons poisoning ascribed to pesticide use.

In the model we included life history traits, mating and reproductive behaviour and winter flock formation to simulate the annual cycle of the birds. The rules of daily choice of feeding grounds and type of preferably collected food were also specified. We included the possibility of pesticide application and so can compute its effects on the woodpigeon population. The emergent properties of the model are statistics describing the population dynamics, including population size, growth rate and recovery time after disturbance.

The results from the model have been compared to the observations gathered during the long-term woodpigeon study in Carlton, Cambridgeshire, UK [3].

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ET11B-5

Towards good modelling practice: TRACE, a standard for documenting ecological modelling in chemical risk assessment

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Ecological models are becoming increasingly important in the context of chemical risk assessments. Due to the interaction of numerous factors, and the extent of temporal and spatial scales of concern, empirical approaches often are too limited to inform decisions or regulations that are aimed at the population or ecosystem level. Ecological models have the potential to bridge this gap. However, no general guidelines exist for the development and use of ecological models. Such guidelines for good modelling practice would be essential for quality assurance of ecological models in the context of chemical risk assessments, and would provide a tool for regulatory agencies to assess the usefulness of models in specific contexts. We present the first step towards the implementation of a good modelling practice: TRACE, a standard framework for the documentation of ecological models and the underlying modelling process. TRACE covers model development, evaluation, and application. By providing a document that applies the proposed framework, modelling projects become transparent, and decision makers as well as potential peer reviewers can assess the quality and usefulness of a model for the problem at hand. Thus, the proposed model documentation framework is the basis for the compilation of an actual guideline for good modelling practice in risk assessment contexts. Using example models, we will demonstrate how TRACE documents are compiled and evaluated.

ET11B-PS

Poster spotlight: Matrix models vs IBMs for the analysis of life-cycle tests and multi-generation tests data

Miscellaneous

Poster spotlight highlighting abstracts TH 149, TH 150:
- Analysis of multi-generation data for Chironomus riparius exposed to uranium-spiked sediments using a DEB-based population dynamics model
- Effects of uranium in Daphnia magna exposed over three successive generations: extrapolation of DEBtox analyses to the population level
- Modelling harpacticoid copepod populations; matrix and individual based modelling

ET12 - Metals and metalloids in the environment: adaptation, bioavailability and speciation

ET12A-1

Key role of the resin thickness on the metal flux and lability degree of complexes measured with diffusion gradients in thin films (DGT)

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Diffusion gradients in thin film devices (DGT) (1-3) has emerged as a powerful technique for in situ measurement of metal fluxes. It consists in a resin layer that strongly binds the metal ions, covered with a gel layer that defines a diffusion domain.

Metal accumulation in DGT devices depends on both the free metal present in the system and the complexes able to dissociate in the time scale of the experiment. A quantitative measure of the complex contribution is the lability degree.

As penetration of complexes into the resin layer is a characteristic feature of the DGT devices, expressions reported for the lability of complexes in voltammetric sensors (4) are, in general, not applicable to DGT

The binding of the free metal by the resin in the resin layer expands the effective reaction layer, i. e., the layer where there is net dissociation. This extension leads to an important increase of both the metal flux and the lability degree in comparison to the values expected when complexes are not able to penetrate into the resin phase. Experimental results of the Cd NTA system confirm these findings.

The limiting case (where metal concentration in the resin layer is negligible) is used to obtain analytical expression for the metal flux and lability degree. From these expressions, the impact of resin and gel thickness, kinetic constants, diffusion coefficients on the DGT response will be discussed, as well as the environmental consequences.

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[3] Unsworth, et al. (2005). Environ. Sci. Technol. 39, 624-630.
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ET12A-2

Development of a reliable and robust method for the detection of ng/L concentrations of Lipid - Soluble Metal Complexes in natural waters

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In natural waters trace metals are present in a wide range of physico - chemical forms or species.

It has been demonstrated that the toxicity of these trace metals to aquatic organisms is related in many cases to the activity of the free metal ion species. However, several studies have shown that the toxicity and bioavailability of Lipid - Soluble Metal Complexes (LSMC), formed by the reaction of metals with synthetic and natural organic ligands, may exceed that of the free metal ion. Surprisingly, there have been very few studies conducted that seek to identify and quantify LSMC in aquatic systems. This is mainly due to the fact that a reliable, robust and sensitive method for the determination of LSMC in waters has not yet been developed. In this research, a sensitive method for the determination of ng/L concentrations of lipid soluble cadmium, copper, nickel, lead and zinc complexes in waters was developed. Waters were extracted under clean room conditions using dialysis cells containing 1-octanol. The metals were then preconcentrated by vacuum distillation, back-extracted into nitric acid and determination and quantified was performed by Inductively Coupled Plasma Mass Spectrometry. By using dialysis membrane the water and solvent are physically separated ensuring the solvent does not become contaminated with colloid material which may positively bias the results. The pore size of the dialysis membrane still allows for the diffusion of LSMC into the solvent. Detection limits for the method were in the low ng/L allowing the developed method to be used to determine the concentrations of LSMC in a range of aquatic environments.

ET12A-3

Effect of organic complexation on copper accumulation and toxicity to the estuarine red macroalga Ceramium tenuicorne: a test of the free ion activity model

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Current water quality criteria (WQC) regulations on copper toxicity to biota are still based on total dissolved (< 0.4 micron membrane filter) copper concentrations with a hardness modification for freshwaters. There are however ongoing efforts to incorporate metal speciation in WQC and toxicity regulations (such as the biotic ligand model-BLM) for copper and other metals. Here, we show that copper accumulation and growth inhibition of the Baltic macroalga *Ceramium tenuicorne* exposed to copper in artificial seawater at typical coastal and estuarine DOC concentrations (similar to 2-4 mg/L-C as fulvic acid), are better correlated to weakly complexed and total dissolved copper concentrations rather, than the free copper concentration [Cu2+]. Our results using a combination of competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) measurements and model calculations (using visual MINTEQ incorporating the Stockholm Humic Model) show that copper accumulation in *C. tenuicorne* only correlates linearly well to [Cu2+] at relatively high [Cu2+] and in the absence of fulvic acid. Thus the FIAM fails to describe copper accumulation in *C. tenuicorne* at copper and DOC concentrations typical of most marine waters. These results seem to indicate that at ambient total dissolved copper concentration in coastal and estuarine waters, *C. tenuicorne* might be able to access a sizeable fraction of organically-complexed copper when free copper concentration to the cell membrane is diffusion limited.

ET12A-4

Toxicity of metal mixtures to Daphnia magna: a test of a multi-metal, multi-site biotic ligand model using Cu and Zn

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For decades, a simplistic summation of toxic units based on concentrations of dissolved metals has been used to semi-quantitatively predict the toxicity of metal mixtures. However, that approach tends to over-predict toxicity. In its place, we have been developing a mechanistic model based on tissue residues of metals and the concept that the toxicity of a mixture of metals can be either dose additive or response additive, depending on the mechanisms of action. To calculate tissue residues and thereby predict toxicity across wide ranges of water quality, we have been developing a multi-metal, multi-site biotic ligand model (MMMS BLM) that concurrently accounts for metal-metal competition for binding on dissolved ligands in the water and at sites of toxicity on organisms. In our initial tests, we exposed *Daphnia magna* to mixtures of Cu and Zn in moderately hard reconstituted water containing dissolved organic (DOC; added as Suwannee River fulvic acid) at 3 mg/L, and compared observed mortality to the response-additive mortality predicted from results of Cu-only and Zn-only toxicity tests. This research has revealed several apparent metal-metal interactions that otherwise might lead to conclusions that metals interact in non-additive ways, yet simple geochemical speciation in the BLM can explain these interactions and reconcile the apparent non-additive toxicity. For example, the toxicity of Cu-Zn mixtures always appeared to be synergistic or additive when based on dissolved metal concentrations, whether Cu was varied while Zn was held constant, or vice versa; whereas in the same tests, the toxicity of the Cu-Zn mixtures always appeared to be antagonistic or additive when based on free-metal-ion concentrations. These preliminary results demonstrate that a MMMS BLM could be an effective tool to help regulatory agencies implement more appropriate methods to regulate metal mixtures than the current default, overly conservative toxic-units approach.

ET12A-5

Effects of chronic nickel exposure on algae, zooplankton and snails in a semi-realistic microcosm

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Until now the aquatic risk assessment for Ni has been based on a large set of single species tests. In order to refine this assessment a microcosm study was performed to evaluate the impact of dissolved nickel on an aquatic community including phytoplankton, zooplankton, periphyton and snails. The study was conducted in 14 microcosms (including a natural sediment layer of 20 cm and an overlaying water volume of 750 L) located in a greenhouse at the Fraunhofer IME in Germany. After a pre-treatment period for establishing the populations, a Ni solution was added to reach concentrations of 6, 12, 24, 48 and 96 µg Ni/L in two microcosms each. Four microcosms served as untreated controls. To achieve constant exposure, appropriate amounts of nickel solution were added over the whole exposure period of 4 months, mostly on a daily basis. Ni concentrations were analytically determined frequently (at least weekly) in each microcosm over the 16 week exposure period. Parameters known to affect Ni toxicity, including water hardness, pH, and dissolved organic carbon (DOC), were also measured represented conditions of high bioavailability. Population and community level indexes were determined for phytoplankton, zooplankton, periphyton, and snails. No adverse effects for these endpoints were found at concentrations below 48 µg Ni/L. Only minor, inconsistent, and/or temporary deviations from controls, i.e., for single sampling dates, were found for a few taxa at concentrations below 48 µg Ni/L. However, these deviations were not related to clear dose-response relationships and not found at the end of the study. Considering the stable exposure over 4 months, these deviations are not seen as adverse effects of Ni exposure. In contrast to this, at 48 and 96 µg Ni/L long-term effects on phytoplankton, rotifers, snails and, due to reduced grazing by snails, indirectly on the periphyton biomass were observed. Thus, the overall NOEC for a 4 months exposure to nickel in this microcosm study is considered to be 24 µg Ni/L.

ET12A-PS

Poster spotlight: Implementation of metal bioavailability in surface water standards

Miscellaneous

Poster spotlight highlighting abstracts WE 148, WE 149, WE 150, WE 151:
- Spatial and temporal variation in bioavailability and species sensitivity of Cu, Ni and Zn in surface waters of The Netherlands
- Delivering a practical methodology to account for metal (bio)availability in the Water Framework Directive - tools and techniques
- Spatial risk assessment of nickel in surface waters of Great Britain
- Water quality standards for silver in Dutch surface waters - A proposal in accordance with the Water Framework Directive

ET12B-1

Hazard assessment of inorganic selenium under REACH: a pragmatic approach

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Selenium is an essential element showing a very narrow margin between dietary essentiality and toxicity. So far, the main focus of the extensive research on selenium has been the environmental fate and effects of the element in the aquatic environment. The available scientific knowledge indicates that diet is the primary exposure pathway for both aquatic invertebrates and vertebrates and that selenium toxicity is primarily manifested as reproductive impairment in egg-laying vertebrates (fish and birds) due to maternal transfer. The severity of the observed adverse effects appears to be more related to tissue concentrations than to aquatic concentrations of the element and selenium toxicity seems to be largely species- and site-specific. Therefore, it is generally accepted that a single predicted no effect concentration (PNEC) based on aqueous Se concentrations would not be appropriate for protecting against selenium toxicity in all systems and that selenium requires site-specific risk assessment to a much greater extent than many other contaminants. However, the European REACH Regulation and other regulatory risk assessment methods traditionally require the derivation of a single threshold concentration of a substance for each environmental compartment (e.g. water, soil, sediment) based on toxicity data for the most sensitive species and ecosystem. For selenium, such an approach would entail the risk of defining PNEC values within the deficiency range for many systems. Since REACH and similar regulations are pushing the limits with regard to timely submission of chemical safety reports, they also force difficult substances such as selenium to be assessed in a pragmatic way using the scientific knowledge already available. A pragmatic approach for the aquatic and terrestrial hazard assessment of selenium under REACH will be presented and the potential for further refinement will be discussed.

ET12B-2

Determination of free metal concentrations with AGNES-SCP

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Gaining access to the free concentration of a variety of metals ions, such as Zn, Cd, Pb or Cu, is key to predict the role and fate of nutrients and pollutant elements in different natural environments like fresh water [1]. Consequently, there is a demand for methodologies that allow the direct quantification of metal ion concentrations under environmentally relevant conditions and at trace level. AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is an electroanalytical technique specifically designed for the determination of free ion concentrations with great environmental interest [2]. Consits of two stages: i) a deposition stage in a mercury amalgam, until equilibrium with the solution is reached; ii) a stripping stage where the element is quantified. Typically, AGNES has been applied with mercury electrodes (HMDE), but, recently, also with screen printed ones (SPE) which could allow to perform in situ measurements due to their small size [3]. In this work, we introduce Stripping Chornopotentiometry (SCP) as the second stage of the technique in order to better deal with possible interferences in complex samples. Suitable experimental conditions have been found for the successful application of the combination of AGNES and SCP both with HMDE and SPE. Calibrations with solutions containing Zn2+, Cd2+ and Pb2+, either alone or the three together, have been carried out satisfactorily. Moreover, speciation studies of Cd2+ in presence of Pb2+ confirm that AGNES-SCP allows to easily determine the free metal concentration in solutions containing interferents. Finally, this approach was tested on a fresh water sample from Garona river taken in Val d'Aran (Spain) and it could be possible to compute the free Zn2+ concentration which was around 30% of the total concentration.

[1] Tessier et al. 1994. Chemical and Biological Regulation of Aquatic Systems. Lewis Publishers, Boca Raton, FL, 197 p. (Chapter 6).
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[3] Parat et al. 2011. Electroanalysis. In press.

ET12B-3

Life-cycle traits of Porcellio dilatatus exposed to different Cd species: effects on survival and reproduction

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The woodlouse *Porcellio dilatatus* (Crustacea) is a suitable model species for the examination of toxic effects following metal assimilation and accumulation. In this study, the influence of cadmium speciation in survival and reproduction of isopods was investigated. Survival and reproductive parameters (pregnancy and abortion occurrence, number of juveniles per female and juvenile weight) were recorded when isopods were exposed to two species of Cd deployed in food: $Cd(Cys)_2$ or $Cd(NO_3)_2$. There was a difference between survival rates of exposed males and females to both Cd species but in the case of $Cd(NO_3)_2$ these differences were more accentuated, with females having higher survival rates. In the presence of both metal species a reduction of pregnancies was observed, but in the case of $Cd(Cys)_2$ all pregnancies were inconclusive. The

number of juveniles delivered per female fed with $Cd(NO_3)_2$ contaminated food was lower than in the control but the juvenile weights were higher. As far as we are aware, the present study is the first toxicity test demonstrating that metal speciation affects reproduction. $Cd(Cys)_2$ showed to be more toxic in this long term exposure and to jeopardize completely the reproduction effort of isopods.

ET12B-4

Solid-phase applications for biosensor deployment in predicting the biotoxicity of heavy metals in soils

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Assessment of bioavailability of contaminants in the environment is important in the context of hazard assessment and remediation. Traditionally, the pollutants have been assessed by using only chemical assays or evaluating physical parameters. To complement this, biosensing systems have been developed to integrate with traditional assays.

In recent years, reporter-gene constructed bacteria (biosensor) for specific pollutants have been successfully applied for heavy metals. The stress induced by toxic compounds can cause disruption to the metabolism of organisms. So once changes occur, it is essential to quantify this impact. In this study, the bioluminescent biosensors produces light which correlates with the dose present in presence of target analytes (Cu and Zn). Understanding the speciation of heavy metals and its relationship with biological responses is an important factor for the soil assessment.

So far, the application of microbial biosensors has relied solely on the extraction of pore water and the subsequent exposure to the test organisms. This fails to consider the complex environment of the soil solid phase which is likely to host most of the labile and bioavailable pollutants and be the more dynamic in both space and time to perturbations.

The deployment to soil will be likely to be coupled with an approach such as "rhizon sampler"where the solution will be extracted from the soil and then assimilated onto the solid phase extraction (SPE) surface. In this study, a range of novel solid phase devices were compared to assess the reproducibility of given assays and their relationship with aqueous phase assays.

In general, the toxicity and bioavailability of heavy metals in solid phase was lower than in aqueous phase demonstrating the need to measure both phases and develop a relationship between them and the likely mobility and partitioning of the target analytes.

Future research will consider the bioavailability of hydrocarbons and in addition to assessing its related toxicity. This will be further inferred to a reliable method of the potential for biodegradation and bioremediation.

ET12B-5

Plant toxicity of 5 years aged Pb salts in soil in relation to recent amendments

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Lead toxicity data derived from soils freshly spiked with Pb salts may overestimate Pb toxicity under field conditions because of a reduced mobility and lower soil solution ionic strength in field contaminated soils. This study was designed to quantify the difference in Pb toxicity between soils dosed with $Pb(NO_3)_2$ left to age 5 years outside with natural leaching, soils freshly dosed with PbCl2 and soils freshly dosed with $PbCl_2$ leached with artificial rainwater. Toxicity was assessed with plant growth (*Lycopersicon esculentum* L.). Leaching of the spiked soils resulted in significantly (p=0.05) higher EC50 values of Pb in soil compared with the EC50 values of Pb of the spiked but unleached soils. Lead in aged soils is still less toxic than in spiked + leached soils, suggesting that ageing does reduce toxicity. To extrapolate toxicity values obtained with laboratory spiked soils to field relevant situations a leaching/ageing factor is needed. This study explain that both processes contribute to the observed discrepancies.

ET12B-6

Trace metal fate and uptake by vegetables grown in close proximity to traffic in Toronto, Canada

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Existing knowledge regarding the processes regulating metal bioavailability under natural conditions and their uptake by plants has been identified as a primary limiting factor in the development of reliable soil quality guidelines. Much of the information about metal dynamics in soils, and the role of various physicochemical characteristics incorporated in more advanced fate models, has been derived from controlled lab studies, using artificially amended soils. Recent evidence suggests that metals present in field soils, which have undergone natural aging processes, are comparatively much less likely to be bioavailable. This highlights the need to further examine the effects of metal aging in soils under natural conditions to ensure that soil quality guidelines are more reflective of the risks trace metals pose in the urban environment.

This research examines the fate of traffic-related trace metal emissions and their uptake by plants grown in Toronto, Canada. Oregano (O. heracleoticum), eggplant (S. melongena) and beets (B. vulgaris) were cultivated at several locations with predicted variable metal inputs over the growing season in 2010. At one location, which is situated at a busy intersection on the St. George campus location of the University of Toronto, the top 30 cm of soil was replaced with an organic, triple mix soil. The same soil was used to cultivate plants in containers at the other two locations. In addition, oregano (O. heracleoticum) plants were planted at three other sites located close to several major roads, which have some of the highest traffic densities in Toronto (planted in existing soil). Preliminary results from the first phase of this study to examine trace element stabilization processes in aging field soils indicate a rapid accumulation of trace metals in newly remediated soils in close proximity to traffic (e.g. Mo, Cd, Pb, V, Cr, Co, Ni, Cu and Zn). At the same time, the rhizosphere soil of cultivated eggplant (S. melongena) has significantly lower trace metal concentrations compared to bulk soil measurements (e.g. Cd, Pb, Cr and V). The physicochemical differences in the bulk soil compared to the rhizosphere soil, and the associated differences in trace metal concentrations, require further attention in attempts to develop soil quality guidelines. Finally, soil contamination is clearly not restricted to urban areas, as is often assumed, but may be more a reflection of regional contaminant levels.

ET12C-1

Metals incorporation and physiological changes in Ulva spp. as responses to the nocturn pulse of metals from sediment in eutrophic systems - a field transplantation experiment

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Coastal lagoons with symptoms of eutrophication often present low oxygenated waters, particularly during the night. Under such conditions, sediment could release metals to the overlying water. Consequently, inhabitant organisms may accumulate metals that were provided from the sediment, which could be on the basis of adaptative responses such as those related with the protection against oxidative stress. Keeping this in view, it is relevant to clarify if the macroalgae Ulva ssp. transplanted in short-time exposures (during 24 hours) respond to the increase of metal availability both in terms of metals accumulation and changes of its physiological status. A field transplantation experiment was performed with Ulva spp. in three short-time exposures (between 15:30-00:30; 00:30-07:30; 07:30-15:30) along a period of 24 hours in summer 2007. This study was carried out in a coastal lagoon previously considered to be a paradigm of eutrophic conditions and moderately contamination by metals (ð'bidos lagoon, Portugal). In summer, dissolved oxygen in a confined branch of the lagoon (Barrosa branch) could fluctuate pronouncedly along a day-night cycle. Ulva spp. was collected at a reference site and transplanted to Barrosa branch and a site near the reference (Lower lagoon) in order to evaluate the uptake of metals and oxidative stress effects. Water quality (including metal levels in water) was also characterised along the 24-hours cycle.

Dissolved oxygen at Barrosa branch varied between 40% and 190% saturation levels, being the most elevated values recorded at daylight hours and lower oxygenation during the night. Increase of ratios to Al of particulate Mn, Fe and Pb during the night revealed an additional enrichment of these elements in the suspended particulate matter. This enhancement pointed to a supplementary input of metals from the sediment occurring during the night. Ulva spp. translocated to the Barrosa branch incorporated significantly higher levels of metals in comparison to Lower lagoon, indicating that the algae respond within hours to the higher metal availability. An induction of SOD and an inhibition of CAT were recorded in Ulva spp. transplanted to Barrosa branch (between 0:30 and 7:30), eventually as a response to the higher incorporation of Mn, Fe and Pb. Ulva spp. incorporated metals and exhibited physiological changes within hours exposures, reflecting the pulse of metals from sediment during the night.

ET12C-2

Kinetics of mercury bioaccumulation in the polychaete Hediste diversicolor and in the bivalve Scrobicularia plana, through a dietary exposure pathway

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Mercury bioaccumulation kinetics of two important macrobenthic species, the polychaete Hediste diversicolor and the bivalve Scrobicularia plana were evaluated following a dietary pathway (i.e. contaminated algae), through a mesocosm laboratory experiment.

Both studied species presented a similar model of Hg bioaccumulation kinetics, a linear pattern of accumulation through time being the mercury accumulation in the organisms proportional to the mercury concentration in the food. Moreover, the mercury bioaccumulation rates were higher in the polychaete H. diversicolor than in the bivalve S. plana, which can be related to their feeding strategies, ingestion rates and assimilation efficiencies.

Comparing the effect of different exposure pathways (food versus sediment) on the mercury bioaccumulation rates, we may infer that mercury uptake via food (i.e. particulated macroalgae) is a major pathway of metal bioaccumulation for the polychaete H. diversicolor, while for the S. plana it seems to be the sediment. Moreover, the mercury bioaccumulation process through the dietary pathway, revealed to be faster than through sediment exposure, especially for the polychaete, which can represent a non-negligible risk for Humans.

ET12C-3

The impact of improved oxygen concentrations on the bioavailability, accumulation and toxicity of sediment-bound metals in freshwater invertebrates

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The present study evaluated the effect of improved oxygen concentrations in overlying surface water on the bioavailability, accumulation and toxicity of sediment-bound metals in the aquatic invertebrates *Lumbriculus variegatus* and *Asellus aquaticus*. A 60 days lab experiment with natural metal-polluted sediment containing high amounts of organic matter and AVS was conducted in 2 different experimental containers. At 6 different time points (after 0, 2, 5, 12, 32 and 54 days) the total metal content, Acid Volatile Sulfides (AVS) and organic matter was measured in the sediment. In addition the metal bioavailability in both water and sediment was measured using Diffusive Gradients in Thin films (DGT) and metal accumulation together with toxicity endpoints was analyzed in the invertebrate species. Our results indicated that SEM_{Tot}-AVS levels in the upper layer of the sediment (0-1 cm) significantly increased after exposure to elevated oxygen concentrations in the surface water. The glycogen content of the organisms in the high oxygen treatment did not significantly differ between the exposure dates and only after 54 days a significant difference in glycogen content between the treatments was noticed. Furthermore no significant correlations could be found between SEMTot-AVS in the sediment and the glycogen content in *Asellus aquaticus*. These results indicate that after almost 60 days of elevated oxygen concentrations in the surface water, metal release from the sediment has not increased enough to induce toxic effects in water-inhabiting invertebrates like *Asellus aquaticus*. Further work of this study will investigate the metal concentrations in DGT and the accumulation in the aquatic invertebrates in order to reveal to which extent metals can be mobilized and accumulated from sediment after increased oxygen exposure.

ET12C-4

Adaptation of copper community tolerance levels after transplantation of biofilms in an urban river

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The aim of this study is to investigate the adaptation of tolerance levels of biofilms transplanted either from a non-contaminated area to an urban-contaminated site or, conversely, from a contaminated area to a non-impacted site. For that purpose, natural fluvial biofilms were collected upstream the urban area of Paris (site A) and transplanted downstream from Paris (site B2), and conversely, biofilms were collected in the urban area of Paris (site B1) and transplanted upstream (site A). Cu tolerance levels of biofilms (both transplanted and original communities) were measured, using a previously developed short-term toxicity test based on the measure of beta-glucosidase activity, just before transplantation and then 15 and 30 days later. Automated-Ribosomal Intergenic Spacer Analysis (ARISA) was used to analyze similarities in both bacterial and eukaryotic community structures of the biofilms.

Biofilms' Cu tolerance levels varied significantly with transplantation: an increase of tolerance was measured for the biofilm TA transferred upstream (site A) to downstream (site B2) and a decrease of tolerance was measured for the biofilm TB1 transferred downstream (site B1) to upstream (site A). Moreover, ARISA fingerprints revealed that both bacterial and eukaryotic community structures were impacted by transplantation. Principal Component Analysis of ARISA profiles grouped upstream (A and TB1) communities on the one hand and downstream (TA and B1) communities on the other hand.
The study shows a fast adaptation of both Cu tolerance levels and community structure of biofilms subjected to a drastic change of environmental surroundings by transplantation.Therefore, transplantation has a predominant impact on periphyton both in terms of community structure and tolerance levels. This study confirms that tolerance measurements are sensitive indicators of metallic exposure levels.

ET12C-5
Exploring genomes to understand how populations are shaped by the environment
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Environmental genomics seeks to understand how gene function is influenced by environmental conditions while accounting for variation that exists within and among natural populations. We apply environmental genomics to link gene-environment interactions to the fitness of individuals and to population-level outcomes in natural populations of Daphnia pulex living near smelters in the Sudbury region of Ontario that have faced severe metal stress for over 100 years. These studies are possible because (1) well annotated genome and transcriptome sequences are available for this species whose ecology is well understood and (2) sophisticated tools for high-throughput biology allow for functional interrogation of the sequence information. We identified genotypes living in lakes from this region that have genetically adapted to cadmium stress. These isolates show no differences in their life history parameters when comparing control and cadmium exposed Daphnia. By contrast, cadmium exposures significantly decrease reproductive success in non-adapted Daphnia. Our studies also indicate that no fitness costs are imparted on these adapted Daphnia in the absence of metals, which differs from our observations of animals physiologically acclimated to cadmium through multiple generations. Adaptation produced different patterns of gene expression in metal exposed Daphnia. To explore the genomic basis for gene-expression differences, gene copy number was mapped across the entire transcriptome of metal-adapted and non-adapted Daphnia and these were compared to the sequenced reference genome. A large amount of copy number variation (CNV) was observed between individuals, including interesting contributions of CNV to the adapted phenotype. For example, cadmium adapted Daphnia contain additional copies of several genes that result in the increased dosage of transcripts for genes that contribute significantly to the adapted phenotype. These studies which begin to detail the genomic basis for adaptation in natural populations both contribute to and benefit from the Daphnia Genomics Consortium.

ET12C-PS
Poster spotlight: Adaptation reactions to toxic trace metals
Miscellaneous
Poster spotlight highlighting abstracts WE 171, WE 172, WE 173, WE 174:
- Multi-generation exposure of the midge Chironomus riparius to three model toxicants
- Can we expect genetic adaptation to metals at conventionally derived HC5 values?
- Sensitivity to cadmium of Carcinus maenas populations from two NW Portuguese estuaries with different levels of contamination
- Multi-generational exposure of Folsomia candida to Cadmium: effects on survival, reproduction and growth

ET13 - Moving towards a systems biology approach to predictive ecotoxicology

ET13-1
Mapping drug physicocChemical features to pathway activity reveals molecular networks linked to toxicity outcome
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The identification of predictive biomarkers is at the core of modern toxicology. So far a number of approaches have been proposed. These rely on statistical inference of toxicity response from either compound features (i.e. QSAR), in vitro cell based assays or molecular profiling of target tissues (i.e. expression profiling). We have previously published an analysis strategy that integrates pathway activity indices derived from gene expression data with physico-chemical features (PCFs) derived using QSAR and its application to a rat model of renal tubular degeneration. Our results indicated the importance of signalling pathways in response to chemical exposure. To identify pathways within a species important in eco-toxicology we have applied this method to a dataset derived from *Daphnia Magna*. Similarly to our previous results in *Rattus Norvegius*, *Daphnia Magna* also seems to respond to chemical exposure by modulating signalling pathways, suggesting an existence of a general toxicity mechanism, shared between the two different species, in synergy with more individually specific single-target based mode of actions.

ET13-2
A network biology approach to ecotoxicology reveals novel pathways linked to environmental chemical exposure
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Omics techniques have previously been applied to study marine pollution, but hitherto linkage to health outcomes and prediction of the composition and molecular mechanisms of action of complex pollutant mixtures has been lacking. To address these challenges, we have used a network inference approach to integrate multi-level datasets representing European flounder fish sampled from seven locations in the Irish Sea and North Sea.
Fish sampled from seven different sites in the North Sea were characterised at molecular and physiological levels using an unprecedented set of assays including histopathology, standard biomarkers, microsatellite markers and hepatic transcriptomics and metabolomics. Encouraged by the observation that the overall molecular state of the fish liver is predictive of the profile of contaminants at different sites and with the purpose of identifying molecular pathways linked to adaptation and adverse outcome, we set to develop an interpretative framework based on inference of a molecular network integrating the multi-level datasets.
This approach allowed the identification of two sub-networks whose activity was predictive of environmental exposure and linked to morphometric indexes such as liver weight. At the functional level these were representing both known and candidate novel adverse outcome pathways. Novel pathways were representative of several aspects of human liver pathophysiology such as liver hyperplasia, fibrosis, and hepatocellular carcinoma

ET13-3
Modeling spatio-temporal dynamics within living cells
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Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP) represent an important class of environmental contaminants. PAHs are ubiquitous contaminants derived from tobacco smoke, automobile exhaust or incomplete combustion of organic matter in general. They exert a wide range of toxic effects including carcinogenic, immunosuppressive or pro-inflammatory responses. PAHs are known ligands of the aryl hydrocarbon receptor (AhR) signalling pathway. AhR is a ligand activated transcription factor important for detoxification of environmental agents, toxicology, the induction of inflammatory signals or the oxidative stress response.
Some central aspects of the AhR signalling pathway are understood: contaminants enter the cell, distribute in the cytosol and different cellular organelles and interact with the cytoplasmic AhR. Binding to the AhR triggers translocation of the receptor/ligand complex to the nucleus, the association with the AhR nuclear translocator and the interaction with xenobiotic responsive elements (XREs) at the DNA. This usually leads to enhanced expression of a number of genes, which are presumed to play a major role in deleterious effects of PAHs. In spite of this general knowledge of AhR-mediated signalling, little is known about the dynamic behaviour of AhR upon activation.
We observe the behavior of the cytoplasmic AhR as well as the AhR-complex inside the nucleus in living cells. We applied the Fluorescence Recovery After Photobleaching (FRAP) method which is a well known and widely used experiment to investigate parameters of motion and interaction. Within this work we present a general method to analyse FRAP data without using pre-assumptions. We applied the method to artificial datasets as well as real measurements on AhR. The analysis of the AhR measurements e.g. lead to predictions on concentration- and time-dependent binding of AhR to the XREs and might help to understand the quantitative relationship betweeen DNA bound AhR and the transcriptional response. Deduced parameters were used to set up a 3D simulation of AhR distribution upon activation based on realistic cell geometries. We introduce a general approach to determine parameters of dynamic processes inside the cell. This approach is applicable to various cellular systems expressing an fluorescent molecule of interest. The parameters deduced can be used for various applications to gain knowledge on the behaviour of cellular systems in different microenvironments.

ET13-4
Predicting the reproductive fitness of Daphnia magna from metabolic signatures
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Molecular biomarkers have considerable potential as diagnostic tools in environmental toxicology and ecological risk assessment. One of the greatest challenges in environmental biomarker research is discovering molecular markers that are truly predictive of whole animal fitness and hence have ecological value, such as growth, energetic status and reproductive output. In this presentation we will describe a series of toxicity studies of Daphnia magna (the water flea). Specifically we have utilised mass spectrometry based metabolomics to measure the metabolic responses of individual daphnids to cadmium, propranolol and dinitrophenol, and in addition have measured their reproductive fitness (in terms of the number of neonates produced over a 21-day period). Multivariate regression analysis was then used to build mathematical models that can predict the whole animal reproductive fitness from the molecular signatures. In addition, multivariate classification methods were used to find metabolites that could predict the metabolic toxicity of these chemicals. Taken together, these findings highlight the genuine possibility that metabolomics can discover biomarkers that provide information on both molecular mode-of-toxicity as well as more ecologically relevant consequences for the whole organism.

ET13-5
Marine diatom Thalassiosira pseudonana towards a system biology for the water quality assessment
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Diatoms are eukaryotic algae, living in oceans and freshwaters. They play a key role in the global carbon cycle, contributing to about 25% to the global primary production .
Genomics and proteomics tools have been used in the diatom T. pseudonana to study the effects of exposure to common aquatic pollutants. The purpose of such studies is to provide i) system biology approach to identify the key pathways linked to the exposure compounds, ii) selection of molecular biomarkers of exposure as early identification of water quality endangerment, iii) a more comprehensive substitute to mere water chemical analysis. We designed and customized a DNA Microarray to investigate the gene expression profile in T. pseudonana and used a quantitative proteomics approach based on isobaric tag labeling to identify and quantify the proteins which are regulated by chemical pollutant exposure. Benzo(a)pyrene, a polycyclic aromatic hydrocarbon (PAH), is one of well-known pollutants widely distributed in aquatic environments and it has been selected for pilot studies using a system biology approach. The diatoms were exposed to Benzo[a]pyrene (BaP) at a concentration which inhibits the growth by 30% . RNA and proteins were extracted to perform either the DNA Microarray analysis or quantitative proteomics. We identified more than 500 genes which were mainly invloved in processes e.g. oxidative stress, transcritpion regulation, lipid transport and metabolism and biosilification. At the protein level, although only 16% of the quantified proteins showed a regulation, five were regulated as well at gene expression level. One of these proteins is the silicon transporter 1 (ST1), an enzyme that is responsible for the uptake of silica, which is down regulated at both gene and protein levels. Furthermore, we showed that the BaP exposed cells reduced the uptake of silica from the media .
Our data shows that one of the processess clearly affected by BaP is the biosilicification process and we could link the gene-protein levels with the physiological state of diatom cells upon exposure to the pollutant. The use of a systems biology approch in diatoms will help to elucidate pathway/metabolic processes involved in the mode of action of pollutants and identitiy molecu-

lar biomarkers of exposure. This information can be used for the assessment of water quality in a more comprehensive way than other established protocols currently in use.

ET13-6

Distinguishing regulatory and toxic transcriptional signatures of xenobiotic compounds

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Elucidating the transcriptional response of cells to chemical exposure is of primary importance for a reliable risk assessment of xenobiotic compounds like environmental pollutants. However, often such compounds trigger a complex cellular response. Dissecting these responses and identifying the transcriptional profiles associated with each individual (sub-) effect is essential for explaining side effects and predicting toxic responses of environmental contaminants.
One of the most studied transcription factors involved in the response to environmental pollutants or xenobiotic compounds in general is the aryl hydrocarbon receptor (Ahr). The Ahr has been studied for decades mainly because of its critical role in xenobiotic-toxicity and carcinogenesis. These well described toxic effects of Ahr stimulation are mainly due to the self induced activation of Phase I/II metabolizing enzymes, creating metabolites mostly responsible for the ultimately toxic response.
Using transcriptome time series of murine hepatoma cells exposed to the environmental contaminant benzo(a)pyrene (B[a]P), we investigate the hypothesis whether time resolved transcriptional signatures of genes that are direct Ahr targets differ from the profiles observed for genes responding to the toxic metabolite of B[a]P benzo(a)pyrene diol epoxide (BPDE).
We demonstrate that machine learning can be used for identifying these characteristic signatures and for subsequently classifying genes as to whether they are direct Ahr-dependent targets or indirectly affected (BPDE-dependent) genes. This is accomplished by training a Random Forest (RF) classifier to learn the difference between genes responding to B[a]P exposure and secondary effects caused by BPDE. The trained classifier is then applied to all genes found to be significantly differentially expressed in our time series as a result of B[a]P exposure, and their roles as direct targets or secondary effects are predicted. In addition, the patterns learned by the classifier are used as a basis for performing weighted clustering.
Introducing a new clustering approach we were able to reliably separate direct Ahr targets, among them twelve transcription factors, from genes activated due to secondary effects. Distinguishing the transcriptional profiles associated with the primary target effect from those acting in parallel is essential for understanding possible toxic side effects of such chemicals.

ET14 - Natural toxins in ecotoxicology

ET14-1

Analysis of microcystin algal toxins in Lake Maggiore water (N-Italy) by SPE-UPLC-MS-MS

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Algal blooms can generally occur in nutrient rich calm fresh waters and low salinity marine areas. Usually these blooms form in mid to late summer, and tend to float near the water surface.
A special type of cyanobacteria (blue-green algae) can produce toxins, called microcystins, which are cyclic peptides. The most important (most often analysed and found) species is microcystin LR.
People (and other mammals) can get sick from microcystin toxins if they have direct contact with a toxic algal bloom by swallowing water, or by having skin contact. Microcystin poisoning can cause breathing problems, stomach upset, nausea, vomiting, diarrhea, headache, fever, allergic skin reactions (a rash or skin blisters), and even liver damage.
Lake water samples (400 ml) were extracted by solid-phase extraction (SPE) at neutral pH using Oasis HLB 200 mg cartridges; elution: 6 ml methanol. Analyses were performed by ultra performance liquid chromatography (UPLC) triple quadrupole mass spectrometry (tq-MS) using a 50 x 2.1 mm (particles 1.7 um) column; eluents: water and acetonitrile (both 0.1% acetic acid); injection volumne 5 ul; Waters Aquity UPLC coupled to an AB Sciex 5500 Qtrap MS-MS; microcystins can be detected in the positive or negative ionization modes.
Very high SPE recoveries of around 97 % (n=4) could be achieved (at neutral pH). UPLC-MS-MS sensitivity is in the low ug/L concentration range, and overall method detection limits (including SPE enrichment) are lying in the low ng/L concentration range.
Some water samples from Lake Maggiore (and other smaller lakes) taken at the end of August and beginning of September 2010 were analysed. Microcystin LR was detected in all samples at low ng/L concentration levels, with a maximum found at ~ 350 ng/L. In this sample, also microcystin RR and LF were detected. Contamination depends strongly on weather (wind) conditions and currents. No microcystin LR was found in tap water.
The formation of microcystins is favoured by high summer temperatures, nutrient-rich (eutrophication) and calm water conditions. In the future, contamination might increase due to changing climatic conditions. More spatially and temporally spread monitoring and research on other algal toxin is necessary.

ET14-2

Trophic transfer of microcystins from Lymnaea stagnalis (Gastropoda Pulmonata) to Gasterosteus aculeatus (Teleostei Gasterosteidae) and impact on the fish

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Due to eutrophication of freshwaters, the frequency of cyanobacteria proliferations is increasing worldwide. From 40 to 75% of cyanobacterial blooms produce hepatotoxins [e.g. microcystins (MCs)], endotoxins released in water during the cell lysis and which constitute a real threat for target organisms as gastropods (intoxication by absorption of toxic cyanobacteria or dissolved toxins). MCs mainly accumulate in the liver (or digestive gland) of metazoans where they interact reversibly (free MCs) or irreversibly (covalently bound MCs) with phosphatase proteins, leading to tissues destruction. We previously demonstrated that the gastropod Lymnaea stagnalis ingested MC-producing cyanobacteria and accumulated free and bound MCs. As bound MCs persisted after a 3-week depuration period and represented up to 90% of total MCs, L. stagnalis is potentially a MC-vector through the food web. The aim of this study is to evaluate if free and bound MCs accumulated in L. stagnalis tissues are transferred to the three-spined stickleback, Gasterosteus aculeatus, and what are the consequences on this latter in terms of: 1) MC accumulation and elimination, 2) histopathology of the liver, 3) oxidative stress response, and 4) behavioural changes. Fish accumulated MCs (up to 3.9 ± 0.1 µg g-1 DW) in the liver, kidneys, muscles, and gills, showed an increased activity of glutathione peroxidase in the liver associated with a moderate pathology, and a decrease of the locomotory activity. During depuration, from 92 to 100% of free MCs were eliminated in all organs excepted in muscles (from 6 to 58%), suggesting that MC accumulation in G. aculeatus after consumption of intoxicated gastropods may induce a second transfer in aquatic and terrestrial food web, and possibly a human contamination.

ET14-3

Is the invasive Dreissena polymorpha better adapted to cyanotoxin exposure than the native Unio tumidus?

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Freshwater mussels as *Dreissena polymorpha* and *Unio tumidus* with high filtration activities may accumulate cyanobacteria and their toxins during cyanobacterial blooms and lysis. Physiological adaptations to cyanotoxins enable organisms to live in cyanobacterial contaminated waterbodies. In contrast to *U. tumidus*, the invasive *D. polymorpha* seems to be a moderate sensitive species able to develop sustainable population in contaminated waterbodies. The population of Unionidae is endangered by water pollution, shoreline construction and competition with *D. polymorpha*. Competition is for food, as the habitats are different: the unionids need soft sediment, whereas *D. polymorpha* attaches to any kind of hard substrate, including shells of unionids.
This study compares the two species with regard to their detoxification capacity for microcystin, the most common cyanotoxin in freshwater. A further aim was to correlate the detoxification attempts to the physiological costs for the organisms. For this, the activities of biotransformation (glutathione S-transferase), antioxidant enzymes (superoxide dismutase and catalase) and energy reservoirs (glycogen-, lipid- and glutathion content) were compared in the invasive *D. polymorpha* and the native *U. tumidus* in response to cyanotoxin exposure (10 and 50 µgL-1 microcystin-LR) for 24 h and 7 d.
Enzyme activities of *D. polymorpha* were measured in whole mussel tissue, digestive gland and gills. Energy reserves were measured in whole mussel tissue. Enzyme activities and energy reserves of *U. tumidus* were measured in digestive gland, gills, mantle and foot. The sGST activities were increased for the whole exposure period in *D. polymorpha* but didn`t changed in *U. tumidus*. The catalase activity was not affected during the whole period in both species. The glycogen content decreased after 24 h in both species indicating the energy requirements due to the stress caused by the MC-LR exposure. We conclude that *D. polymorpha* is capable of detoxification of MC-LR but at expense of energy. The results suggest that *U. tumidus* is less able to detoxify MC-LR via biotransformation enzyme GST. Nevertheless, there was an enhanced requirement for energy, as indicated by reduced glycogen contents in both mussel species. Compared to *U. tumidus* the invasive *D. polymorpha* seems to be better adapted to cyanotoxin exposure.

ET14-4

The use of single ecosystemfunction for a sustainible removal of cyanobacterial toxins from water

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Cyanobacterial blooms are a worldwide problem due to the eutrophication of water bodies. Most the cyanobacteria known today are producing toxins, from which the microcystins are the most common detected. Over 70 congeners of microcystins have been isolated (Carmichael, 1997). Microcystins are known to be potent inhibitors of protein phosphatase 1 and 2A and also known to be tumor promotors. During senescence of cells the toxins are released into the water in high amounts. The microcystins itsself are very stable molecules and highly persistent in the water (DeFigueiredo et al 2004).
In many countries, namely the semi-aride and aride ones, freshwater lakes are used for aquaculture, spray irrigation of agricultural plants and also as a source for drinking water (Nimptsch et al. 2008). There are several studies on how to remove cyanobacteria respectively their toxins from the lake water. Some of these studies are using clay particles, or hey bale to get rid of the cyanobacteria. But in most cases the toxins itsself stay in the water body.
Aim of our research is to identify and use single ecosystem functions, which can help in remidiation of cyanotoxins from lake water in a sustainible way. The single ecosystem function are e.g. the biology of aquatic macrophytes and their ability to take up toxic substances.

ET14-5

Occurence of biotoxins in mussels from different coastal locations and risk management

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The Biotoxins (Red Tide) is an ecological and economic problem concerning aquaculture and tourisim in the coastal areas world wide. The neurotoxic, immunotoxic and genotoxic effects of domoic acid produced by the diatom *Pseudonitzschia multiseries* and saxitoxins (STX) paralytic shellfisch poisoning -PSP) produced by *Alexandrum catenella* and *Alexandrum ostenfeldii*. In addition the flagellate *Dinophysis acuta* produces the Diarrhetic Shellfish Poisoning (DSP). The need for increasing quality control at different critical steps of the food production chain has to be addressed by developing a set of rapid biomarker and sensor systems.

ET14-PS

Poster spotlight: Natural substances - new emerging contaminants in our environment

Miscellaneous

Poster spotlight highlighting abstracts TU 247, TU 248, TU 249:
- Epigenetic toxicity of cyanobacterial extracts: effects of ozonation and chlorination
- Acute and sub-chronic toxicity studies from Cereus jamacaru ethanol and aqueous extracts
- Identification of Protein Biomarker(s) Associated with Pacific Ciguatoxin (P-CTX-1) Exposure in Grouper (Epinephelus coioides)
- Effects of combined mixture of microcystin-LR and cylindrospermopsin on the growth of Chlorella vulgaris

ET16 - Soil ecotoxicology and quality assessment

ET16-1

Effects of slurry from sulfadiazine (SDZ) treated pigs on the structural diversity of microorganisms in rhizosphere soil

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Efficient agricultural production frequently includes the use of veterinary antibiotics, consequently, manures from antibiotic-treated animals are disposed on arable soils. Antibiotics, co-applied with nutrient sources like manure, are increasingly reported to change soil microbial community structures in un-rooted bulk soil. However, the antibiotic effects in the rhizosphere soil compartment are not well understood. Therefore, a 60-d mesocosm study with maize plants in Luvisol, treated with sulfadiazine (SDZ) by a single manure application was performed. We hypothesized, that compared to bulk soil, antibiotic effects are dissimilar in different soil microcompartments and even more pronounced in soil affected by the presence of maize roots. In contrast, while antibiotic effects on community changes were temporally significant in bulk soils, effects in rhizosphere could not be revealed, using endpoints such as total community DGGE or PLFA patterns. This finding was related to stronger dissipation, and thus lower bioaccessibility of SDZ in the rhizosphere. However, antibiotic effects in rhizosphere soil were detected when investigated on specific pseudomonas community level. These results show that it is not sufficient to investigate homogenized soil material but the heterogeneity of soils must be considered in ecotoxicology testing, which leads to a diverse effectiveness of contaminants.

ET16-2

Changes of metal availability over time in sludge-amended and metal-spiked soils. Does earthworm activity influence those changes?

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Total metal concentration is used to regulate sewage sludge application on soils. However, the real toxic risk posed by metals is determined by the fraction that is biologically available for the organisms, which is dependent on matrix of contamination (e.g. via sludge or metal solution) and various physical and biological processes (e.g. earthworm activity) which vary with time. Monitoring metal fate in soil over time using chemical extractions and measuring body residues in earthworms allows a clear understanding of changes in bioavailabilty and a better prediction of their effects. Comparative evaluations of changes in the availability over time in the presence and absence of earthworms of a certain pool of metals originating from sludge or from a metal spike in soil were performed. The objectives of this study were i) to evaluate changes in the availability of metals over time in metal-spiked and sludge-amended soils on short-term (under laboratory conditions) and long-term basis (under field conditions); ii) to assess the influence of the activity of a realistic density of earthworms on changes of total and extractable metal concentrations; and, iii) to measure metal uptake by earthworms from different test matrices. With these purposes, a laboratory and a field experiment were conducted. Treatments consisted of dose gradients of a sludge mainly contaminated with chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn; sludge-amended soils), and similar concentration gradients of the same pool of metals applied directly to the soil (metal-spiked soils). Half of the treatments were inoculated with a density of 500 Dendrobaena veneta per m2. Total and 0.01 M CaCl2 extractable metal concentrations were measured in soil samples and internal metal concentrations in the earthworms. Results demonstrated that the sludge matrix generally contributed to increased adsorption and reduced mobility of metals in soil. Environmental factors (e.g. plant roots and generality of edaphic fauna activity) induced decreases in metal extractability and the activity of a realistic density of D. veneta did not affect metal availability in test treatments over at least 12 months. Ni bioaccumulation was influenced by the test matrix and D. veneta was able to regulate its Zn internal concentration.

ET16-3

Evaluation of soil compaction effects on soil organisms and soil biological processes

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Investigations on soil compaction focused mainly on effects on soil physical parameters and on plant growth. Nevertheless, a substantial number of papers deal with effects of soil compaction on soil organisms (soil fauna, soil microorganisms) and biologically driven processes in soils (e.g., macropore formation, respiration rates, N-mineralisation). In view of soil protection and the protection of soil functions, there is an essential need to identify soil compaction threshold values with respect to soil biota and soil biological processes. No such values are currently available. Thus the aim of our study was to evaluate literature on the effects of soil compaction on soil organisms and soil biological processes (e.g., respiration, nitrification); to identify relevant parameters which are helpful for assessing soil compaction from the soil biological point of view; and to find out whether threshold values of soil structure parameters proposed by soil physicists correspond to harmful impacts on soil organisms and biological processes in soils. Our literature review showed that due to the high variability of experimental situations and conditions in the evaluated papers, especially in papers describing field investigations, no general effect of soil compaction was found. Negative and positive effects occurred with slight compaction as well as with strong compaction. A verification of the thresholds published to date for soil compaction was not possible based on the data evaluated. However, the fact that above an effective bulk density of 1.7 g cm-3, only negative effects on microbial biomass and C-mineralisation were found confirms this value, proposed by soil physicists, also from the soil biological point of view.

ET16-4

Biomarkers and energetic reserves in isopods: the effects of long-term exposure to dimethoate

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The aim of this work was to evaluate the biomarkers activity and energy reserves content in a long-term exposure to the pesticide dimethoate, followed by a recovery period.
Organisms were exposed to soil contaminated with dimethoate in concentrations of 0.4 mg/kg soil (real case scenario) and 10 mg/kg soil (below EC50 value), plus a control. Replicates of 5 animals were collected at time 0, 24h, 48h, 96h, 7-days, 14-days, 21-days and 28-days. After this period animals were changed to boxes with clean soil and replicates were collected after 7-days and 14-days of recovery.
Biomarkers acetylcholinesterase (AChE), lactate dehydrogenase, lipid peroxidation, glutathione S-transferases, glutathione peroxidation, catalase, glutathione reduced and glutathione oxidized along with energy content (lipids, sugars and proteins), energy consumption and cellular energy allocation were measured.
As expected a strong inhibition was observed in acetylcholinesterase as the main target of the pesticide. Although a previous study had stated that organisms have low survival chances under neurotoxic regimes i.e. where AChE inhibition is higher than 80%, we observed a 7 day survival period with AChE inhibitions higher than 90%. Other biomarkers as lipid peroxidation presented significant differences when compared with the control at periods where high mortality rates were counted.
Energy reserves content, energy consumption and cellular energy allocation rate significantly fluctuated along time exposure and recovery.
The results show not only correlations between several biomarkers, the energy reserves and mortality, but also with other detoxification processes not related with neurotoxicity.
The present work showed that several sub-individual biomarker activity can be used as early warnings for Environmental Risk Assessment, and that sub-individual effects can be linked with ecologically relevant parameters (e.g. mortality and energy reserves content)

ET16-5

Development of a new plant-based biotest to assess trace element phytoavailability in contaminated soils - Selection of target-plant species for standardisation

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Concerning the threat of soil contamination, the assessment of trace element phytoavailability at an operational level still requires the identification, the development and the standardisation of a set of biological methods (i.e. biotest). The present abstract introduces the first step in the development of a new plant-based biotest, the RHIZOtest, focused on the selection of the target-plant species suggested for the standardisation of the RHIZOtest.
The RHIZOtest is notably based on a complete physical separation between plant and soil compartments enabling an easy, fast and clean recovery of the roots. The RHIZOtest was deployed for ten plant species commonly used in agriculture and on three soils exhibiting a broad range of pH and a high concentration in several trace elements. The measurement of trace element phytoavailability was finally achieved as the mean flux of trace element to the plants during the exposure of the plants to the soil.
Seven out of ten plant species exhibited homogenous growth of roots and shoots and consequently can be used adequately in the RHIZOtest experimental procedure. As expected, plant uptake flux of trace elements varied significantly and many-fold among the ten plant species tested. However, trace element phytoavailability also broadly varied among trace elements and soils. Finally, a procedure of ordination and scoring enabled us to select three plant species that maximised trace element phytoavailability according to a precautionary-like principle. This three plant species will be suggested for further standardisation of the RHIZOtest.
This study supports the requirement of biological methods that enable to encompass the biological diversity in the assessment of trace element phytovailability whereas chemical methods are not able to take it into account. Such kind of validation procedure for a biotest is the unique opportunity for achieving operational methods based on a hard scientific background that could be standardised for the assessment of trace element phytoavailability.

ET16-6

Use of the Comet and micronucleus assays in the detection of genotoxic effects in Eisenia andrei coelomocytes after exposure of the earthworms to dioxin and B[a]P-spiked soils

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Many organic pollutants bring about not only general toxic effects, but are also genotoxicants, meaning they can alter both the structure and the integrity of DNA, either directly or indirectly. The evaluation of genotoxicity biomarkers is of particular importance, considering the relevance of these data in terms of possible carcinogenic risk not only for edafic communities but also for humans. In this study, earthworms of the species Eisenia andrei were exposed for 10 and 28 days to different sublethal concentrations of Benzo[a]Pyrene (B[a]P) (0.1, 10, 50 mg/kg) and 2,3,7,8 tetrachlorodibenzodioxin (TCDD) (1x10-4, 1x10-5, 2x10-3 mg/kg) mixed into a standard artificial soil. The selected B[a]P and TCDD concentrations were, beginning with the lower ones, the Italian law limit for residential areas, for industrial areas, and 5 and 20 times higher respectively. The level of DNA damage, using the Comet assay, was assessed in earthworm coelomocytes. Taking into account the relevance in assessing the chromosomal damage to provide evidence of fixed lesions to the genetic material, in the same cells, the induction of cytoplasmic micronuclei (MNi) was also studied by cytochemical staining of DNA by the fluorescent dye DAPI. MNi were scored according to standardized criteria. In addition, the presence of the contaminants in the earthworms was investigated by chemical and immunohistochemical analyses. Cryostat tissue sections were reacted with specific anti-B[a]P and -TCDD antibodies. The results obtained showed that, although no effect on mortality was found, the Comet and MN assays were able to reveal genotoxic effects. Also, and in particular, the two lower concentrations of the chemicals utilised, induced, just 10 days after the exposure, both DNA and chromosomal damage in earthworm cells. In the treated animals, the immunohistochemical staining revealed the distribution of the pollutants both in the coelomocytes and in the different tissues. In this work we demonstrated that the Comet assay and MN test in E. andrei coelomocytes should be considered sensitive biomarkers of genotoxicity within an environmentally realistic range. The use of immunohistochemistry for detecting environmental contaminants like B[a]P and TCDD is valuable in demonstrating a potential link between the presence of the chemicals in the organisms and the occurrence of toxic/genotoxic effects and in addressing chemical analyses in greater depth.

HM01 - Characterisation and remediation of contaminated soils and sediments

HM01-1

Phytoremediation: simultaneous calibration of contaminant sorption and plant uptake

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Phytoremediation is an emerging technique that stands out for its simplicity and low cost. Compared to traditional techniques, phytoremediation leads to slower and generally more uncertain results because of the many complexities affecting the soil-plant-atmosphere environment. This study focuses on numerical simulations of phytoremediation data of metal polluted soils using the Hydrus-1D software package, a potentially useful tool for evaluating the efficacy of alternative remediation techniques. Data used for the analysis were derived from experimental phytoremediation study using pots planted with Vetiver grass, and with the soil contaminated with Ni^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}. Available data were basic soil physico-chemical properties, meteorological information, the applied irrigation scheme, and metal concentrations in the plants. Variably-saturated flow was described using the standard Richards' equation, and solute transport using the advection-dispersion equation. Sink terms in the governing flow and transport equations accounted for root water and solute uptake, respectively. Plant growth and compartmentalization of the contaminants within the vegetation, were not considered. Sorption of metals by the solid phase was described using linear isotherms. Numerical simulations showed hydraulic conditions consistent with the experimental data. Three calibrations were considered (passive metal uptake, passive metal uptake with sorption adjustments, and active metal uptake). Simulated metal accumulations in the dried mass of the plants were compared to phytoextracted data. Passive uptake simulations showed good agreement with the experimental data for Pb^{2+} and Zn^{2+} (R^2 values were 0.7435 and 0.8526, respectively) but they significantly overestimated data for Ni^{2+} and Cd^{2+} (up to five times). For these metals it was necessary to use an active solute uptake model (yielding R^2 values 0.9884 and 0.9911 for Cd^{2+} and Ni^{2+}, respectively). Improved simulations of Pb^{2+} and Zn^{2+} uptake were further possible by adjusting the linear distribution coefficient for sorption. This study shows that it was possible to achieve good calibration of the metal phytoextraction process by Vetiver grass as observed by a series of controlled pot experiments. The adopted numerical approach for analyzing phytoremediation appears very promising for application to more dynamic field conditions.

HM01-2

Evaluation of arsenic phytoextraction efficiency of Pteris vittata under field conditions

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The Chinese brake fern *Pteris vittata* is a well known arsenic hyperaccumulator plant and possesses features that can make it an ideal species for phytoremediation of As contaminated sites: fast growth rate, high biomass production and a high efficiency of As translocation from roots to fronds. Extensive research has been carried out using this plant under laboratory and greenhouse conditions, while more limited information is available on the behaviour of P. vittata when plants are grown directly in the field.

This study summarizes and compares the results of field experiments conducted with this plant in contaminated sites located in northern Italy, characterized by different levels of contamination and soil characteristics. The research was aimed not only at estimating As extraction efficiency, but also at evaluating the most critical parameters for plant growth, since *P. vittata* is not a native species. Various experimental conditions have been tested, such as plant age, presence of other contaminants, influence of agronomic practices. In addition, the effect of mycorrhizal symbioses on plant growth and on arsenic phytoextraction efficiency was also investigated.

P. vittata showed in general a high phytoextraction efficiency, but plant growth and survival were strongly affected by adverse pedoclimatic conditions. Mycorrhization had a positive effect on plant growth; however, it was not possible to point out a clear influence of mycorrhization on As phytoextraction.

HM01-3

Assessing the impact of sediment contamination on benthic macroinvertebrates in freshwater tidal ecosystems

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The Rhine-Meuse delta is located in the south-west of the Netherlands, and contains the largest freshwater tidal ecosystem in the Netherlands. Historic pollution of the rivers Rhine and Meuse has resulted in the presence of different kind of contaminants (PAHs, PCBs, trace metals) in the delta sediments. The objective of this study is to present an approach and a system for evaluating sediment contamination using benthic macroinvertebrates. Data on habitat characteristics, sediment contamination and macroinvertebrate community structure were available for > 500 locations in the delta, in the period 1993 - 2006. The data were analyzed using various multivariate statistical analyses. This resulted in the construction of two metrics to be used within the Water Framework Directive in the Netherlands. The newly developed metrics correlated individually better than any of the existing metrics, combining both new metrics they performed much better than the combination of three existing metrics. The two metrics are now implemented in the WFD assessment of the Dutch R8 river type. A first validation of the metrics to new data showed that they performed quite well. Further testing of these metrics on other areas should show if the metrics should be adjusted using a traits-approach.

HM01-4

Sediment remediation with activated carbon: Modeling the trade-off between PAH toxicity reduction and negative activated carbon effects on macroinvertebrate populations

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Activated carbon (AC) addition to contaminated sediments leads to reduction of bioavailable fractions of hydrophobic compounds (HOCs), and thus, toxicity of sediment-bound HOCs. However, it has been shown that AC itself may cause some negative effects on aquatic organisms, e.g. mortality, growth and ingestion rate inhibition. The aim of this study was to simulate the combine effect of polyaromatic hydrocarbons (PAHs) and AC amendment on biomass development on two benthic species, Gammarus pulex and Asellus aquaticus. Dose-response relations derived from a series of laboratory experiments were used to calibrate and evaluate the model. AC addition has no effect on biomass development of A. aquaticus but addition of AC>3% leads to inhibition of biomass development of G. pulex by 50%. In polluted sediments without AC, the effect of PAHs was severe and led to extinction of both species population within several days. Addition of AC drastically reduced mortality rates leading to growth of the populations. In summary, at higher concentrations, AC might have an effect on biomass development of some sensitive species like G. pulex. However, overall effects AC addition to PAH contaminated sites are predicted to be positive as long as AC is added at optimum concentration (3%).

HM01-5

Towards the phytoremediation of mining explorations - a NATO SfP project

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In different NATO and Mediterranean Dialogue countries the mining exploration was or still is, an important economic activity. Along with it, is left a legacy of contamination problems represented by the exposure to metal and radionuclide compounds which become a risk to the aquatic, terrestrial and human health. This Science for Peace NATO project gathers Ecotoxicologists, Microbiologists, Molecular Biologists, Chemists and Plant Physiologists to develop and improve eco-friendly techniques for the remediation of contaminated sites in Tunisia, Morocco and Portugal. More specifically, it intends to implement and optimize the phytostabilisation of metals and radionuclides based on the knowledge attained from plant-bacteria interactions in each country area. The first attempts regarded the application of a risk assessment framework to establish priority areas for further intervention. Secondly, it was isolated metal resistant strains of endophytic and rhizosphere bacteria from native plant roots collected in contaminated sites. Those strains have been characterized through the use of molecular techniques. The next step is to inoculate the roots of small plantlets from selected species with bacteria endophytes and assess their ability to promote plant growth in contaminated soils, under green house and in situ experiments. For that, it is also needed the assessment of plant species that could grow under such contaminated sites, in order to be a successful target for using in the phytoremediation process. The present communication aims to perform an overview of the project goals as well as to explain the experimental steps done so far and the preliminary results obtained.

HM01-6

Characterizing contaminant residuals from environmental dredging using chemical and biological metrics

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Environmental dredging is a common remedial action for managing contaminated sediments. However, post dredge concentrations in surface sediment are difficult to predict prior to initiating dredging actions. In some cases, post surface concentrations have been higher than anticipated. This research focused on methods to characterize post dredge residuals and develop an approach to predict post dredging residual contaminant characteristics and concentrations.

In Northeast Ohio on Lake Erie, the Ashtabula River was contaminated by a number of industrial sources over years of runoff and discharges. In 2006-2007, 1.1 miles of the river was dredged under the authority and leveraged funding of the Great Lakes Legacy Act. The dredging operation carried out over two fields season resulted in 540,000 cyds of contaminated sediments being dredged, dewatered, and placed in a CERCLA landfill. The primary contaminant driver for the removal action was PCBs at concentrations up to 600pm but also included radionuclides, metals, and other organic contaminants

EPA's Office of Research and Development conducted an interdisciplinary research study in association with the dredging operations on the Ashtabula River. The research had two primary goals: 1) quantifying the extent and characteristics of the residuals that remained following dredging in a selected river reach, and 2) characterize the ecosystem exposure that occurred during and following dredging on the entire dredged area. ORD's National Risk Management Research Laboratory (NRMRL) and National Exposure Research Laboratory (NERL) collaborated to leverage resources and expertise toward these goals along with the Great Lake National Program Office. Results of the study are presented on the approaches evaluated to characterize the post dredge residuals. Several approaches were evaluated to quantify and characterize the residuals remaining in two distinct dredge areas, dredging to soft sediment and dredging to bedrock. Comparison will be made between the two areas dredged, as well an evaluation of the methods for the dredge residual characterization. Biological indicators of system wide recovery will also be presented in relation to the dredge residuals.

HM02 - Impact and remediation of wastewater

HM02A-1

Xenobiotic removal efficiencies in wastewater treatment plants: residence time distribution as a guiding principle for sampling strategies

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The effect of mixing regimes and residence time distribution (RTD) on pollutants in wastewater treatment plants (WWTP) is well understood in hydraulic engineering. Nevertheless, it is often neglected in sampling design and data analysis investigating xenobiotic removal efficiencies in WWTPs. Most investigations on the latter rely on 24 h composite samples and the mean hydraulic retention time (HRT) used as time offset to match influent and effluent loads. However, disregarding the mixing regime characteristics in evaluating xenobiotic removal performances may lead to biased estimations or even negative mass balances.

This study aims to develop an approach to estimate xenobiotic removal efficiencies from monitoring data taking the hydraulic residence time distribution (RTD) in WWTPs into account. For this purpose, a completely mixed tanks-in-series model was used to address hydraulic mixing regimes in a Luxembourg WWTP. Emission predictions of the WWTP were validated with

measured diurnal effluent data for the pharmaceuticals carbamazepine and diclofenac as well as the complexing agents NTA and DTPA.
Influent loads (of 24 h) were found to be released as effluent distributions that clearly expand over more than one day. A 24 h effluent sampling period with an optimal offset (18 h) between influent and effluent recovered only half (55.6 %) of the influent load in a dry weather scenario and consisted largely of fractions from previous and even fractions of the following day(s). Knowing the influent concentrations and flows of 3 (or 4) consecutive measurement days, their fraction released during the chosen measurement period can be determined allowing to estimate the overall removal performance.
The presented study demonstrates that hydrodynamics are crucial for elimination efficiencies of xenobiotics in municipal WWTPs. The HRT is of limited used only since it actually contains no information about mixing behavior. Commonly reported negative mass balances for xenobiotics might therefore also be a consequence of biased sampling schemes. The optimal sampling setup for full-scale mass balancing at the selected WWTP was determined to be a coverage of 3-4 consecutive days in the inlet and a single day sampling at the outlet. This constellation allowed to capture more than 83 % of the incoming water under realistic conditions. Due to the influent variability elimination rates of less than 5-10 % are probably impossible to track in full-scale investigations.

HM02A-2
Restricted elimination of the non-ionic surfactant TMDD in WWTPs
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2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD) is a non ionic surfactant that has been found in different rivers in Germany with concentration up to 4.25 µg/L. The compound is a high production volume chemical which is used in diverse applications such as colours, inks, adhesives and coatings but until now the sources of TMDD in the aquatic environmental are unknown. Wastewater treatment plants (WWTPs) were suggested to be the dominating sources for TMDD in rivers, but information neither about the TMDD concentrations nor about its elimination rates in WWTPs are available so far. Therefore, detailed sampling campaigns at 4 WWTPs in Germany were carried out. The results indicate that TMDD is introduced by the sewage with loads fluctuating between 10.1 g/d and 1142 g/d and it is discharged with effluents loads between <LOQ to 434 g/d into the rivers. Based on the load analysis, the elimination rates of TMDD in the studied WWTPs varied between 33% and 68%. The elimination rates in each treatment stage of one WWTP were also determined. The results indicate that TMDD in preferably degraded in the biological stages under aerobic conditions.

HM02A-3
Distinct transformation pathways of opium alkaloids in biological wastewater treatment
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Recent studies confirmed that many organic micropollutants are transformed into unknown products during biological wastewater treatment. The emission of transformation products (TPs), which often exhibit only a slightly modified molecular structure and a higher polarity and persistency, has raised increasing concerns, since they are prone to contaminate drinking water resources and might exhibit a similar or even higher biological activity than the parent compounds. In this study, the aerobic transformation pathways of 4 natural opium alkaloids (codeine, morphine, dihydrocodeine and morphine) were elucidated using batch experiments with activated sludge. The complementary use of high-resolution mass spectrometry (HR-MS) and nuclear magnetic resonance (NMR) allowed for the identification of several up to now unknown transformation products (TPs). In total, chemical structures of 18 TPs of codeine, 17 TPs of morphine and 2 TPs of dihydrocodeine and hydrocodon were proposed. Elucidation of the transformation pathways in sterile (autoclaved) and non-sterile sludge suspensions revealed that the transformation of codeine and morphine is characterized by a variety of chemical (non-enzymatic) and biological (enzymatic) reactions. The development of a sensitive analytical method for quantification of several TPs of codeine in wastewater enabled to confirm that the results from the batch experiments can be transferred to full-scale WWTPs. The results of the study indicate that structurally closely related compounds such as the opium alkaloids might undergo distinct transformation pathways and highlight the importance to consider both chemical and biological reactions when predicting the transformation pathways of organic micropollutants in biological wastewater treatment.

HM02A-4
Are organic micropollutants more bioavailable in wastewater than common models predict?
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The fate of micropollutants within the aquatic environment is influenced by their interaction with dissolved organic carbon (DOC). Only the freely dissolved concentration is bioavailable, while the fraction sorbed to DOC is not bioavailable. While most studies within the literature have focused on reference or natural DOC as a surrogate, very little is known regarding micropollutant interaction with wastewater derived DOC. An understanding of this interaction is important as there is increasing interest in recycling secondary treated effluent for non-potable and indirect potable applications, and the interaction with DOC may not only influence the bioavailability but also retention during membrane treatment processes. The aim of this study was to quantify dissolved organic carbon-water partition coefficients (K_{DOC}) for both reference and wastewater derived DOC with a range of micropollutants using a polydimethylsiloxane depletion method. The results indicated a significant difference in K_{DOC} between reference and wastewater derived DOC for moderately hydrophobic micropollutants. For the most hydrophobic compounds, such as benzo(a)pyrene, sorption to wastewater derived DOC was over 1000 times lower than to reference DOC. The interaction of the estrogenic micropollutant nonylphenol with wastewater derived DOC from different stages of a wastewater treatment and advanced water treatment train was studied, but little difference in K_{DOC} was observed. Analysis of the studied reference and wastewater derived DOC revealed that they have very different properties due to their different origins. The wastewater derived DOC was less aromatic and had a lower molecular weight due to microbial activity and this reduced the sorption capacity. In conclusion, a larger fraction of hydrophobic micropollutants are expected to be freely dissolved and thus bioavailable in wastewater than predicted using K_{DOC} measured with reference DOC.

HM02A-5
A toxicity monitoring of industrial effluent: implications in the implementation of toxicity-based discharge limits in Korea
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In this study, the acute toxicity and feeding rate tests were applied to periodically monitor the toxic effects of effluents from a wastewater treatment plant (WWTP) using *Daphnia magna* (reference species) and *Moina macrocopa* (domestic species). Acute toxicity of industrial wastewater toward *D. magna* and *M. macrocopa* was largely reduced by primary biological treatment, thus subsequent effluents (PE, SE and FE) and stream waters (US and DS) showed either no acute toxicity or toxicity values less than 1 TU. In addition, the toxicity of final effluent (FE) varied over different sampling events, and *M. macrocopa* appeared to be more sensitive than *D. magna*. Among toxic metals, Cu concnetrations of FEs range from 0.002 - 0.067 mg L^{-1}, which are higher than EC_{50} values (0.022 and 0.011 mg L^{-1} for *D. magna* and *M. macrocopa*, respectively). However, acute toxicity more than 1 TU was not observed even in those samples. Considering concentrations of dissolved organic carbon (15.25 - 29.70 mg L^{-1}) in FE, reduction of free Cu concentration by complexation appeared to responsible for that. The feeding rates of *D. magna* and *M. macrocopa* were significantly reduced when compared to the control though acute toxicity was not observed. The feeding rate of *D. magna* was inhibited by the average of 50.8, 23.3 and 22.2% for the FEs, DSs and USs, respectively, while it was inhibited by the average of 58.6, 45.2 and 36.0%, respectively, when *M. macrocopa* was evaluated. The feeding inhibition of the DS over the overall sampling period was more significant than that of the US, suggesting that effluent from the PJ-WWTP likely had an impact on the S-stream. These findings suggest that a periodic monitoring of effluent with a domestic species (i.e. *M. macrocopa*) is desirable in the implementation of toxicity-based discharge limits in Korea. In addition, both the lethal and sublethal toxicity tests should be used to evaluate the impact of low levels of toxic chemicals in effluent.

HM02A-6
Whole effluent assessment of urban discharges
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The European Water Framework Directive and the Environmental Quality Standards Directive lay down a framework for maintaining or obtaining good ecological and chemical status of European surface and coastal water bodies by the year 2015. The aim of this work was through Whole Effluent Assessment (WEA) to identify problematic urban discharges, e.g. stormwater, municipal wastewater, combined sewer overflow (CSO), industrial wastewater. Samples from around Copenhagen were therefore tested in the Larval Development Ratio (LDR) test using the marine crustacean Acartia tonsa. The number of non-hatched eggs, nauplii and copepodites was determined in controls as well as in samples and the effect was calculated as the ratio between the number of copepodites and the sum of the numbers of nauplii and copepodites. Ratios below or above 1 significantly (t-test) differing from the control group are considered inhibited or stimulated, respectively, both indicating an effect of the stressor. Except for the stormwater, which did not show any effects at a concentration of 72%, all samples need to be diluted to more than 72%. Compared with the control group, the CSO from Belvedere needs to be diluted between 1,000 (P=0.001) and 10,000 (P=0.1736) times, the concentrate from the PP needs to be diluted around 1,000 (P=0.126) times and the outlet from the WWTP Damhusåen needs to be diluted at least 200 (P=0.001) times before the development of the organisms are comparable with the control group.

HM02B-1
Optimisation of Fenton reagents using central composite design for hybrid treatment of recalcitrant metal-working fluid wastewater
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Disposal of operationally exhausted metal working fluids (MWF) biologically is an attractive option, since it is effective and has low energy demands. However, it is enormously challenging since they are chemically complex including the addition of toxic biocides which are added specifically to retard biodegradation whilst operational. In this study the feasibility of employing a sequential Fenton-biological oxidation for the treatment of recalcitrant components of MWF wastewater which were resistant to bacterial treatment was investigated. A statistical experimental design using Central Composite Design was employed to address Fenton reagent [H2O2, Fe2+] dose optimisation which ensured minimal concentrations of the reagents, thus making the treatment environmentally and economically viable. The results demonstrated that Fenton pre treatment of the recalcitrant MWF wastewater achieved an overall improvement in biodegradability (BOD5/COD increased from 0.160 to 0.538) with 92% and 85% reduction in COD and TOC respectively.

HM02B-2
Ozone and activated carbon treatment of sewage effluents: toxicity removal vs. toxicity increase
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Ozonation and activated carbon filtration provide effective barriers to a wide range of organic pollutants and can thus reduce the emission of hazardous contaminants via sewage treatment plant effluents. However, concerns arose about potential hazardous oxidation products occurring as a result of ozonation. Consequently, for a risk-benefit analysis, an extensive ecotoxicological evaluation of advanced treated wastewaters is essential. The presented work is part of a comprehensive study within the Neptune project (www.neptune-eu.org), covering in vivo tests with six different test organisms and a variety of in vitro bioassays.
For a comparative in vivo toxicity analysis, test organisms were exposed to raw wastewater from different sampling points onsite at two treatment plants in a flow through test system. Test organisms: Lemna minor, Chironomus riparius, Lumbriculus variegatus, Potamopyrgus antipodarum, Dreissena polymorpha and Oncorryhnchus mykiss. For the in vitro analysis, WW was solid phase extracted before toxicity testing. Toxicity endpoints using yeast based bioassays: (anti-) estrogenicity, (anti-) androgenicity, AhR agonistic (dioxin like) activity; tests on genotoxicity/mutagenicity: SOS/umu test, Ames fluctuation assay using different tester strains; test on non-specific toxicity: cytotoxicity assay using a rat pituitary cell line.
In vivo toxicity is increased after ozonation due to byproduct formation, whereas sand filtration appeared as an appropriate post treatment for byproduct removal/degradation. Solely activated carbon treatment revealed a significant reduction of in vivo non-specific toxicity compared to conventional treatment. Endocrine activity, genotoxicity and cytotoxicity of wastewater are effectively reduced with ozonation and activated carbon filtration, indicating the removal/degradation of causative contaminants. A consistent mutagenicity increase after ozonation confirms the for-

mation of toxic oxidation products in vitro and hence further research on byproduct formation and removal effectiveness of post treatments is desirable.

HM02B-3

Evaluation of bioassays and wastewater quality: In vitro and in vivo bioassays for the performance review in the Project 'Strategy MicroPoll'

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The goal of the project "Strategy Micropoll" of the Swiss Federal Office for the Environment was to evaluate the efficiency of two advanced wastewater treatment technologies: (i) ozonation followed by sand filtration (SF), and (ii) powdered activated carbon addition followed by ultrafiltration (PAC-UF) in eliminating micropollutants from wastewater treatment effluent. Two large-scale pilot studies were conducted at the wastewater treatment plant (WWTP) Wüeri Regensdorf (Switzerland) and the WWTP Vidy Lausanne (Switzerland). The studies were done in close collaboration with experts from research, practice and with personnel and financial support of cantonal water protection agencies and the operators of the WWTPs.

Samples before and after each treatment step were analyzed for organic micropollutants and ecotoxicological effects, in order to gain insight into the efficiency of the different treatment steps. The focus lay on studying the effects of the advanced treatment technologies ozonation-SF and PAC-UF on the removal efficiency of polar, persistent and bioactive substances as well as possible by-products.

It was demonstrated that ozonation-SF and PAC-UF treatment are useful measures to reduce the effects of micropollutants in waterbodies. The overall elimination rate regarding specific effects was generally above 80%. Overall advanced treatment led to lowered risk quotients, i.e. the ratio between measured concentrations and environmental quality standards, as well as reduced toxicity in bioassays, and thus lowered risk of adverse effects. There was no evidence of toxic effects due to the formation of stable transformation products in the ozonation. However, a final filtration step with a biological activity (e.g. sand filter) after ozonation is recommended in order to reduce possible risks of transformation products released into water bodies.

In both pilot studies, the application of bioassays for the performance review of advanced wastewater treatment has proven to be relevant and useful. In general, *in vitro* bioassays were deemed most promising for the routine monitoring of the performance of advanced treatment in WWTPs, however only specific effects are assessed in those tests. Certain *in vivo* bioassays could also show the beneficial effect of ozonation and PAC-UF treatment such as the fish early life stage test (FELST) with *Oncorhynchus mykiss*. The quality of treated effluent was significantly improved, leading to improved surface water quality.

HM02B-4

Using multiple lines of evidence to determine the major factors affecting ecosystem health in an urban stream

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A study was conducted on an urban stream in Melbourne, Australia, to identify the major sources of pollution impacting aquatic ecosystem health. Sediment pollution was identified as a major threat to benthic macroinvertebrates and sewage spills were identified as a potential threat to fish communities. Sediments were analysed for pesticides, heavy metals, petroleum hydrocarbons and nutrients and sediment toxicity was assessed using *Chironomus tepperi* and toxicity identification evaluation. The gastropod *Potamopyrgus antipodarum*, the Poeciliidae *Gambusia holbrooki* and goldfish (*Carassius auratus*) were examined to determine whether there was evidence of exposure to endocrine disrupting chemicals. A variety of pollution point sources were found, ranging from heavy metals, synthetic pyrethroids and fungicides. Toxicity identification evaluation tests confirmed that organic and heavy metal pollution contributed to sediment toxicity. Sewer spills did not appear to impact fish communities compared to those present in the rural headwaters of the creek. This study provides clear information to assist environmental managers understand what are the priority pollution issues that need to be addressed to protect aquatic ecosystems.

HM02B-5

Biodegradation of non-steroidal antiinflammtory drugs (NSAIDs) and their non-halogenated analogs in nitrifying batch reactors

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The non-steroidal anti-inflammatory drugs (NSAIDs) have received considerable attention by the scientific community due to frequent detection in monitoring surveys on sewage-impacted surface waters, associated with high consumption rates and low removal efficiencies during conventional activated sludge treatment (AST) in WWTPs.Despite the interest in the fate of these polar compounds in engineered and environmental systems, it has been marginally considered. Little evidence has been published as regards metabolic pathways in microbial communities like those encountered in the aeration tank of the AST. Previously, nitroso (TP324) and nitro (TP340) compounds of diclofenac (DCF) were identified.Given the potential ecotoxicological risks of nitrated derivatives, the production of these compounds is an environmentally relevant matter of concern.This study aimed to investigate uncovered aspects in environmental fate in WWTPs of the NSAIDs:DCF, meclofenamic,flufenamic and tolfenamic acids, and lumiracoxib. Biodegradation experiments were performed under controlled laboratory settings in order to gain further insight into the biodegradability and metabolic pathways of these compounds.Samples from the biodegradation studies were screened for the presence of stable intermediates and these were characterized by hybrid quadrupole-time of flight (QqTOF)-MS in combination with H/D-exchange experiments leading to the discovery of unusual microbial transformation products.To evaluate the biodegradability of target compounds, the structurally related compound 2-anylinophenylacetic acid (APAA) was used.Biodegradation experiments in batch-reactors loaded with mixed liquor demonstrated that degraded faster than DCF. After one day, 50% of APAA (228 Da) was degraded and the formation of one transformation product was confirmed. Structure elucidation by means of ultra performance liquid chromatography-electrospray ionization-hybrid quadrupole-time of flight-mass spectrometry in conjunction with H/D-exchange experiments identified it as deriving from nitrosation of the hydroxyl group in the carboxylic acid moiety (256 Da).Although the contribution of nitrifying bacteria to the biomass in the mixed microbial community of the AST tank in WWTPs is less than 5 %, the operational conditions of the lab-scale reactors were favorable for the growth of nitrifiers in terms of oxygen supply, and temperature and pH of the mixed liquor.

HM02B-6

Post-ozonation in a municipal wastewater treatment plant improves water quality in receiving stream as indicated by the abundance of species at risk

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Removal of organic micropollutants from wastewater by post-ozonation has been investigated in a municipal wastewater treatment plant (WWTP) upgraded with ozonation, followed by sand filtration, as an additional treatment step of the secondary effluent. Here, the SPEAR (Species At Risk) indicator was used to analyse macroinvertebrate abundance data that was collected in the receiving stream to investigate whether ozonation followed by sand filtration improved the water quality. Before ozonation no significant difference between upstream and downstream SPEARpesticides could be observed. During ozonation the SPEARpesticides values indicate a better water quality downstream of the WWTP. There are 9% more vulnerable species present in the downstream samples. The comparison of the downstream macroinvertebrates before ozonation with those during ozonation exhibits an even clearer improvement of the water quality through ozonation (13% more vulnerable species). The SPEAR concept, originally developed to indicate pesticide stress, also appears to indicate toxic stress by micropollutants other than pesticides. The responsiveness of the SPEARpesticides indicator does not necessarily mean that the stressors are pesticides; rather it means that those macroinvertebrates that are vulnerable to pesticide pollution are also vulnerable to pollution by micropollutants from WWTPs. The change in SPEAR in the macroinvertebrate community downstream the WWTP indicates that toxicity by pollutants decreased by more than one order of magnitude during ozonation. Ozonation followed by sand filtration has favourable impacts on the composition of the macroinvertebrate community which indicates a significant and relevant improvement of the water quality in the receiving stream.

HM03 - Integrated long term monitoring as a tool for the global assessment of POPs

HM03-1

5 Years of POP air monitoring in Germany: recent data from the air pollution monitoring network of the German Federal Environment Agency

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The directive 2004/107/EC requests indicative measurements of several polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene (BaP) at background sites. In Germany, these measurements are performed in the air pollution monitoring network of the German Federal Environment Agency (Umweltbundesamt, UBA). Additionally, these monitoring efforts are coordinated with the European Monitoring and Evaluation Programme of the UN/ECE (EMEP). Therefore, the UBA monitoring programme includes other persistent organic pollutants like polychlorinated biphenyls (PCB) or several organochlorine pesticides. Their routine determination started in 2007. Here, we will look back at 5 years of POP air monitoring in the UBA air pollution monitoring network. We will provide an overview about ambient air concentrations of selected POPs at four rural background monitoring sites in Germany (Zingst, Westerland, Schauinsland, Schmücke) and discuss them in terms of temporal and spatial variations. Challenges and difficulties encountered during the monitoring programme will be discussed in the presentation.

HM03-2

Global-scale passive air sampling of legacy and emerging chemicals under the GAPS Network: recent progress and application of the 'integrated approach'

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The Global Atmospheric Passive Sampling (GAPS) Network is the only global-scale program that contributes information on air concentrations of persistent organic pollutants (POPs) to the Global Monitoring Plan (GMP) of the Stockholm Convention on POPs. Under Article 16 (Effectiveness Evaluation) of the Convention, monitoring data for air is required to assess effectiveness of control measures on POPs and investigate their regional and global transport.

Quarterly sampling at more than 50 sites under the GAPS Network using polyurethane foam (PUF) disk samplers is now in its 6th year and has generated the first ever global data set of POPs in air with seasonal resolution. This includes polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs, e.g. a-HCH, g-HCH, chlordanes, endosulfans). The availability of this type of data has contributed to an 'integrated approach' to understanding chemical sources, fate and transport. This involves integrating measurement data with transport models and emissions inventories.

A pilot study completed in 2009 tested a new type of PUF disk sampler that is impregnated with XAD to increase its sorptive capacity, referred to as the sorbent-impregnated PUF or SIP-disk sampler. Co-deployment of PUF disk and SIP disk samplers at a subset of 20 GAPS sites has shown good agreement between the two sampler types for PCBs. The SIP disk samplers also generated the first comparable, global-scale data for several classes of priority chemicals including polyfluorinated chemicals (PFCs), siloxanes, alternative flame retardants and current-use pesticides (CUPs).

HM03-3

Assessing emerging organic pollutants in air in the Canadian Arctic and the Great Lakes regions

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The Northern Contaminants Program (NCP) and the Integrated Atmospheric Deposition Network (IADN) conduct measurements of persistent organic pollutants (POPs) and other priority organic chemicals in the Canadian Arctic and the Great Lakes regions since the 1990s. Both programs have been actively screening for and adding new POPs and emerging organic pollutants to their target analyte lists in support of national and international control initiatives. An update on the long-term atmospheric trends of polybrominated diphenyl ethers (PBDEs) in air

at the Canadian High Arctic station of Alert and at two Canadian IADN master stations, namely Point Petre and Burnt Island, will be presented. Preliminary time trends of four perfluorinated compounds measured at Alert indicate ubiquitous presence of these compounds in Arctic air. Recent air measurements of emerging pollutants, such as non-PBDE flame retardants (FRs) and current-use pesticides (CUPs) in these two remote regions imply long-range atmospheric transport potential of these compounds; indicating the need to further investigate their environmental fate and toxicity.

HM03-4

Comparison of long-term trends in concentrations of persistent organic pollutants in ambient air derived from passive and active sampling techniques.

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The Global Monitoring Plan (GMP) is an activity developed under the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) with a purpose of providing a tool for effectiveness evaluation of the SC measures. First global assessment of current baseline levels of POPs in core matrices (ambient air and human milk) was completed in 2008. Majority of data on ambient air levels of POPs was collected using the passive air samplers (PAS). Even though passive samplers only provide semi-quantitative information on the atmospheric pollution, they still offer a valuable tool to study spatial and temporal variability of the POP levels in the atmosphere. Such data are very valuable when it comes to assessments of the fate of persistent compounds in the environment - thein cycling between compartments, long-range transport or accumulation in various matrices - as well as for the validation of various distribution and transport models.
In 2009, the Conference of the Parties of the SC decided that levels of POPs in core matrices will be assessed every six years. Question remains whether the six year period proves to be sufficient for establishment of the trends, and whether these trends will correlate with those derived from conventional high volume sampling.
Observatory of the Czech Hydrometeorological Institute in Košetice is a site where background POP monitoring program of the European Monitoring and Evaluation Program (EMEP) has been active since 1988. Simultaneous employment of PAS and high volume samplers at this site since 2003
provides necessary information on the performance of passive samplers and allows for intercalibration of these two techniques. At the same time, it is the only site with the history of six years of on-going monthly PAS measuremens where the observed temporal trends can be compared to the long-term trends derived from two decades of continuous ambient air monitoring using the high volume air samplers.

HM03-5

Twenty years of monitoring of persistent organic pollutants in the United Kingdom: sources, fate and time trends.

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The Centre for Chemical Management (CCM) in Lancaster, under the support of the Department for Environment, Food and Rural Affairs (DEFRA) has been running a long-term air monitoring program in the UK called TOMPs (Toxic Organic Micro Pollutants), since the beginning of the 1990s. In the frames of this project, air samples (gas and particulate phases) have been collected from a number of urban and rural/semirural areas and analysed for PCBs, PAHs, PCDD/Fs and PBDEs.
The occurrence of the afore-mentioned chemicals throughout these two decades, the changes in their levels and their fate are investigated. Various widely accepted tools like the PAH molecular diagnostic ratios, isomers and congeners profile analysis and other statistical approaches are used to reveal sources and seasonal variations
The atmospheric concentrations are compared with existing emission inventories and the latter are evaluated.
The results throughout these two decades are absolutely comparable, because the same methods/techniques have been used and clearly these results constitute one of the longest time series ever published.

HM03-PS

Poster spotlight: Monitoring POPs in different matrices

Miscellaneous

Poster spotlight highlighting abstracts WE 206, WE 207, WE 208, WE 209:
- Sampling rates when measuring gas phase POPs air concentration with passive air samplers.
- Monitoring the continental and intercontinental background of persistent organic pollutants in Africa
- Are the Mt. Qomolangma regions in Himalayas (Nepal) really remote areas for persistent organic pollutant contamination?
- Long-term trends of POPs in human milk in Czech Republic

HM04 - Sorption and bioavailability of organic chemicals: mechanisms relevant for toxicity and bioremediation

HM04-1

PAH extraction recovery from granular activated carbon

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River and marine sediments are contaminated with a wide variety of hydrophobic organic compounds (HOC), which are persistent and tend to concentrate as they further proceed in the food chain. Currently, a new risk based approach accomplished by measures of freely dissolved pollutants in the pore water is in focus of international research. It has been shown that the fraction of HOCs that desorbs sufficiently fast and equilibrates to the freely dissolved concentration in the pore water is more relevant for risks to aquatic biota, than the total concentration in the sediment. In the past years many studies demonstrated the potential of activated carbon (AC) amendments for sediment associated organic pollutants and thus availability reduction. Whereas activated carbon already has been applied to immobilize HOCs in bed sediments, not that much attention has been dedicated to granular activated carbon. We therefore conducted experiments where GAC was used to concentrate aqueous phase PAHs from sediment-water slurries. Polluted GAC was further used for testing different solvent (mixtures) and extraction techniques for the recovery studies of bound PAHs.

HM04-2

Effect of the soil amendments activated charcoal, biochar and compost on desorption and biodegradation of phenanthrene

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Soil and groundwater remediation is aimed at reducing levels of pollutants to below regulatory thresholds. Polycyclic aromatic hydrocarbons (PAHs) are an important class of soil pollutants. Often, a large portion of the PAHs are degraded by soil microorganisms within short times (<100 days), and this is often followed by slower degradation, resulting in a non-degradable residual fraction.
Soil amendments such as activated charcoal (AC), biochar and compost can reduce the aqueous concentration of PAHs. On the one hand this might limit the bioavailability and uptake by organisms leading to reduced toxicity, but on the other hand this might also decrease biodegradation.
This study investigated the abiotic desorption of [9-^{14}C]phenanthrene, as a model PAH, from the three soil amendments, and compared this to the biodegradation of the sorbed phenanthrene.
The total abiotic desorption of phenanthrene from the soil amendments in different sterile aqueous solutions (MilliQ water, fertiliser and broth) to an infinite silicone sink was measured. The total amount of phenanthrene desorbed was 12 to 46% for AC, 66 to 86% for biochar and 98% for compost, with higher desorption in the presence of broth.
This was compared to the biodegradation of phenanthrene sorbed to the soil amendments in fertiliser and broth by *Sphingomonas* sp (DSM 12247). Phenanthrene was completely degraded in all the soil amendment suspensions, and the total amount mineralized was 43 to 75 % for AC, 50 to 82% for biochar and 56 to 74% for compost, with higher mineralization in the presence of fertiliser.
Therefore, a low abiotic desorption of phenanthrene (as with the AC) does not lead to a low biodegradation. Our results suggest that adding soil amendments to contaminated soil can reduce phenanthrene mobility without affecting the biodegradation and mineralization.

HM04-3

Dispersal networks for enhancing bacterial degradation under heterogeneous environmental conditions

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Successful bioremediation of organically polluted soil sites depends on the bioavailability of pollutants to catabolically active microorganisms. In particular, the heterogeneity of environmental conditions often limits bacterial access to pollutants. Experimental and modelling studies revealed that fungal networks can facilitate bacterial dispersal and may thereby improve pollutant bioavailability. Here we investigate the influence of such bacterial dispersal networks on biodegradation performance under spatially heterogeneous abiotic conditions using a process-based simulation model. To match situations regarded as being typical in polluted soils, two types of abiotic conditions are studied: heterogeneous bacterial dispersal conditions, i.e. a mix of areas where bacterial movement is efficient or restricted, and heterogeneous initial resource distributions, i.e. a mix of areas with high or low pollutant concentrations. Under these conditions, the simulation model predicts that networks facilitating bacterial dispersal can enhance biodegradation performance for a wide range of spatial heterogeneities. Additionally, the time horizon over which this performance is assessed and the networks' spatial configuration are key factors determining the degree of biodegradation improvement by dispersal networks. Our simulation results strongly support the idea of stimulating the establishment of fungal mycelia for enhanced bioremediation of polluted soils.

HM04-4

A self-dying reporter bacterium determining the bioavailable fraction of phenanthrene in soil

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When a hydrophobic organic pollutant is released to soil, sorption/desorption is the most important process for the bioavailability and toxicity of the compound. With time, the contaminant would migrate into hydrophobic organic matter phase, and its bioavailability and toxicity may decrease. If a remedial goal is established based on the total extractable concentration of the contaminant in the contaminated site, the risk and the expenses may be overestimated and dissipated. Therefore, the assessment of the bioavailability and toxicity of an organic pollutant in soil seems to be worthwhile for setting up the reasonable remedial plan. The aim of the present study is to investigate the performance and feasibility of a self-dying reporter bacterium visualizing and quantifying the bioavailability and toxicity of phenanthrene in soil. Time-dependent aging phenomenon of the contaminant in soil was also considered. A self-dying reporter bacterium harboring a cell-killing gef gene (named strain S) was designed to die and lose its green fluorescence on the initiation of phenanthrene degradation. To analyze the aging effect of phenanthrene in soil by the reporter bacterium, phenanthrene-spiked soil was incubated for 18 months and the fluorescence response of the reporter bacterium was measured. No significant change was observed in strain S with Ottawa sand with the increasing aging time, while the reporter's response in soil drastically changed after 3 months. The available fraction measured by the other physico-chemical methods (i.e., hydroxypropyl-beta-cyclodextrin extraction and desorption with Tenax bead) tended to overestimate the availability of phenanthrene in soil. Our data show that the reporter bacterium determines the living organism-based availability and toxicity, which are based on the interaction between phenanthrene, soil, and bacterium. In conclusion, the residual toxicity and bioavailability in soil with increasing aging time and the soil properties was able to be visualized and quantified by using the novel reporter bacterium developed in this study. In addition, comparing the strain S to a traditional reporter bacterium, the strain S has better resolution and feasibility to soil. This study supports the potential of the reporter strain for applications in the visual monitoring of the bioavailable phenanthrene and real risk in contaminated sites. It can also be applied to set a risk-based cleanup level and a contaminant site management plan.

HM04-5

Sorption of phenolic acid anions by black carbon is accompanied by release of hydroxide into solution

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Black carbon occurs naturally in soils, and certain types of engineered carbons, such as biochar

and activated carbon, have been considered for their potential to enhance soil fertility or reduce the bioavailability of soil contaminants. While adsorption of hydrophobic compounds to black carbon has been studied intensively, little attention has been paid to ionizable compounds which are common among the myriad of natural and synthetic compounds that come into contact with soil. We studied the sorption of cinnamic and coumaric acid anions by commercial biochar prototypes. These compounds belong to the class of plant root exudates known as "phenolic acids"that play important roles in agricultural and ecological dynamics as allelopathic agents. Their structures also represent some of the aromatic substructures of humic and fulvic acids, which are well known to adsorb to black carbon surfaces. Cinnamic and coumaric acids (pKa 4.4 and 4.7, respectively) are appreciably ionized in soil pore water at ordinary pH values. We focused on conditions favoring the anion. Sorption in phosphate-buffered systems at pH 6.9 was highly nonlinear and trended with the specific surface area of the biochar. Sorption is remarkably strong: the Kd (Cs/Cw; L/kg) values, which ranged from 500-40000 depending on biochar and phenolic acid concentration, are several orders of magnitude greater than the corresponding calculated octanol-water partition coefficient (Kow) of the organoanions (0.38 and ~ 0.1 L/L, respectively). Sorption was unaffected by Ca2+ or Mg2+ up to 0.1 M, ruling out an influence of surface charge or the involvement of bridging of carboxylate groups by metal ions. Isotherms in pH 6.9 buffered and non-buffered systems diverge as phenolic acid loading increases, the non-buffered showing reduced sorption relative to the buffered system. Accompanying loading in the absence of buffer is an increase in the pH, indicating that adsorption of the phenolic acid takes place simultaneously with the release of hydroxide ions into solution: RCO_2^- + H2O + BC --> $(RCO_2^-(H^+)$-BC + OH^- . This represents a novel mechanism for sorption of carboxylate ions and has significant implications for natural and synthetic entities (molecules or substructures) having a pKa in an intermediate range (e.g., 3-8). A possible driving force is the hydrophobic effect of the undissociated acid, which may be strong enough to overcome the unfavorable energy required to ionize water.

HM04-6

How to model the sorption affinity of cationic organic compounds in natural organic matter?

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Using a highly consistent experimental data set for 70 compounds, we examined how the molecular structure of organic cations affects the sorption affinity to natural organic matter (NOM). Positively charged organic compounds, such as cationic surfactants, fluoxetine (prozac) or methamphetamine, are sorbed to negatively charged sites in NOM. This sorption process occurs mainly through an ion-exchange process with other sorbed cations, such as sodium or calcium. Previous studies and our recent work showed that the affinity of organic cations for NOM increases (i) if salt concentration decrease, and (ii), for organic cations with larger nonpolar side chains. Thus, the ion-exchange affinity of organic cations on NOM involves both ionic and non-ionic interactions. For a proper risk assessment of organic cations, it is highly desirable to predict the influence of ionic and nonionic interactions from the molecular structure. Testing sorption at controlled pH and ionic strength creates constant influence of ionic interactions. Remaining differences between organic cations will then be related to differences in nonionic interactions. The observed differences in ion-exchange affinities for organic cations are not explained by calculated differences in octanol-water distribution coefficients (Kow), as recently proposed in risk assessment procedures. Other single-parameter regressions also appear to be unsuccessful. We demonstrate that multiparameter models can, as far as we studied, at best partially explain differences in sorption affinity between compounds, because the selection of the suitable descriptive molecular parameters is highly challenging. New insights in the ion-exchange affinity of organic cations and advanced sorption models are highly needed for further improvement of risk assessment procedures.

LC01 - Developments in Life Cycle Impact Assessment (LCIA)

LC01A-1

Fate modelling of chemical compounds with incomplete data sets

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Impact assessment of chemical compounds in Life Cycle Impact Assessment (LCIA) and Environmental Risk Assessment (ERA) requires a vast amount of data on the properties of the chemical compounds being assessed. These data are used in multi-media fate and exposure models, to calculate risk levels and other indicators. ERA typically addresses one specific chemical, but in an LCIA, the number of chemicals encountered may be quite high, up to hundreds or thousands. This study explores the development of meta-models, which are supposed to reflect the "true"multi-media fate and exposure model in an approximate way. The idea is that not all data needed in a multi-media fate and exposure model are completely independent and equally important, but that there are physical-chemical and biological relationships between sets of chemical properties. A statistical model is constructed to underpin this assumption, and to provide simplified proxies for the more complicated "real"model relationships.
In the presented study two approaches for the reduction of the data demand associated with characterization of chemical emissions in USEtox™ are tested:
The first approach yields a simplified set of mode of entry specific meta-models with a data demand of app. 63 % (5/8) of the USEtox™ characterization model.
The second yields a simplified set of mode of entry specific meta-models with a data demand of 75 % (6/8) of the original model.
The results of the study indicate that it is possible to simplify characterization models and lower the data demand of these models applying the presented approach. The results further indicate that the second approach relying on 75 % of the original data set provides the meta-model sets which best mimics the original model. An overall trend observed from the 75 % data demand meta-model sets, is that except for fate factors covering the route from soil emission (natural and agricultural soil) to aquatic compartments, good correlation between the predicted fate factors (derived from the meta-models) and the observed fate factors (modeled in USEtox™) are obtained. The regression coefficients obtained for the predicted fate factors plotted against the observed fate factors, excluding the fate factors covering the route from emission to soil to aquatic compartments, were all in the range 0.6818≤ R2 ≤0.9470

LC01A-2

Variability and spatial distribution of chemical's removal rates: implication for spatial resolution within Life Cycle Impact Assessment

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Spatial differentiation is a topic of increasing interest within Life Cycle Impact Assessment (LCIA). A key issue to be addressed in the LCIA methods, models and corresponding characterisation factors is the level of spatial detail required and uncertainties related to the use of generic characterisation factors when the exact location of the activities is unknown. For ecotoxicity and human toxicity impact categories, some preliminary evaluations were done in order to assess the relative influence of substance properties and environmental paramenters on the variability in the fate of chemicals in air, water and soil. In this work a methodology was developed as a guideline to decide the appropriate spatial resolution to address the environmental fate as first step of impact assessment of chemicals. The methodology was tested on a set of 34 representative organic chemicals, clustered according to their physical and chemical properties. The test set took into account chemicals having a large diversity of properties in order to be representative, as far as possible, as well as their potential differences in the environmental fate.

LC01A-3

Development of a new modelling framework to address issues of metal fate and effects in LCIA

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Life Cycle Impact Assessment (LCIA) models currently estimate fate and toxicity assuming that all dissolved metal species are equally bioavailable and can cause toxicity in ecological receptors. This treatment of metals, similar to organic chemicals, introduces a significant error mainly because of metal speciation and non-degradability that affect their bioavailability and fate. The goal of our project was to incorporate state-of-the-art science into a consistent, generic framework for adjudicating chemical hazard with a view to addressing the criticisms raised for metals.
We recently proposed a new modelling framewok that introduced a Bioavailability Factor (BF) to the calculation of Comparative Toxicity Potential (CTP) for use in Life Cycle Impact Assessment (LCIA). The method uses (1) USEtox for environmental fate, (2) WHAM 6.0 for metal partitioning and speciation in aquatic systems, and (3) Biotic Ligand Model (BLM) for calculating average toxicity or Effect Factor (EF). We also proposed to apply modified Free Ion Activity Model (FIAM) to calculate EFs for metals for which Biotic Ligand Models are not available. While arriving at a set of CTPs, we explored the sensitivity of factors like variability in the chemistry of receiving environment on metal fate and toxicity. We showed the implications of choosing a watertype by applying the model to Cu, Ni and Zn for 100 frehswater systems of world's largest lakes and rivers with varying aquatic chemistry. Results of model applications consistently showed that the greatest contributor to variability in CTP was BF followed by EF. A large range of ~4 orders-of-magnitude in estimates of BFs and EFs often changed the relative hazard of metals based on numerical ranking of CTPs. However, the current LCIA practice has been to use generic environmental data to typify a single "evaluative environment."To address this issue, we then analyzed the individual effects of water chemistry parameters, such as pH, DOC and hardness, to derive generic freshwater archetypes to be used in LCIA.
The successful outcome of this project is a new modelling framework for metals, which is consistent with that for organic chemicals, to provide equal and fair evaluation of environmental impacts of their emission. We report a large range of individual metal CTPs that relate mainly to variability in ambient chemistry or archetypes.

LC01A-4

Comparison of metal toxic impacts between aquatic and terrestrial organisms: is the free ion concentration a sufficient descriptor?

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Characterization of metal toxic impacts in comparative risk assessment and life cycle impact assessment (LCIA) should take into account metal speciation and interactions with soil/water organic constituents, because these mechanisms control metal bioavailability and may influence their toxic properties. In a comparative context we are faced with the need to characterise thousands of substances, but the limitation of the available data calls for reliable indicators suitable for extrapolation from the limited data that is available. Indeed, free metal ion concentration has in some cases been shown to be a sufficient indicator of metal toxicity for both aquatic and terrestrial species. With the aim of deriving extrapolations to predict terrestrial toxic impacts of metals from aquatic effect data, we compared copper toxicity of aquatic organisms with that of terrestrial organisms, testing the hypothesis that the free metal ion is an appropriate "general"descriptor of metal toxicity.
Results for 128 laboratory tests on Daphnia magna exposed to copper ions (Cu2+) in water show that variation of several orders of magnitude are observed between the toxicity tests. These variations may be a result of the inability of the free metal ion concentration to reflect toxicity, as the presence of protons and other cations reacting with biological binding sites has been shown to affect the toxicity of copper to D. magna. Similar patterns, albeit with smaller variations, are observed for terrestrial organisms. Up to three orders of magnitude difference occur for the extreme case of barley (Hordeum vulgare).
Given the scarcity of terrestrial effect data compared to aquatic data, reliable and transparent, mechanistic-based predictions of terrestrial toxic impacts from aquatic effect data would be an important step ahead in the context of LCIA or comparative risk. Here we demonstrate that the overall ability of the free metal ion to reflect toxicity of metals for aquatic and terrestrial organisms is limited. This has consequences if potential terrestrial toxic effects are based on extrapolations from aquatic data, because the use of more sophisticated models such as the Biotic Ligand Model (BLM) would be required. However, extrapolation models based on an improved free ion approach might still be a good proxy, particularly when the comparative nature of life cycle assessment is taken into account.

LC01A-5

Including ecotoxic effects on warm-blooded predators in life cycle impact assessment

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In current Life Cycle Impact Assessment (LCIA) of ecotoxicity the focus is on cold-blooded species. This paper describes a method to calculate Characterization Factors (CFs) for the assessment of potential toxic impacts of chemical emissions on warm-blooded predators in freshwater food

chains. The method was applied to 329 organic chemicals. The CF for warm-blooded predators was defined as a multiplication of the chemical-specific Fate Factor (FF), Exposure Factor (XF), Bioaccumulation Factor (BF), and Effect Factor (EF). FFs and XFs were calculated with the multimedia fate and exposure model USES-LCA 2.0. BFs were calculated with the bioaccumulation model OMEGA, for chemical uptake via fresh water, food and air. EFs were calculated based on median lethal doses (LD50-values), obtained from literature. The chemicals' Concentration Built-up (CB, i.e. the product of FF, XF, and BF, summed for the different routes of exposure) showed a range of 7 to 9 orders of magnitude, depending on the emission compartment. EFs displayed a range of 7 orders of magnitude. Characterization factors ranged 8 orders of magnitude for an emission to rural air, fresh water, and agricultural soil. The contribution of the different uptake routes to CB were 1% (90%-CI 0-2%) for uptake via air, 43% (90%-CI 11-50%) for uptake via water, and 56% (90%-CI 49-87%) for uptake via food, after emissions to fresh water. Uptake via air was mainly relevant for emissions to air (on average 42% with 90%-CI 5-98%). Our results showed that the chemicals' CB were positively correlated with their Kow, irrespective of the emission compartment. In conclusion we can say that we were able to calculate CFs for the impact of toxic emissions on ecosystems. Uptake via air, fresh water, and food may all be relevant, depending on the emission compartment and the properties of the chemical. The inclusion of biotransformation was highly relevant, but uncertain at the same time. The poster will further discuss the practical implications of our results for the assessment of toxic emissions in a life cycle context.

LC01A-6

LCA of contaminated site remediation - integration of site-specific impact assessment of local toxic impacts

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The environmental impacts from remediation can be divided into primary and secondary impacts. Primary impacts cover the local impacts associated with the on-site contamination, whereas the secondary impacts are impacts on the local, regional and global scale generated by the remediation activities. Although two different remediation methods reach the same remedial target with time, their timeframes can be substantially different and lead to a difference in the local toxic impacts over time. By including primary impacts in the LCA of remediation this quality difference is accounted for.

Primary impacts have typically been assessed using site-generic characterization models representing a continental scale and excluding the groundwater compartment. Soil contaminants have therefore generally been assigned as emissions to surface soil or surface water compartments. However, such site-generic assessments poorly reflect the fate of frequent soil contaminants such as chloroethenes as they exclude the groundwater compartment and assume that the main part escapes to the atmosphere. Another important limitation of the generic impact assessment models is that they do not include the formation of metabolites during biodegradation of chlorinated ethenes, of which particularly vinyl chloride is problematic due to its toxic and carcinogenic effects. In this study, the assessment of local toxic impacts with the USEtox model was therefore combined with site-specific reactive transport modeling of the contaminant mass discharge to groundwater. The exposure via contaminated groundwater was subsequently estimated using exposure parametres representing the local groundwater body.

The developed methodology for a site-specific impact assessment of primary impacts is tested on two case localities contaminated with chlorinated solvents. Secondary and primary impacts of a number of remediation options for the two sites are evaluated and compared. The results show that especially vinyl chloride, which is an intermediate product during biodegradation of trichloroethene, contributes significantly to the human toxicity of bioremediation scenarios (86-98 % of the human toxicity impacts at Site 1). The inclusion of primary impacts in the environmental assessment of remediation alternatives gives a more complete basis for comparison of technologies with substantially different timeframes and efficiencies.

LC01B-1

Classification of pollutants into emission based impact categories : a site specific conceptual frame

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The classification step is systematically neglected in the calculation of LCIA indicators: for several direct contribution of one pollutant to several impact categories, the distribution between these impact categories is not accounted. The proposed approach of a non-redundant classification is based on the probability that an emitted substance and its chemically degraded forms are involved into several environmental impacts.

The so-called zone classification is based on a classification coefficient alpha and requires categorization of chemical pathways (reactive, suspensive, direct, indirect). It is made of two steps: i) the first step requires defining an impacted zone around the source, inside which the emitted chemicals are expected to majorly diffuse or spread; ii) in the second step, the score of the chemical is set according to the occurrence of the chemical target inside the impacted zone.

The method is applied on a case study of NOx emissions in Paris. The choices of impacted zone and of chemical targets are discussed with the aim to meeting paradoxical constraints: local parameters must be sufficiently detailed in the majority of cases, but generic enough to avoid time consuming researches. The availability of data is also discussed as well as the possibility to include indirect impacts into the environmental system.

The conceptual frame suggested for classification of pollutants into emission-based impact categories, is a first approach for a scientific question that was raised in the 90's. It has important consequences in terms of data collection, system boundaries, and only practise will show its actual feasibility.

LC01B-2

Toward consistent Emergy calculations with LCI databases: perspectives on characterization factors

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Emergy, a method developed by Howard T. Odum during the eighties, is defined as the quantity of energy (usually solar energy, calculated in solar em-joules; i.e. seJ) directly and indirectly necessary to support a given system and its level of organization. It provides a measure of the global natural efforts required (i.e. memorized) to make a product or service available. Its boundaries of calculation can be very large, including for instance many of the ecosystem services not captured, at present, by other life cycle oriented approaches. Aim of this work was to develop a consistent set of characterization factors that may enable for a comprehensive calculation of emergy in the Ecoinvent LCI database. These factors are identified in Unit Emergy Values (UEVs) of primary natural resources (e.g. mineral, metal, fossil, water, etc.), and are calculated in MJse/unit of resource (e.g. MJse/kg, MJse/MJ, MJse/m3). In conventional emergy evaluations, a starting reference baseline, represented by the sum of solar, tide, and Earth deep heat energies (annual emergy budget; e.g. 9.44E24 seJ/yr), is used to estimate the average annual emergy flows of wind, water, and land systems, and therefore all other resource and product UEVs in cascade. This traditional approach (i.e. 'top-down') presents several sources of uncertainty. We propose here a uniform 'bottom-up' procedure that may allow for a more consistent quantification of primary resource UEVs. In this case, only strictly necessary quantities of sun, tide, and deep heat energies are directly and independently included in natural resource productions. Results, in particular for fossil resources (i.e. gas, oil, coal), showed that 'bottom-up' based-UEVs are slightly higher than previous 'top-down' based-ones, due to an increase of calculation details. Advances have been made in re-calculating UEVs for a larger number of Ecoinvent elementary flows (e.g. for minerals, land occupations, biomasses). The 'bottom-up' approach should be further expanded to include all resource category groups, providing regionalized factors according to new LCI database versions possibly being available in the future. As discussed, re-calculating 'bottom-up'-based UEVs is one of the essential step toward a consistent implementation of the emergy method in LCA, giving added value to existing methods in the sphere of assessment for resource consumption and ecosystem quality.

LC01B-3

The Water Impact Index: a stand-alone metric for water footprinting

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The decrease of freshwater resources availability is recognized as one of the major environmental issue of coming decades. Within Life-Cycle Assessment, several impact categories already aim to tackle impacts of entropic activities on water bodies, both from the quality aspect (i.e. aquatic ecotoxicity, eutrophication), but also from a quantity aspect. However, decision makers often have to deal with a high number of indicators, being environmental but also economical or social. Therefore, the number of environmental indicators considered by decision makers is often limited, leading to the avoidance of important environmental issues.

In order to reduce the number of environmental indicators related to freshwater aspects, the Water Impact Index: a "stand-alone"metric for assessing impact of entropic activities on freshwater resources has been developed. This indicator accounts for the reduction of freshwater availability for different users (both humans and ecosystems). For any product or process, the physical water balance is weighted by a quality index and a water stress index. This methodology is applied with a Life-Cycle thinking, taking into account both direct but also indirect water uses of any process. A case study on municipal water services shows how this indicator could be applied in order to help decision makers in a better understanding of water use impacts. Regarding drinking water production, the Water Impact Index is driven by direct water abstraction, as the quality of the resource used is very high. The sewage system contributes to reduce the Water Impact Index: the quality of wastewater collected is improved and brought back closer to environmental requirements. The study of different scenarios, such as overflows management solutions or potential water treatment processes improvements, has highlighted that the direct Water Impact Index could be easily decreased, with a low impact on indirect Water Impact Index. Finally, some crossed carbon footprint / Water Impact Index studies have shown that even if some change in water treatment solutions can lead to impact trade-off, some win/win solutions remain possible.

This study shows how some pieces of existing LCIA methodologies for assessing water use could be combined in order to create a simplified methodology to assess impacts of water use.

LC01B-4

Urban-wide change in albedo: a multi-scale approach to LCA. The case study of New York City.

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In last decades the urban heat island phenomenon is becoming more frequent than in the past. The exposure to extreme temperatures is the primary cause of weather-related mortality in the United States especially among old people or people with social or physical vulnerability. Mitigation and adaptation strategies are necessary to reduce the impact of the extreme temperaures on human health. One of the possible mitigation strategy is the increase of the urban albedo. Typically, the life cycle assessment (LCA) evaluation of a building components settled in urban contexts considers just partially the interactions between the components and the environment. In this study we considered the effects of the city-wide conversion of traditional black roofs into white roofs through the use of a LCA multi-scale approach. In a 'traditional' LCA approach the impact relative to the substitution of city-wide black roofs with white roofs would have been evaluated as the product of a functional unit white roof per the number functional units of roofs. Although this approach is widely applied it is not always able to explain the relationships between roofs and urban context. In this study we evaluated the avoided impact on human health due to the decrease of the summer temperatures obtained substituting the city-wide black roofs with white ones. The avoided damages on human health have been calculated using epidemiological data and considering an end-point approach to the LCA. The avoided deaths for heat strokes have been translated - through the use of statistical data - into Disability Adjusted Life Years (DALY). The total number of DALY has been divided per the number of square meters of white roofs, in order to have the equivalent amount of avoided impact on human health relative to each square meter of surface. The avoided impact on human health has been considered in the LCA evaluation of maintenance phase of a square meter of white roof. It has resulted that the avoided damages on human health determine a decrease by 69% in the final eco-score of a square meter of white roof. In this study we broadened the application of LCA to the urban context deepening the parameter albedo and we suggested a possible way to overcome some of the limits of the traditional LCA approach especially when it is applied to the building sector. Furthermore, this study provides useful information for decision-makers and policy-makers about environmentally preferable choices.

LC01B-5

Including noise impacts on human health in LCA

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Important methodological improvements are still needed for the development of characterisation models and factors to be used in LCA practice. In most cases the introduction of new impact categories would sensibly change the results of many completed LCA studies. LC-IMPACT three years' project focuses on the development and application of methods, characterisation and normalisation factors for categories not commonly included in life cycle impact assessment studies, taking the ILCD and SETAC/UNEP recommendations as a starting point. Our research within the project contributes to the development of a method for the inclusion of noise impacts on human health in LCA. To date, sound and noise are seldom present in LCI. Impact assessments have been conducted with regard to noise exposure. The few attempts available in the literature developed methodologies which are far from having the needed generalisation which would make them applicable to large systems, on a European or World level, or the flexibility for expanding them from a noise source (e.g. transportation noise) to another and from a subject (e.g. humans) to others. Our study aims at expanding from the knowledge acquired in the field of transportation noise, which seems to have attracted most of the research focus, to developing a method to include in LCA generic sources of noise which have a quantifiable impact on human health and which are of interest in the LCA practice.

LC01B-6
Biome-specific effect factors for terrestrial acidification at the global scale
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Up to now, life cycle impact assessment (LCIA) does not encompass regionalized and global scale effect factors for terrestrial acidification. The major increases in acid deposition currently occur in soils that are not only the most sensitive to acidification but also the ones that hold the highest share of world's biodiversity. Thus it is important that LCIA is performed at the global scale. One of the outcomes of soil acidification is the decrease of soil pH. Given that the performance of plants depends on this soil property, changes in soil pH will affect plant diversity. This research consisted on an extensive literature review in which the potentially non-occurring fraction (PNOF) of plant species relative to soil pH was determined for the fourteen world biomes (e.g. tundra, deserts). The literature review yielded occurrence and non-occurrence data on over 5000 plant species; and 182 studies were included. Next, we computed biome-specific effect factors corresponding to changes in PNOF following a marginal change in soil pH. This study also considered the uncertainty of the effect factors within each biome. The results of this work provide regionalized biome-specific effect factors for terrestrial acidification at an endpoint level and at the global scale.

LC02 - Developments in Life Cycle Sustainability Assessment (LCSA)

LC02-1
Towards a life cycle sustainability assessment of products
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Taking the life cycle approach means going beyond the traditional focus on production site and manufacturing processes and including the environmental, social, and economic impact of a product over its entire life cycle. In order to put life cycle thinking into effective practice, the United Nations Environment Programme (UNEP) and the Society for Environmental Toxicology and Chemistry (SETAC) launched an International Partnership, the so-called 'Life Cycle Initiative'. The Life Cycle Initiative contributes to the 10-Year Framework of Programs in order to pursue Sustainable Consumption and Production (Marrakech Process). The Project Group of the Life Cycle Initiative on Social Life Cycle Assessment started its work in 2005. Following the completion of the UNEP Guidelines for Social LCA of products [1], this Project Group has the aim to interlink current LCA tools and provide a triple-bottom-line sustainable development toolbox.

LC02-2
Life Cycle Costing - the second pillar in Life Cycle Sustainability Assessment (LCSA)
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Sustainability was adopted by the UN in Rio de Janeiro (1992) as the main political goal for the future development of humankind. It should also be the ultimate aim of product development. According to the well known interpretation of the original definition given in the Brundtland report, sustainability comprises three components: environment, economy and social aspects. These components or "pillars"of sustainability have to be properly assessed and balanced if a new product is to be designed or an existing one is to be improved.
For the environmental pillar there is already an internationally standardized tool (ISO 14040+14044: 2006): (Environmental) Life Cycle Assessment (LCA). Environmental Life Cycle Costing (LCC) is the logical counterpart of LCA for the economic assessment of products and Social Life Cycle Assessment (SLCA) for the social pillar. LCC surpasses the purely economic cost calculation by taking into account the use- and end-of-life phases and hidden costs. An LCC Code of Practice has been developed and recently published by SETAC. This code gives advise how to conduct an environmental LCC in accord with LCA. It is a very important point that different life-cycle based methods for sustainability assessment use consistent (ideally identical) system boundaries. This also includes the use of the physical life cycle in this "LCA type"LCC. Only in this case, LCSA can be defined and quantified according to the following general formula:
LCSA = LCA + LCC + SLCA
Weighting between the three pillars should be avoided so that an excellent economic performance cannot outweigh the environmental and social components. It should be noted that LCC is also a very useful stand-alone method for the estimation of the costs of a product over the whole life cycle. The full power of LCC is shown, however, in combination with LCA and SLCA.

LC02-3
Economic material availability as a new area of protection for life cycle sustainability assessment
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The assessment of material use and availability is of major importance to secure future resource supply. Especially with regard to new vehicle concepts, resource questions are of increasing relevance as supposedly scarce metals are used.
So far, the utilization of resources along the life cycle of vehicles is assessed with indicators like primary energy demand and abiotic depletion potential. However, those indicators are usually dominated by the usage of fossil energy carriers. Materials which are commonly perceived as critical, like rare earth metals or lithium, do not contribute to the results of these indicators in a noticeable manner. The reason for this is that indicators like abiotic depletion potential only focus on geological extraction - reserve - ratios and partly use rather theoretical stocks like ultimate reserves (total material stock in the earth crust). Hence, they deliver no conclusion about the real availability or criticality of metallic resources.
In this work we introduce a new area of protection, the economic material availability. Several geological and economic criteria are identified and selected according to their potential and relevance for quantifying economic material availability. Different options for assessing these indicators are modeled and tested with regard to their usefulness and applicability within life cycle assessment.
In order to illustrate the effects of the new area of protection and the corresponding indicators, a comprehensive case study is accomplished. The life cycle inventory of a product from the automotive industry is assessed by means of conventional abiotic depletion potentials and the newly developed economic material availability indicators.

LC02-4
Social LCA of an ecolabeled notebook
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The demand for electronic products increases continuously, especially portable devices as laptops gain in popularity - irrespective of their impacts on environment and society. Computers are very complex products with global value chains: They are assembled in Asia, pre products come from Korea, Thailand, China, India, Mexico, or the Philippines, raw materials originate from all over the world. In addition, the majority of e-waste is recycled illegally in Asia or Africa. Strong competition moves the production of electronic devices and modules to low wage countries with low social and environmental standards, thus notebooks cause along their life cycle many negative environmental as well as social effects.
The presentation will focus on a study where we analyzed social, socio-economic, and also environmental impacts of an ecolabeled notebook of the Taiwanese company ASUSTeK, over its entire life cycle. The study was conducted from June until October 2010. The social and socio-economic impacts were modeled following the method of social LCA (S-LCA), according the UNEP/SETAC guidelines for S-LCA of products. The newly developed methodological sheets for the S-LCA method were considered as well.
We will present our modeling approach, the indicators / subcategories / stakeholder framework developed for the S-LCA, data sources for supply chain modeling and for assessing social and environmental impacts, practical problems in the modeling process, solutions, and also lessons learnt from the case study.
Considering methodological aspects is rewarding as we have conducted one of the first real case studies for social LCA; we will therefore report "how it was to apply the method".
Little explanation is probably needed on why it is interesting to look at the outcomes of the case study. With a consistent consideration of social and environmental impacts of a notebook over its entire life cycle, the case study is able to show hot spots for the environment and regarding social impacts that are related to the notebook. The study is also able to relate social benefits to environmental impacts. We will therefore present results of the case study, and of recommendations that can be derived, on a policy, company, and private consumer level.

LC02-5
Societal perspective in S-LCA: a parallel to welfare maximisation in economic theory
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Social life cycle assessment (S-LCA), as defined in the UNEP/SETAC Guidelines [1], is increasingly emerging as a method for evaluating social performance of products/systems. The framework, which follows the LCA structure closely, does not pose limitations on the possible applications that might range from products to complex systems (e.g. single technologies or systems like waste management systems, etc.). The assessment is conducted from the perspective of different stakeholders, whose relevance is determined by the object and goal of the analysis. However, some critical points have been identified when the framework is applied to the evaluation of a (new/innovative) technology: what perspective should be adopted and, consequently, how the most appropriate indicators are identified.
In present applications, a company perspective is normally at the core of the method. Social impacts in S-LCA are related to the way a specific company manages social aspects of concern. Preliminary discussions by the authors highlighted that a company perspective should be left aside in favour of a societal one when a technology is the object of the analysis. The societal perspective should consider how the adoption of the technology would affect the social structure in which it will be embedded. To characterize the different perspective, the definition of a new stakeholder is proposed, which has foundations in economic theory

LC02-6
Life Cycle Sustainability Assessment of multifunctional land-use systems: a consequential modelling approach
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Concerns over global climate change, decreased provision of ecosystem services and biodiversity loss have led to interest in using land in ways that mitigate these threats, particularly in using various forms of bioenergy to displace fossil fuels or to sequester carbon. In view of the competing

demands on land to feed people adequately, sustain biodiversity and ecosystem services and mitigate climate change, there is a clear need for a systematic basis for allocating land use with respect to economic and environmental objectives.
This study formulated an integrated environmental and economic assessment of the global consequences of changing current land use in UK with different land-use strategies for food, feed, fibre, fuel and carbon sequestration. Novel operational approaches are proposed for resolving methodological issues, and are applied in the characterisation of the main land-use strategies in the UK: diversion, intensification and extensification of existing cropland; and expansion onto set-aside and grassland. 224 scenarios were devised to reflect the different uses of the considered crops: wheat, oilseed rape, barley, sugar beet, Miscanthus, willow, and 9 forestry crops.
The results indicate that diverting food to either feed or fuel generally does not deliver improvements in all three criteria (i.e. of mitigating climate and biodiversity impacts, while creating extra economic value), but generally results in greenhouse gas (GHG) emission savings, mainly due to indirect land-use change (iLUC) effects.
Intensification in all land uses almost never saves GHGs. Conversely, extensification in all land uses almost always saves GHGs, but results in higher impacts to ecosystems. Expanding arable crops onto grassland never results in GHG savings, and only a few combinations of crops on set-aside will save GHGs. Only intensive strategies present Pareto improvements. Forestry crops always perform well. With very few exceptions, it is best to leave grassland untouched.
Expansion onto set-aside is undesirable by arable cropping, but desirable by energy and forestry crops. The former are best used for electricity whereas the latter as carbon sinks, even though no economic value is generated in the foreground system.

LC03 - Increasing robustness of LCA methodology

LC03-1
Development of product category rules for a sector environmental product declaration
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Systems for Type III Environmental Product Declarations (EPDs), relying on LCA methodology, are gradually becoming more known and operational on the market. Many industrial associations show growing interest on "product-type EPDs", in contrast to product specific EPDs. For this reason, the International EPD® system introduced a new feature, i.e. the Sector EPD. This tool shows a sort of average environmental profile of the product, based on the typical processing adopted in the local context and representative of the production offered by the sector. For this application, specific rules [Product Category Rules (PCR)] have to be issued to ensure comparability among different declarations within the same service group. The aim of the present study is to describe the particular choices in the development of PCR for a Sector EPD applied to cement production, as a case study.
PCR 2010:9 has been developed in order to define the requirements, based on environmental parameters, that should be considered in the LCA and EPD for the production of cement. The document was issued in an open and participatory process between companies and organizations having a good knowledge of the specific environmental aspects of the process.
Since the EPD is a concise document that outlines a simple profile of the environmental performances of a product, including objective, comparable and credible data, the definition of an appropriate methodology must be performed with particular rigour. The process of development of this methodology for a Sector EPD includes the definition of required data, data collection procedures, sample selection criteria, etc. The research highlights that a targeted analysis of the sample representativeness is required to support the robustness of the selection in view of the successive verification process. At the same time, some precise methodological choices have to be established in order to define the procedural criteria on the base of the transition from a product-specific to a product-type declaration. In this way, a sector EPD can find application in the use as a sector bench-mark for separate producers manufacturing or offering products and services in the same product category. It can also be used as a guidance tool indicating the general environmental performance of a product category, i.e. for planners, designers and architects in the construction sector not needing information from specific products from selected suppliers.

LC03-2
Assessing temporary carbon sequestration and storage with dynamic LCA
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Life cycle assessment (LCA) is a preferred tool to assess greenhouse gases (GHG) mitigation projects as it considers the entire life cycle of the studied system and it allows looking at different environmental impacts in addition to global warming. However, to assess temporary carbon sequestration and storage projects, it is important to take into account temporal aspects, which current LCA methodology does not allow to do. Indeed, delaying a GHG emission or sequestering a certain amount of carbon and releasing it back to the atmosphere several years later will not have any effect on LCA results as emissions are aggregated in the inventory without any consideration for their timing.
Levasseur et al. have developed a dynamic LCA approach which includes the temporal distribution of the emissions in the inventory, and then uses dynamic characterization factors to determine the time-dependant impact of every emission according to the moment they occur. Dynamic characterization factors have been developed for the global warming impact category by integrating over the time the Absolute global warming potential (AGWP) defined by the Intergovernmental Panel on Climate Change (IPCC) for each GHG [2]. In this paper, the dynamic LCA approach is applied to a 70-year life afforestation project meant to compensate for the impact of an initial 1,000 kg CO2-eq pulse-emission, and evaluates whether the benefits of this temporary carbon sequestration and storage project indeed mitigates the impact of the initial emission. This mitigation is evaluated for several sequestration scenarios.
Dynamic LCA is a flexible approach to consistently consider the timing of the emissions while assessing the impact on global warming of any type of product system. Therefore, it allows evaluating whether a temporary carbon sequestration and storage project compensates for a given GHG emission. Dynamic LCA also allows decision makers to test the sensitivity of the results to the choice of a time horizon for the analysis.

LC03-3
Methods addressing water use in LCA: review and recommendations
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Stress on global water resources is recognized as an important issue. Although the impacts related to water use on human life, and on biotic and abiotic environments can be considerable, such impacts have only recently been assessed in LCA.
The present project aims at reviewing and performing a systematic qualitative analysis of existing methods linked to assessment of water use. This project is part of the work undertaken by the « Water Use in LCA » working group of the UNEP-SETAC Life Cycle Initiative. The methods are assessed according to a set of pre-defined criteria (completeness of scope; environmental relevance; scientific robustness and certainty; documentation, transparency and reproducibility; applicability and potential stakeholder acceptance) and a procedure as proposed by the ILCD Handbook.This work looks at similarities and differences between methods, identifies key elements and provides indications for deriving operational characterization methods and factors to assess water use in LCA. Interim recommendations on water use modeling, inventory database development and impact assessment methods are formulated to support practitioners in their short term application. The methods evaluation as well as the outcoming recommendations will be presented as the results of this work.
The methods assessed are: Inventory databases: ecoinvent; GaBi; Quantis (in development), WFN database (in development) Inventory methods: WBCSD 2007 (Global Water Tool); Chapagain and Hoekstra 2009; Vince 2007; Boulay 2010; Bayart 2008; Milà I Canals 2009; Peters 2010; Quantis 2010 (in development). Midpoint impact assessment: WFN 2009; Ridoutt and Pfister 2010; Frischknecht 2006; Pfister 2009; Milà I Canals 2009; Boulay 2010;Veolia 2010, Bayart 2008. Damage impact assessment: Pfister 2009; Motoshita 2010; Boulay 2010; Maendly and Humbert 2010; Verones 2010; van Zelm 2010; Bösch 2007 (CExD).

LC03-4
Enhanced structure path analysis: a new method to create spatiotemporally defined Life Cycle Inventory
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Refinements to the Life Cycle Assessment (LCA) methodology are required to obtain realistic and scientifically sound LCA results. We propose to contribute to this goal with the creation of spatiotemporally defined Life Cycle Inventory (LCI).
A fully spatiotemporally defined LCI will ultimately enable its comparison with extractions/emissions (identified as Pressures in the DPSIR framework) from direct environmental analysis models. It then might be possible to validate the same LCI through a comparison with real world observations of substances concentration in the environment.
A method has already been proposed to create LCA results which can be used with spatially resolved impact analysis factors. This method use the same calculation structure as the standard LCA calculation but will encounter difficulties to create temporally resolved LCI.
We propose a new method based on the Structure Path Analysis (SPA) methodology. Here we are mostly interested in the SPA method capacity to identify the linkage between technological processes in a supply chain. This characteristic should enable the use of temporal functions to describe the moments of emissions/extractions for any process of the supply chain. We will present how we define the limits of this calculation and the database requirements for using temporal functions.
The method can work with different level of precision on the spatial and temporal information. This means that LCI results could be assessed by different spatially resolved impact analysis method. The only requirement for this step is to create a translator for varying spatiotemporal information.
This new method called ESPA is a first step to create LCA results which should be more realistic and which might be compared in the future to real world observations first at Pressure level and then at State level by using appropriate models.

LC03-5
Re-engineering LCA for simplicity and flexibility
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With the recent availability of the ecoSpold 2 data format it has become possible to realise the full potential of the design stratgey for database flexibility proposed by Weidema (2003). This design strategy has now been applied to the ecoinvent database, resulting in an LCI database with comprehensive coverage and full flexibility for different applications, for example attributional models with any desired allocation key, and consequential models with any desired level of constraints. At the same time, this design flexibility leads to a reduction in the efforts needed for the maintenance of the database, since allowing many different models to be constructed from the same basic unlinked unit process datasets means that only one dataset needs to be maintained for each unit process, and the maintenance of each dataset can be made independent from the maintenace of all other datasets. Flexibility and simplicity are therefore not contradictions but rather simultaneous results of the new design strategy. The philosophy behind the strategy is explained and several details of the technical implementation are described.

LC03-PS
Poster spotlight: How to communicate LCA results?
Miscellaneous
Poster spotlight highlighting abstracts TU 267, TU 268, TU 269:
- The translational roles of LCA studies and practitioners
- The EPD® approach as meeting point between robustness and communicability of LCA studies: the certification of an internal LCA process
- How potential carbon policies could effect producer grain cultivar selection: a LCA analysis of USA rice
- Accuracy vs. robustness: challenges of including LCA into legislations/standardization schemes for sustainable biofuels

LC04 - Life Cycle Inventory modelling and attributional/consequential issues (LCI)

LC04-1
Consequential LCA, attributional LCA and scenarios
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Consequential LCA (CLCA) has become a popular mode of LCA modeling. Starting from the

beginning of this century it has significantly grown in number of case study applications. The search term "consequential"yields for the the International Journal of Life Cycle Assessment 77 hits. Now, at SETAC Europe 2011, there is a separate theme "Life Cycle Inventory modelling and attributional/consequential issues (LCI)". This paper discusses the role of scenarios in relation to LCA, consequential or attributional. In doing so, we will focus on the LCI phase.
CLCA builds up a consequential product system from product bottom-up. Consequences all over the world in terms of displaced land, water, energy production, etc. are all modeled from a single product's perspective. Upscaling the results of bottom-up CLCAs independently is very likely to give inconsistent and impossible results. We therefore argue that such bottom-up scenarios may not be the best way to quantify direct and indirect effects for product systems related to main sustainability (transition) problems like energy, land use, water and material/resources use, transport systems etc. For these problems, it might be more appropriate to develop top-down scenarios (from global scenarios to single product consequences, e.g., from global energy scenarios to single product's consequences). Research in this area still needs to be developed further. Some first (incomplete) examples of top-down scenario approaches for LCA are provided.

LC04-2
Scope dependent inventory models: a proposal for the choice between attributional and consequential models
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Three basically different models exist in life cycle inventory analysis: the attributional, the consequential and the decisional model. We propose criteria that help to classify typical LCA questions regarding real business cases and find the most appropriate LCI model. The framework is applied to a case study of an LCA where attributional and decisional LCI datasets of European electricity mixes are described in order to show the differences of the environmental impacts depending on the modelling approach selected.
Individual decision with comparatively small consequences can be modelled under ceteris paribus (other things being equal) conditions. Decision situations with medium to large potential consequences should be modelled under the conditions of mutatis mutandis (the necessary changes being made). The key question is how to distinguish between small, medium and large consequences. We recommend using the relative economic size to classify objects of investigation and the LCA goals related to them into three groups to which the most appropriate LCI models are assigned.
The attributional approach is sensible for environmental reporting and product labelling and declaration where the relative economic size of the object of investigation is small. The decisional approach is sensible for LCAs of product and process development, as well as site and supplier evaluation carried out by private companies in case the relative economic size of the object of investigation is medium. The consequential approach is of relevance for policy support of governments and international organisations as well as for strategic decisions of companies, where the relative economic size of the object of investigation is large. The European attributional and decisional electricity supply mix causes greenhouse gas emissions of 554 g CO2-eq./kWh and 473 g CO2-eq./kWh, respectively.
The criterion "relative economic size"helps to better decide on the appropriate LCI model to be applied in decisional LCA case studies. The delimitation values proposed (<0.1 %, between 0.1 % and 1 %, >1 %, respectively) are still preliminary and show a certain degree of ambiguity. Nevertheless, the criterion proves to be both practical and potentially relevant. The case study of the European electricity mixes shows that a distinction of different decision contexts is required and feasible. It is recommended to apply the "relative economic size"criterion in the goal and scope phase of any LCA.

LC04-3
Operational LCA guidance for fuel cells: methodological challenges
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The main critics addressed to LCA are the lack of robustness, which affects the comparability among different studies on the same product, and the complexity of the method, which hampers its applicability in the industrial context. The subjectivity linked to some methodological choices (e.g. allocation, system boundary definition, modelling, etc.), together with a general inhomogeneity of LCI databases are the main critical aspects. They require a deep knowledge of the technical system analysed as well as of the LCA method, and cannot be referred to the company itself. There is large agreement, not only in the LCA community, that companies need tailor-made provisions, rules and data, which support a life cycle analysis in the sector of interest. In order to meet this urgent need, the JRC-IES has recently published the International Reference Life Cycle Data System (ILCD) Handbook that offers a step-by-step guidance for LCA practitioner. However, the Handbook is applicable to a wide range of different decision-contexts and sectors and therefore needs to be translated to product-specific criteria, guidelines and simplified tools to foster LCA applications in the industry sectors.
This paper presents the first example of a sector-specific operational guidance document on the product group: on "Hydrogen and Fuel cells"(FCs), highlighting the fuel cell part. The Fuel Cells and Hydrogen Joint Undertaking (FCH JU) of the European Commission funds this work, as part of the project FC HyGuide. The approach, "translating"the general ILCD-Handbook into a tailor-made specific guidance document is introduced, showing main methodological aspects choices that have to be addressed.

LC04-4
Electricity trade analysis and marginal technologies in consequential LCA: the case of Quebec's Hydropower in the Northeastern American Market (2006-2008)
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Establishing valuable data on marginal energy production technologies is of vital importance in consequential LCA (C-LCA). In electricity production studies, coal or natural gas are often assumed to be the marginal technologies because of generally not publically available specific information due to its strategic value. Moreover, marginal technologies are not static and change as a function of time due to electricity market price and fuel market price volatilities. In the absence of full dispatch information, a procedure to estimate the marginal energy production technologies as a function of time is crucial for more accurate C-LCA results.
The objective of this study is to illustrate the usefulness of the electricity trade analysis in identifying a complex set of hourly**1** marginal technologies and providing reliable data to future C-LCA studies. To achieve this objective, this study focuses on the province of Quebec (Canada), which exports hydropower to its Northeast American neighbors (New Brunswick, New England, New York and Ontario). Over the period 2006-2008, Quebec hourly exchanges of electricity with its adjacent jurisdictions are examined as they increase or decrease their local productions. Moreover, hourly marginal electricity production technologies are identified and validated using the Ontario hourly output per power plant and information released in the Quebec adjacent system operator reports.
Results show that marginal electricity production is characterized by a complex set of technologies able to meet the demand hour-by-hour and not solely by one typical marginal technology (i.e. coal or natural gas). Also, according to our approach, the percentage of hours when hydropower, coal, natural gas or oil are designated as the marginal technology differs on a yearly and jurisdiction basis. These results are crucial to methodological discussion on the identification of the marginal electricity production technology, as it is the case for C-LCA in connection to electricity.
In conclusion, the use of electricity trade analysis as part of the consequential LCA methodology is a cornerstone for establishing, on a yearly average basis, valuable data on the marginal electricity production, as it is the case for Quebec adjacent markets.
1. Even if in some cases, such as Ontario, five-minute data would be available, the time unit used was the hour because it is the minimum length at which all data are available.

LC04-5
European energy policies analysis with general equilibrium model GTAP in a consequential and prospective life cycle assessment framework
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Consequential life cycle assessment (C-LCA) has been developed to study environmental consequences caused by changes occurring in life cycles. Most of the C-LCA have been conducted on small systems affected by marginal variations. Basic economic models and elasticity of offer and demand have been used to assess expected consequences of life cycle marginal changes on economic markets. In this study, C-LCA approach has been applied to a large system of life cycles facing significant variations over 2 decades: economic consequences on world markets of a bioenergy policy applied inside the European Union (EU) between 2005 and 2025 have been modeled with general equilibrium economic model GTAP. Environmental impacts of EU bioenergy policy have been compared to a situation where an EU business as usual energy policy was applied (base situation). Potential environmental impacts attributed to the variation of production caused by EU bioenergy policy on each economic sector in all regions of the word (indirect impacts) and potential environmental impacts attributed to EU energy production (direct impacts) have been calculated. Direct and indirect environmental impacts have been expressed for each 5 years period, per region and per economic sector. Results show that, at world scale, over the whole period, EU bioenergy policy and its indirect consequences would cause fewer impacts on human health, resources and climate change than the base situation but damages on ecosystems would be more important. As expected, coal and wood sectors are significantly affected by EU bioenergy policy. Indeed, EU bioenergy is mostly produced from wood biomass and used to substitute coal in electricity generation. During 2005-2010, benefits of the EU bioenergy policy for human health, resources and climate change are balanced by rebound effects, especially in coal extraction sector. Sensitivity analysis shows that macroeconomic parameters (gross domestic product, population, capital, labor force) and technology innovation used as inputs in the GTAP simulations are quite sensitive parameters that affect the results of the study. This highlight the necessity to develop methods to manage uncertainty related to the use of prospective data and economic model.

LC04-PS
Poster spotlight: Further on LCI methodology
Miscellaneous
Poster spotlight highlighting abstracts WE 226, WE 227, WE 228, WE 233:
- Decision contexts, application contexts, and LCI modeling approaches
- Inclusion of land use changes in LCA on bioenergy: parameterization of land use change patterns
- Partial and General equilibrium modelling to integrate consequential effects of indirect land use changes (ILUC) in LCA of biogas
- A constraint-based method for performing life cycle inventory analysis using the matrix model

LC05 - Life Cycle Management approaches for different industrial sectors (LCM)

LC05A-1
Life cycle management capability framework: a new approach for sustainable value chains
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There is a broad consensus that large multinational corporations (MNCs) should assume responsibility for and manage the social and environmental impacts of their products and services, including the activities of their suppliers. There is also a broad consensus that the best way to hold corporations accountable is to require public reporting of quantitative measures of sustainability performance. Unfortunately, there is less agreement on what exactly constitutes sustainable business performance. Diverse stakeholder interests have resulted in a laundry list of performance measures, recommended practices, and desired outcomes. Capability maturity models have a long history in process quality improvement initiatives. The models provide a structured sequence of activities for improvement. The life cycle management (LCM) capability framework uses a focus on decision- making to provide a simple and practical basis for assessing organizational maturity. The goal is to make decisions with a full awareness of the future and remote consequences of a choice on stakeholders and the natural environment. There were several key lessons learned from Phase 1 which will be discussed in the presentation.

LC05A-2
Rethinking water policy in water-scarce countries: lessons learned from a life-cycle water

footprint perspective

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As water resources are unevenly distributed and, in some regions scarcity and droughts are increasing both in frequency and intensity, concerns about them are also becoming more and more important on the political agenda. In this context, the United Nations Environment Programme (UNEP) as part of the UNEP's umbrella project entitled 'Water Footprint, Neutrality and Efficiency' (WaFNE) is addressing the growing need to further enhance water efficiency and to improve water quality more holistically, by applying harmonized concepts in water-intensive industries and water-stressed areas especially in the developing world. The final objective is to improve water governance through the engagement of the public and private sectors (business and industry, including financial services) in collaborative work with UNEP. In the same line, developments and discussions on the water footprint indicator are being held under different forums, such as the UNEP/SETAC framework for life cycle impact assessment, the Water Footprint Network and the ISO 14046 Water Footprint Initiative Subcommittee.

Traditionally, governments responsible for water resources management have targeted their policies towards direct water users (such as farmers, industries and households). Recently, however, it has been shown that this approach is limited. Indirect water users and managers, such as final consumers, retailers, traders and all sorts of businesses active along the supply chains of final consumer goods remain out of the scope of governmental policies aimed at mitigating water scarcity and pollution. All water use in the world, however, is ultimately linked to final consumption by consumers. It is therefore interesting to analyze these new multi-sectorial policy aspects and multi-actor approach that have the potential contribute to a better management of water resources.

LC05A-3

Life Cycle Assessment as a decision support tool in the waste management sector: a critical review

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The purpose of the paper is to present the most recent studies, published in the leading scientific journals, concerning with the application of the Life Cycle Assessment methodology in the solid waste management sector.

First of all, there is an introduction of the current trends of the life cycle analysis in this field, coming from a detailed survey on the LCA studies published in the last five years in the main scientific journals dealing with LCA methodologies and waste management.

The methodology is to identify and describe, for each article, the aim and purpose, the chosen approach, the data and models used, the results obtained and finally the conclusions. For a greater clarity the literature review is made by dividing the LCA studies into different areas: studies of the solid waste management in a broader sense, studies on thermal treatment of waste, studies of specific fractions of waste and studies related to specific processes.

After a presentation of the main common characteristics of every category of articles, a summary is given in order to highlight some common suggestions in the conduction of a life cycle assessment study, coming from this analysis, according to the different aspects: typology of the study (namely if it is a single process-oriented analysis rather than at strategic level), methodology (avoided impacts approach, hypothesis and assumption, data) and results (including the main remarks coming from the life cycle assessment).

As a result the literature review shows that conducting a LCA study in the waste management sector is a still time demanding and complex task, because of the complexity of the systems and the different aspect that have to be taken into account. To conclude some remarks are given on how to perform an LCA study in this sector, with regard to the system boundaries (need to include as much operations as possible, namely, collection, transport, typology of garbage bins), data (prefer site-specific data, including a waste analysis in different times during a year in order to define a medium composition), functional unit (which should be accurately defined in accordance with the scope of the study, considering the possible future variations), hypothesis (which should be accurate with special regard to the National energetic mix, the quality of the waste) and finally methodology (optional step in LCIA phase, sensitivity analysis, including economical considerations).

LC05A-5

LCA software in the analysis of municipal waste treatment technologies

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Due to the multidisciplinary character of the LCA it has already been successfully applied in different areas of our life. The significant number of publications shows that LCA is a well-known and accepted method used also for the purposes of municipal solid waste (MSW) management. Within the last years there has been a number of LCA software e.g. EASEWASTE, ORWARE developed to calculate potential emissions within the waste management systems or chosen waste management technologies and to define the environmental impacts of the analysed systems or objects.

The study did not intend to compare MSW management scenarios but only specific landfill and incineration technologies. The analysis was performed within the defined system boundaries and based on the chosen functional unit and with a use of three LCA software: GaBi, SimaPro and EASEWASTE.

The aims of the work were to perform: 1) an analysis of chosen incineration plants and a landfill site, 2) a comparison between the analyzed objects (MSW management technologies), 3) a comparison of different software used for calculation of the environmental impacts of MSW technologies.

The results show that landfilling of MSW has a higher potential environmental impact than incineration. Additionally, the calculations that base on the same system boundaries and input data but obtained with alternative LCA software give different values, but keep the same evaluation of analysed objects.

LC05A-6

A comparison among different Automotive Shredder Residue treatment processes

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About 10 million tonnes of waste are generated in Europe from ELVs management chain every year. Currently, 80% of the ELV total weight is recycled during the end-of-life treatment phases, namely: pre-treatment, dismantling, and shredding phase. The remaining 20% is called Automotive Shredder Residue (ASR) and at present it is disposed by landfilling in most European Countries. The complexity of ASR composition, due both spatial and temporal variations, as well as the different source materials shredded (i.e. vehicles and white goods) puts several limits over material recycling processes. ASR valorisation treatments are necessary according to the limit of 13 MJ/kg stated for waste disposal by the Directive 1999/31/EC. Furthermore, the Directive 2000/53/EC claims for ELVs target by the year 2015 (85% recycling rate and 95% recovery rate). The reduction of the current percentage by recovering materials and energy is thus a major challenge for European Community.

In this study a comparison among five ASR management strategies is carried out with a characterisation and a quantification of environmental impacts related to each scenario investigated by means of a Life Cycle Assessment (LCA) approach. These scenarios (landfilling, landfilling after nonferrous metals removal, incinerating, mechanical recycling and gasification) have been compared according to the functional unit of 1 tonn ASR. According to transparency requirements for the sources of data in LCA studies, limits and assumptions of the study are declare. Furthermore, the "Data quality pedigree matrix"was adopted herein in order to declare the quality of data collected. Ecoindicator'99 method has been adopted to assess the main impacts on climate change, carcinogens, respiratory diseases from organic and inorganic substances, acidification/eutrophication effects, ecotoxicity, land use, mineral and fossil fuels depletions.

The results show that industrial processes aimed at matter recovery are the options that can obtain greater environmental benefits compared to present practises. A sensitivity analysis by Monte Carlo approach have been applied in order to evaluate on statistical basis the result. Further improvements will be achieved only by integrating end-of-life treatments into eco-design strategies aiming at a more efficient separation of high value-added materials such as plastics and metals, and leading to a reduction of waste outputs from ELVs management chain.

LC05B-1

Seven barriers to reliable life-cycle assessments for biofuels

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To address energy security and climate-change concerns, substitutes are needed for petroleum-based transportation fuels. In seeking transportation fuel alternatives to petroleum, policy makers need information to support timely and effective decision-making. In their efforts to provide this information in a reliable and timely manner, life-cycle assessors face a number of barriers. Here we identify and discuss seven barriers that must be addressed in applying LCA to effectively assess the relative sustainability of biofuels as liquid transportation fuels. These barriers include (1) understanding farmers, feedstock options, and land use; (2) mapping out biofuel production technologies and practices; (3) characterizing use-phase emissions and their health consequences; (4) addressing spatial heterogeneity; (5) accounting for time in allocating impacts; (6) assessing transition paths as well as end states; and (7) confronting uncertainty and variability. Recognizing LCA as a process and not a product is key to meeting these challenges. Barriers to LCA arise because many stakeholders expect LCA to provide a clear and final answer"'the "truth". This serves only to highlight the flaws and uncertainties of LCA and fails to take advantage of the true power of LCA as an ongoing process that organizes both information and the process of prioritizing information needs. Addressing the world's need for near-term, cost-effective, and reliable production systems for biofuels requires that the technological, social, economic, and environmental challenges be addressed in parallel.

LC05B-2

Spanish IO-LCA vs. process LCA: application to bioethanol production in Spain

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LC05B-3

Design for environment conceptual implementation plan for tanneries, dairy, meat processing and electroplating sectors in Argentina

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The Design for Environment (DfE) conceptual implementation plan was a World Bank sponsored research project applied to the Argentina Industrial Sustainable Development program. The objective of this program is to provide technical and financial assistance to small and medium enterprises of four different industrial sectors (tanneries, meat processing, dairies, and electroplating) to improve their environmental and competitive market challenges. West Texas A&M University was called upon to provide technical assistance in developing the DfE approach and model to identify sustainable alternatives to typical industrial processes. The DfE approach was proposed to develop detailed re-design recommendations for each of the four industrial sectors. In this effort, twelve DfE evaluations and recommendation reports were completed. New markets, regulation needs, and products were identified. Also, WTAMU developed a loan/grant program based on the assumption that improvements to market-competitiveness would be funded by repayable loans guaranteed by the World Bank through local banks. WTAMU selected a loan/grant approach used by the United States Department of Agriculture Natural Resources Conservation Service (USDA-NRCS) as a model for Argentina.

This project provides the approach, design and implementation of DfE to meet Argentine/World Bank objectives. The effort included development of detailed process flow diagrams, technological evaluation to meet and exceed Argentine regulations, estimates of market-competitiveness versus environmental option costs, an examination of feasibility, a comparison of market-competitiveness to environmental goals, and recommended grant-to-loan allocations. The outcome of the project identified alternatives capable of improving competitiveness and attainment of national and government environmental standards.

LC05B-4

LCA and environmental product declaration of an immunological product (vaccine) for boar taint control in male pigs

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1. Introduction

Agriculture, animal farming and related sectors are facing the environmental sustainability challenge by means of advanced analysis tools such as Life Cycle Assessment (LCA) with the goal of understanding the origin of their environmental burden and to propose solutions for impacts mitigation. In 2009, Pfizer Animal Health (USA), in cooperation with Life Cycle Engineering

(Italy), started to apply the LCA methodology to some innovative products, with a first case-study on ImprovacTM, an immunological product (vaccine) for male pigs that, by providing farmers with an alternative way to avoid the problem of boar taint, also allows them to increase the efficiency of male pig production, which may in turn provide considerable life-cycle environmental benefits.
Physical castration (currently the most common method of controlling boar taint) represents the benchmark system. It produces a negative effect on the growth performance of pigs: compared to entire male pigs (boars), castrated (barrows) are less efficient at converting food into body weight and tend to have lower carcasses quality.
The use of Improvac replaces physical castration and allows male pigs to grow as boars for most of their lives: Pigs on an Improvac program naturally eat less feed, create less waste (manure) and have carcasses with a greater percentage of lean meat than barrows. This provides both environmental and market benefits.
2. Objective
This session aims at presenting the results of the LCA study on the complete ImprovacTM system, including the upstream processes in which semi-products and patented medicine components are realised, the core process where the vaccine is manufactured and the downstream processes where the vaccine is used at animal raising level, following through to the slaughterhouse where the meat is produced.
3. Results
The benefits of the Improvac technology can be identified in a reduced consumption of feed, representing around 20% of Global Warming Potential, and a reduced production of slurry, that accounts for about 40% of the total GWP. The LCA study showed a clear environmental benefit in terms of all the main impact indicators of the Improvac system vs. the baseline scenario (physical castration): the conservatively estimated reduction in carbon footprint is near to 4% on a live weight basis (27 kg C02 equivalent for a typical pig), which corresponds to a reduction of about 6% on a one kg of lean meat basis.

LC05B-5
Evaluation of environmental impacts of Epson Italy company through LCA methodology
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The LCA evaluation of environmental impacts of Epson company was focused on two main aspects: transport (private and commercial) and services (consumption in the building and the actions of employees). The evaluation was carried out with a questionnaire for the employees and by analysis of data on consumption (electricity, gas, water) and transport (including air travel). LCA was performed with the software GaBi4, to highlight and quantify the environmental loads and energy and was expressed as amount of CO2 emitted.
A questionnaire to all employees was designed to analyze which potential environmental impacts could have their behaviours and determine actions to improve the system. The questionnaire contained 3 sections: transports for employees (full-time workers in Epson building), transports for sales representatives (company car provided) and behaviours in office (for all workers).
Some data coming from transports analysis for employees were: type of vehicles used (private/public), displacement, pollution class and type of fuel; average distance between home and work, average time in minutes to get to work from home.
The parameters tested for the behaviours in office were: heating and air conditioning system, waste and energy consumption, use of resources.
For LCA evaluation we integrated the data collected by the survey (for transport) and those on consumption provided by the company. The balance of the system was determined by impact evaluation method CML2001. We only report the results related to Global Warming Potential, expressed in Kg CO2 equivalent.
The total amount of CO2 equivalent produced annually by Epson results to be 714.26 tons, which means 5.49 Ton CO2 per employee due to the quantity of time spent in office and the journey between home and work. The value resulted to be in the media. In Italy each year are produced 9.5 tons CO2 eq / year per capita (7.6 tons CO2 net - in Lombardy 9.9 Ton CO2 Eq).
The final assessment was related to the amount of CO2 equivalent and GWP impact category, since these parameters are actually the most significant ones for communication. The questionnaire resulted to be very useful for the perception of employees on environmental policy of the company.
Epson has used these results to make important choices: reduce air travel, promotion on video conference, replacement of conventional bulbs with LEDs.

LC05B-6
Food miles in retailers decision making processes
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Several European studies have shown that food production and consumption generates 10 - 30 % of the total environmental impact of consumption. About 25 % of Swiss diet consists of fruit and vegetable intake. Most of these products are sold by retailers that can manage their assortment and supply chains with high environmental leverage. In this study it could be shown that detailed LCA analyses of food products, carried out in collaboration with retailers and implemented into the companies' decision making can substantially reduce the impact of food consumption by optimizing where to source vegetables from and how transport them to the final destination.

NM01 - Detecting, quantifying and characterizing engineered nanomaterials in the environment and in biological systems

NM01A-1
Nanoparticles in food and biological samples
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A variety of nanomaterials are being used in consumer products, in food packaging materials, in some food supplements and even some food products; e.g.silica, silver, titanium, copper, gold and zinc. As a consequence, direct and indirect consumer exposure to nanoparticles is likely. Detection and characterization of nanoparticles in products, food and biological samples of toxicology tests is an essential part of understanding the potential benefits as well as the potential risks of the application of nanoparticles. Single particle ICPMS was used as a screening tool for nanoparticles in food and biological samples. Chromatographic methods in combination with ICPMS have been developed as a confirmation method and have recently been used to show the presence of nano-sized silica in food and drinking water.
In single particle ICPMS nanoparticles in the sample are introduced into the ICPMS plasma producing a plume of analyte ions resulting in a signal spike in the mass spectrometer. From this the size of the particles, their concentration and their size distribution can be calculated. Using the NIST gold reference materials 8011, 8012 and 8013, the method was tested and its performance characteristics determined. This screening method was used in practice for the determination of gold and silver nanoparticles in food supplements and in simulated digestion experiments. In addition, the method was used to detect silver nanoparticles in the livers of rats exposed to silver nanoparticles through their food. The detection of nanoparticles in the liver indicates the actual uptake of such particles from food.
Hydrodynamic chromatography combined with ICPMS was used as a confirmation method to determine the actual size of inorganic nanoparticles. This method was used to determine silica nanoparticles in food items and drinking water. Silica is added to food items as an anti-caking agent known as E551. While this normally exists of larger than nano-sized particles the material has a nano structure with primary particle sizes in the nano-range. Using HDC-ICPMS the presence of nano-sized silica in several food items could be shown. In addition, tap water and several mineral waters were also shown to contain nano-sized silica.

NM01A-2
Characterization of engineered nanoparticles in consumer products
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Consumer products with engineered nanoparticles (ENPs) are available on the market today in various industrial sectors. The most common consumer products are cosmetics and personal care products, textiles and household products, usually including zinc oxide (ZnO) and/or titanium dioxide (TiO2) as UV-filters or silver (Ag) as an antimicrobial agent. Because consumer products with ENPs are intended to be applied during everyday life, it is expected that human as well as environmental exposure to ENPs is occuring already today and might increase in the near future. Most methods, such as X-ray diffraction or dynamic light scattering, however, work best for the characterization of ENPs with similar shape and narrow size distribution. In case of ENPs in consumer products or environmental samples, the morphology of particles is not known beforehand and ENPs may show a wide size distribution, any agglomeration status or various shapes. The presence of other chemicals or particles in products is an additional challenge.
We have therefore investigated which analytical methods can be used for the characterization of consumer products. TiO2 and ZnO ENPs in sunscreens were investigated with the conventional methods transmission and scanning electron microscopy (TEM and SEM, respectively). Further, we used wet SEM (WetSEMTM) as a new technique. This allowed us to directly analyze the ENPs in pure samples in the state they are applied by consumers, thus avoiding drying artefacts. Additionally, four spray products were analyzed. First, the ENPs in the dispersions (before spraying) were analyzed by TEM in combination with EDS. Elemental analysis was carried out by inductively coupled plasma mass spectrometry. Second, the aerosols generated by the spray vessels were analyzed for ENPs with a scanning mobility particle sizer in combination with TEM analysis.
We conclude that analytical methods that are used for the characterization of nanoparticular powders are not necessarily applicable for the characterization of ENPs in complex media such as consumer products. Generally, several analytical methods have to be combined in order to achieve a comprehensive analysis of the respective ENPs. Focus must further be set on the sample preparation in order to ensure that ENPs are analyzed in the state that is considered relevant. Without previous information on the ENP characteristics, analytical methods have to be chosen carefully to ensure a representative sampling and analysis.

NM01A-3
Determinations of manufactured and incidental nanomaterials in highway runoff waters
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In the environment there are three main sources of nanomaterials; natural, incidental and manufactured. The relative proportion of these is unknown but believed to vary in space and time. Little is known about the environmental impacts of these classes, and new methods that can selectively determine them are needed. We have focused on highway runoff waters where especially incidental and manufactured nanoparticles should be significant.
There is a challenge to quantitatively analyze and size nanoparticle in such complex samples and even more difficult to selectively discriminate between different classes or types. We have addressed these problems by developing several approaches and methodologies: i) inductively coupled plasma masspectrometry (ICP-MS) capable of detect and semiquantitatively size single nanoparticles, ii) field flow fractionation to size sort nanomaterials only based on hydrodynamic forces, and iii) optimize an Environmental SEM methodology.
Although most of the mass of metals are transported on larger particles >5 µm, there is a significant fraction also carried on incidental nanoparticles for some metals. The concentration and size distribution trends can be related to rain pattern and traffic intensity.
Some manufactured nanomaterials have been identified in the stormwater runoff and on tunnel wall dust, and the significance of these will be discussed.

NM01A-4
The application of field-flow fractionation for the characterization of nanoparticles in complex samples
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The thorough analysis of nanoparticles comprises a sequence of detection, identification, quantification and if possible a detailed characterization. In a complex sample as soil, sediment or sewage sludge, each step of this sequence is currently still a challenge, and, given a suitable sample preparation, Flow- and Sedimentation-Field Flow Fractionation (Flow-FFF, Sed-FFF) are two of the most promising techniques currently developed for these tasks. The objective is to present sample preparation strategies for natural, silver and gold nanoparticles, principles of method development for proper FFF separation of nanoparticles from complex samples and results obtained

from mixtures of ENPs (Ag and Au) and natural particles. We are demonstrating the potentials and limitations of FFF to distinguish engineered from natural particles.
Metal nanoparticles as citrate stabilized gold nanoparticles do not always intensively interact with typical natural nanoparticles which are in stable dispersion. Conventional ICPMS coupled to FFF seems to be suitable for complex samples containing certain ENPs which are composed predominantly out of elements having a low background in the environment. It is possible to create analytical procedures suitable for the parallel FFF fractionation of natural and gold nanoparticles.

NM01A-5

The silver nano washing machine. Nanoparticle release from a consumer product.

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Due to their special physical and chemical properties nanoparticles are increasingly manipulated in industry, research and medical applications. Furthermore, the number of nano-enabled consumer products is rising rapidly, and, since some consumer products were previously seen to release nanoparticles, they are a possible source for nanoparticles to the environment. Routine methods for nanoparticle detection or even quantification in environmental samples are still missing, and environmental concentrations have to be estimated from nanoparticle production and use rates. Studies investigating nanoparticle release can therefore add valuable information for future risk assessments. This study characterizes the effluent from a commercially available silver nano washing machine, investigating the released silver concentrations, number and characteristics of the released nanoparticles and the effect of the effluent on a natural bacterial community.
Average released silver concentration were 11 µg L-1, as determined by inductive coupled mass spectrometry (ICP-MS). Ion selective electrode (ISE) measurements and filtration studies suggested the ionic fraction to be marginal. The presence of silver nanoparticles (AgNPs) was confirmed by single particle ICP-MS and an average number of 80 Mio AgNPs mL -1 was measured. The AgNPs size was determined to be 10 nm and <20 nm with transmission electron microscopy (TEM) and single particle ICP-MS, respectively. Nanoparticle tracking analysis (NTA) measured the particles size to be 60 - 100 nm. The washing machine effluent was shown to have a detrimental effect on a natural bacterial community as it clearly reduced the bacterial abundance. If AgNPs producing washing machines will become a common feature of households, the wastewater will contain significant loadings of AgNPs, which might be released into the aquatic environment.

NM01A-6

Characterization of CeO2 and ZnO nanoparticles in the framework of an eco-toxicological study

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Due to their unique physical properties, engineered nanoscale materials are increasingly being used to impart enhanced functionality and improved performance to a wide range of products. As a consequence, human and environmental exposure to these materials is also increasing. For this reason, there is an urgent need to establish robust methodologies for characterizing nanomaterials' properties and assessing their interaction with living organisms.
In this work, we characterize the physico-chemical properties of seven ZnO and CeO2 industrially manufactured nanoparticle (NP) samples in the framework of an eco-toxicological study. We developed methodologies for characterizing NP properties including size, size distribution, aggregation, surface charge and surface chemistry. Substantial effort was dedicated to the development of experimental protocols for the study of NP-catalyzed photo-production of harmful reactive oxygen species (ROS). The NPs were characterized in powder form, as well as after dispersion in DI water, seawater and medium employed in in vitro experiments. The effects of exposure to ZnO and CeO2 NPs on the freshwater Daphnia organisms were also assessed.
Particle size as reported from SEM analysis generally was larger than the relative crystallite size measured by XRD, indicating NP polycrystallinity. NPs with larger sizes were associated with smaller surface areas. When dispersed in media, all NP dispersions were unstable, with tendency to aggregate and sediment. All the NP samples catalyzed the photo-production of ROS, with the largest production observed for the particles smaller in size. The type of media surrounding the NPs was found to affect ROS production. One type of ZnO NPs exhibited a rate of superoxide radical photo-production ten times higher than anatase NPs, which were used as reference materials for photo-catalytic studies. Both CeO2 and ZnO NPs were located in Daphnia organisms after exposure. However, while acute exposure (48h) of Daphnia to CeO2 NPs had no effect on mortality at concentrations up to 100 mg/L, exposure to ZnO NPs caused a concentration-dependent increase on mortality with LC50 of 1.55mg/L.
This study aimed at providing a detailed characterization of the phisico-chemical properties of NPs, to set the basis for understanding NP fate in the environment, their modalities of interaction with living organisms and their toxicological effects.

NM01B-1

ZnO nanoparticle dissolution: measurement with AGNES and modeling

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The growing concern on the ecotoxicological issues related to engineered nanomaterials requires the adaptation and developing of analytical and physicochemical techniques in order to gain knowledge on the characteristics of the nanoparticles (NPs) and their behaviour in aqueous dispersions. A distinctive feature of the ZnO nanoparticles is that they can dissolve in aqueous solution in a relatively large proportion [1,2]. Moreover, there are conflicting reports on whether the toxicity of ZnO nanoparticles can be traced back to their nanoscopic properties or rather to the simple fact of their relatively high solubility in natural waters producing a large concentration of Zn(II).
The emerging electroanalytical technique AGNES (Absence of Gradients and Nersntian Equilibrium Stripping) was specifically designed for free metal concentration determination [3]. Moreover, the favourable timescale of this technique allows the measurement in situ of the changing Zn(II) resulting from the dissolution of ZnO nanoparticles. The experimental time evolution of the free Zn concentration when ZnO nanoparticles are dispersed in a medium or when the pH of the medium is modified, allows the study of the kinetic process up to the achievement of the thermodynamical equilibrium or a metastable state. A physicochemical model, which combines surface reaction and diffusion of Zn(II) from the NPs, has been developed to account for the observed kinetic behaviour. Conclusions on the time scale and reversibility of the solubilization process, the key role of the aggregation phenomena and the determination of parameters characterizing the dissolution at different pH values will be shown and discussed in the presentation. The impact of primary size, pH and nanoparticle concentration on the dissolution rate will be discussed, as well as possible environmental consequences.

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NM01B-2

Influence of serum protein concentrations and particle characteristics on gold nanoparticle uptake in mammalian cells

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There are currently over 1000 consumer products on the market that contain or utilize nanomaterials. This number is expected to increase exponentially in the near future in the absence of detailed knowledge concerning the interactions of such materials with biological systems. Furthermore, while nanoparticles have been shown to cross cellular membranes, little research has examined the influence of nanoparticle characteristics on membrane transport. The goal of this project was to characterize the influence of particle size, shape, and surface chemistry on the movement of gold nanoparticles across mammalian cell membranes, as well as the influence of serum protein concentrations on this uptake. Preliminary experimentation has examined the uptake of 4 nm, 18 nm, and 50 nm citrate capped gold nano-spheres in A549 carcinomic human alveolar cells at increasing particle concentrations with a constant concentration of serum proteins, as well as at increasing protein concentrations with constant particle concentrations. Cells were plated in 12-well plates at 100,000 cells per well and exposed in fresh media 24 hours after plating. Accumulation was monitored following 2, 6, 12, and 24 hours. Bioaccumulation of nanoparticles at each time point was quantified by inductively coupled plasma-mass spectrometry (ICP-MS), and movement was visualized using dark field microscopy. Results at a constant protein concentration followed typical dose-response relationships as particle uptake increased with an increase in particle exposure concentration. However, when exposed at the same particle concentration, uptake decreased as serum protein concentrations increased, revealing a negative relationship between these factors (R2=0.92). It is believed that this decrease in uptake is a function of the over-saturation of particles with serum proteins in the media, thus causing competition at the receptor site for receptor mediated endocytosis.

NM01B-3

Characterising the behaviour of engineered nanoparticles in sediment-water systems

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Novel applications of nanotechnology may lead to the release of engineered nanoparticles (ENPs) into the environment resulting in concerns over their potential environmental impact. To determine their potential ecotoxicity, environmental fate and behaviour, it is essential to be able to detect, quantify and characterise ENPs in the environment. However, the characterisation of ENPs in complex matrices is extremely challenging and the analytical approaches need to be able not only to determine the size and size dirstibution of nanoparticles, but also to differenciate between naturally existing nanoparticles in the environment and the target ENPs. The aim of this study was to invesigate the fate of ENPs in sediment-water systems focusing on the identification and application of suitable analytical techniques for ENP characterisation which overcome the difficulties mentioned above. Partitioning studies of 1 µg ml-1 nano Ag and 1.5 µg ml-1 Au in either artificial or natural sediment were conducted. The 24 h equilibrium partitioning percentage as well as the size distribution of partitioned ENPs were measured using nanoparticle tracking analysis, inductively coupled plasma mass spectrometry (ICP-MS) and transmission electron microscopy (TEM).
This newly developped nanosight method is capable of subtracting the naturally occuring particles and hence estabilish the number concentration of the study ENPs over time as well as the size distribution. It was achieved via the pretreatment of the sediment by vacuum filtration before ENP spiking, centrifugation after equilibration and even dilution before video analysis. Natural sediment-water system was also run in order to correct the interferences from re-released natural NPs into the ENPs spiked system. These results confirm that the partition behaviour of each ENP to the different sediment depend on the nature of functional groups, e.g. citrate coated Au which containing "Na+"accounted higher partition percentage (56%) than the mercaptoundecanoic acid coated Au (35%) to the artificial sediment, Toyopearl SP-650M resin, which is a strong cation exchanger. The study also proved that NTA was capable to provide quantitative information of ENPs regarding the size distribution and number concentrations in complex matrices after appreciate sample preparation. ICP-MS analysis and TEM anlysis of sediment samples is underway.

NM01B-4

Stability of nanoparticles in aqueous dispersions: effects of nanoparticle morphology and surface chemistry

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Analyzing the physico-chemical properties of engineered nanomaterials (ENMs) as they are dosed to organisms, released into the environment, or taken up by organisms remains one of the important challenges in conducting meaningful studies of their environmental behavior and toxicology. Released ENMs can enter drinking water, food materials, or organisms. Understanding the stability of ENMs in aqueous dispersions is essential to obtaining reliable environmental impact and toxicological results. This work relates the stability of metal oxide nanoparticles in aqueous dispersions to their morphologies and surface chemistries. Liquid phase compositions include some buffer systems as well as dilute protein solutions. A set of complementary analytical tools (transmission electron microscopy (TEM) with electron energy loss spectroscopy (EELS) and energy-dispersive x-ray (EDS; scanning electron microscopy (SEM); dynamic light scattering (DLS); z- potential; Fourier transform infrared spectroscopy (FTIR); thermogravimetric analysis (TGA); x-ray photoelectron spectroscopy (XPS); and X-ray diffraction (XRD)) were used to evaluate the complex morphologies and surface chemistries that can occur. These ENMs are 'monodisperse' ceria samples with primary particle sizes similar to those now being used to reduce soot and improve efficiency during the combustion of diesel fuels. The ceria samples in this study had primary particles known to be unaggregated. These materials have high purity (XRD

and EDS), no internal porosity (BET), and are highly crystalline (TEM), leading to controlled surface chemistries (confirmed by EELS under HRTEM, FTIR, TGA, and XPS). The surfaces of these ENMs can sorb metabolic salts, e.g. the citrate ion, and proteins (such as globular proteins) in aqueous systems. In some cases, buffer salts can cause agglomeration or precipitation of the nanoparticles. Dispersion stability has been evaluated using DLS and z-potential. Surface sorption of metabolites and proteins can occur in 'layers' (confirmed using FTIR and TGA techniques), which leads to changes in the z-potential curves for the nanoparticles. These findings have relevance to understanding the behavior of ENMs in the environment and in organisms (including human health).

NM01B-5

Transformations of silver nanoparticles at environmentally relevant concentrations in fresh and saline surface waters

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Predicting the fate of silver engineered nanomaterials (ENMs) in surface waters requires an understanding of how the size distributions of different silver ENMs are affected by water chemistry. Conventional techniques for measuring nanoparticle size distribution, such as electron microscopy or light-scattering methods, are not applicable for measuring size distributions at environmentally relevant silver nanoparticle concentrations in media containing background nanoparticles of other material. Field flow fractionation (FFF) coupled with inductively coupled plasma mass spectrometry (ICPMS) is one approach for such measurements, but FFF-ICPMS is limited to silver concentrations above about 10 µg/L, and it cannot distinguish between silver-containing particles of even greatly differing elemental composition. We apply an alternative approach, called single particle ICPMS (SP-ICPMS), to study the effect of water chemistry and silver nanoparticle concentration on transformations of silver ENMs in synthetic and real surface waters. Silver nanoparticles in NaCl suspensions with similar salinity to open seawater undergo aggregation, in agreement with established theory and previous studies at higher concentration. They also undergo size reduction consistent with dissolution. The dominant process (aggregation or dissolution) depends on particle size and concentration. Size transformations in suspensions of silver nanoparticles in real surface waters are also studied. The efficacy of SP-ICPMS in measuring nanoparticle transformations in these complex media is evaluated as a function of plasma and data acquisition parameters, and optimal conditions are described. Studies of a range of nanoparticle sizes and environmentally relevant concentrations are presented. The effect of natural organic matter on transformation kinetics and equilibria is presented.

NM01B-6

Absorption of functionalized single-walled nanotubes across Daphnia magna gut tract

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Engineered carbon nanostructures, such as single-walled carbon nanotubes (SWNTs), are inherently hydrophobic and are not stable in aqueous systems. However SWNTs can be surface modified with functional groups that will affect aqueous stability. Furthermore, the aqueous stability and bioavailability of these nanotubes may be influenced by the water quality parameters such as ionic strength, pH, and natural organic matter (NOM). NOM may interact with the functionalized surface of SWNTS resulting in increased aqueous stability. This enhanced stability is likely to lead to an increased residence time in the water column and increased exposure times for pelagic organisms. While our previous work demonstrated that multiwalled carbon nanotubes (MWNT) stayed within the gut tract of D. magna, some researchers have suggested that the small diameter of SWNTS may allow them to cross cell membranes. In the present study NOM from Suwannee River (SR-NOM) increased the stability of OH, SiO2, polyethylene glycol (PEG), and polyaminobenzene sulfonic acid (PABS) functionalized SWNT suspensions. Standard 96 hr static renewal D. magna toxicity tests were conducted to evaluate the acute toxicity of these functionalized SWNTs. Particles were characterized using zeta potential, dynamic light scattering, and transmission electron microscopy (TEM). Confocal microscopy, micro Raman spectroscopy, and TEM coupled with electron emission loss spectroscopy (EELS) were also used to inspect the gut tracts of D. magna to determine the fate of ingested functionalized SWNTs. Functionalized SWNT exposure to D. magna caused little acute toxicity compared to published data on MWNT. Confocal microscopy and micro Raman techniques provided evidence that functionalized SWNTs greatly impact the gut tract once ingested, but were unable to work at the scale needed to determine material absorption. TEM coupled with EELS provided the best evidence for material absorption across the gut tract. The data suggest that SWNTs surface modified with more basic functional groups, OH and SiO2, may have more of an ability to absorb across the gut tract than those surface modified with larger PEG and PABS functional groups.

NM02 - Fate and effects of nanoparticles

NM02A-1

Assessing ionic silver availability to algae from differently coated silver nanoparticles

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Because its biocide properties, silver nanoparticles (AgNP) are present in numerous consumer products. Toxicity of AgNP to organisms is related with both the formation of ionic silver (Ag^+) and interactions between AgNP and Ag^+ with cell membranes. At present methodological limitations result in underestimation of readily bioavailable ionic silver, especially if ionic silver is formed at nanoparticles-cell interface and is immediately taken up by cells.

In this work we propose a method to examine the role of Ag^+ in explaining toxicity of AgNPs to algae, by using cysteine to assess Ag^+ bioavailability. We assessed the toxicity of AgNPs coated with 5 different chemicals (carbonate -CO3-, polyetheleneglycol -PEG-, lactate -LAC-, chitosan -CHI- and polyvinyl pyrrolidone -PVP-) on the photosynthesis of Chlamydomonas reinhardtii. Based on total Ag concentration, AgNP EC50 ranged from 0,78 µM (PVP) to 2,98 µM (CO3). In a subsequent experiment cysteine completely abolished AgNP toxicity, indicating that toxicity was mediated by Ag^+ and at the same time that different coatings were not toxic to photosynthesis. Hence, it was expected that EC50 values calculated as a function of the Ag^+ present in the experimental suspensions (and assessed using chemical analysis) would converge to similar values. But, these values showed again a wide range of variation, from 0.03 µM Ag+ (CO3) to 0.2 µM Ag+ (PEG).

Then, we considered the possibility that Ag^+ concentration used to calculate EC50 was underestimated. We calculated the cysteine concentration required to completely abolish toxicity of AgNPs EC50, as an estimate of bioavailable Ag^+ (cysteine binds Ag+ in 1:1 stoichiometry). Results shown that all EC50 AgNP required similar values of cysteine (from 0,405 to 0,500 µM) to completely abolish toxicity to photosynthesis. Hence, Ag+ bioavailable to algae was in all cases around 0,4-0,5 µM. Results indicate a) the formation of Ag^+ at the nanoparticles-cell interface, b) cysteine allows estimating Ag^+ concentration bioavailable in experiments with algae and c) cysteine also allowed concluding on the potential toxicity of coatings.

NM02A-2

Toxicity of silver nanoparticle to freshwater algae and cyanobacteria

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Nanoparticles (NPs) display special chemical properties because of their size, shape, composition and electronic structure. These properties lend NPs their functionality, but may also led to toxic effects. Due to their widespread use in consumer products an exposure of the aquatic environment to NPs is anticipated and already proven in first analytical surveys. Especially metal/metaloxide NPs are widely used, of which silver nanoparticles (AgNPs) have gained considerable attention due to their broad microbiocidal properties. This implies a specific hazard for exposed aquatic primary producers (algae). In order to identify the most suitable test species for the hazard assessment of AgNPs, we determined the toxicity of two AgNPs (10 nm nominal size, uncoated and 40nm size, citrate coated) to the green algae Pseudokirchneriella subcapitata and Chlamydomonas reinhardtii, the diatom Cyclotella meninghiana and the cyanobacterium Synechoccocus leopoldensis. To test if the toxicity was particle related or caused by free Ag+ ions only, we tested AgNO3 in the same total silver concentrations. All studies were accompanied by chemical analytics: TEM, ICP-MS (total silver content and Ag+ ions), ultrafiltration/centrifuge-aided membrane filtration (Ag+ ions) and Nanoparticle Tracking analysis (NTA) to determin the particle size distribution (NanoSight system). A difference of approximately one order of magnitude was found for the EC50 values of the two AgNPs for Chlamyodomnas. For all other species the EC50 values for the two AgNPs were similar. The sensitivity of the tested species was clearly different, the EC50 values differed by a factor of 8 for AgNP40 and 14 for AgNP10. The lowest EC50 was always observed for the cyanobacterium Synechococcus. However, different shapes of the concentration-response curves led to a more complex picture for the low-dose range (NOECs). Here the green algae were more sensitive. AgNO3 proved to be most toxic for all species, approximately one order of magnitude more toxic than AgNPs. The ecotoxicological results will be presented and discussed in relation to the analytical data.

Further studies are necessary to identify the causes for the differences in species sensitivity and curve shapes. Hence, within the next couple of months we will investigate the modes of action of the AgNPs and AgNO3, in particular the formation of ROS (reactive oxigen species) and effects on membrane integrity by applying various fluorescence methods.

NM02A-3

Fabrication and ecotoxicological assessment on microalgae of coating magnetite nanoparticles with humics

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The research was aimed to fabricate of magnetoactive composite based on coating Fe3O4 nanoparticles with humic substance using mechanochemical technique and to estimate the toxicological potential of nanocomposite fabricated. The nanocomposite obtained were characterized by XRD, Mössbauer, SEM and TEM studies. It has been demonstrated Fe3O4 nanoparticles stabilized by humic substances (Fe3O4/HS) have mean size of the particles - 7-16 nm.

The response of test-organisms, green alga Scenedesmus quadricauda to nanocomposite Fe3O4/HA was investigated in laboratory experiments. The testing of Fe3O4/HA toxicity by measuring fluorescence parameters of microalgae revealed a marked suppression of photosynthetic activity of the algal culture at concentration of aqeous suspension of the composite 0.1%. In samples of algae, grown in nutrient depleted medium (culture water), the lethal concentration was equal to 0.01%, which is by an order of magnitude lower, than that in alga grown in the Uspensky medium.

Analysis of fluorescence characteristics in algae showed a decrease in the quantum yield of photochemical conversion of light energy in photosynthesis and in the relative rate of the noncyclic electron transport in algae under the effect of Fe3O4/HA. The shape of fluorescence induction curve changed in the presence of Fe3O4/HA, which could be seen in the changed ratio of different induction peaks. Fe3O4/HA was found to exert acute toxic effect on Scenedesmus at concentration 0.01%.

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NM02A-4

Mixed toxicity in an aqueous algae-nanoparticle-pollutant laboratory system

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The forecasted augmentation in the manufacture and use of engineered nanomaterials (ENM) makes it likely that increasing human and environmental exposure will occur. Carbon nanotubes (CNT) are one major building block of nanotechnologies and their environmental and health effects are currently under heavy investigation. CNT may have direct ecotoxicological effects on organisms. However, they are also strong adsorbents for many organic compounds and may thus influence their bioavailability. In the natural environment, ENM will always occur together with a great number of organic micropollutants. The fate and the toxic effects of this kind of mixtures

on organisms are today not characterized.
The aim of our study was therefore to systematically quantify the interactions in an aqueous ternary biota-nanoparticle-pollutant laboratory system. Experiments were performed with the green alga *Chlorella vulgaris* as model organism, different multi-walled CNT as nanoparticles and the herbicide diuron as a model pollutant. Effects on *C. vulgaris* were determined as in vivo photosynthetic activity. In parallel, the aqueous diuron concentration was measured with a Varian LC-MSMS system. The binding affinity of pollutants to the CNT, the impact of CNT on the bioavailability of pollutants, and the ecotoxicological effects of CNT on algal photosynthesis were determined.
The results show that the presence of CNT drastically reduced the dissolved diuron exposure of algae. Therefore, the primary effect was that CNT reduced diuron toxicity. For instance, in the presence of 10 mg CNT/L, a nominal concentration of 150 µg/L of diuron caused a 50% inhibition of the photosynthetic activity only. For comparison, the EC50 of diuron without CNT was around 35-90 µg/L and comparable to literature data. However, the toxicity of diuron in the presence of CNT was significantly higher than predicted from the dissolved diuron concentration alone, especially after longer exposure of the algae to the mixture. The comparison of the photosynthetic activity measurements and the LC-MSMS measurements of dissolved diuron shows that the presence of 10 mg CNT/L increased the diuron toxicity by a factor of roughly 3.5 compared to the same diuron concentration without CNT, especially after longer exposure of algae to the mixture. This enhanced toxicity might be explained by a enhanced availability of diuron due to high levels of CNT near the algal surface.

NM02A-5
Sublethal effects of a set of nanoparticles of industrial interest on different experimental systems from protozoa to human cells
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In this research the environmental impact of nanostructured composites have been investigated. We have carried out in vitro toxicological tests in order to evaluate possible noxious effects of nanomaterials selected to develop new nanocomposite products.
We have used HaCaT cells, a line of immortalized keratinocytes representing an in vitro model of human epidermis. Hence, our in vitro model allows to assess potential toxic effects of dermal exposure to nanomaterials. The skin is a preferential route by which nanomaterials come in contact with the human body.
In addition, to evaluate the possible effects at the environmental level, the model organism *D. discoideum* has been utilized. This social ameba has been used to study nanoparticle effects on mortality and reproduction rate, and to analyze sublethal endpoints such as endocytosis rate, lysosomal membrane stability (LMS), and genotoxicity.
In order to highlight the cytotoxic effects of nanomaterials on HaCaT keratinocytes, cells have been incubated for 24h with each material at increasing doses of 0.5, 10 and 100 µg/mL. The comet assay has been performed by using 10 and 100 µg/mL only, due to negligible effects observed with the lowest dose in preliminary tests.
The results have shown that sepiolite is the most toxic compound, since it has induced both apoptosis and necrosis starting from the lowest concentration of 0.5 µg/mL, and moreover is genotoxic starting from 10 µg/mL. Next follows cloisite, which induces apoptosis and necrosis at all concentrations, but is genotoxic only at the highest dose of 100 µg/mL. Two other compounds, OC and POG, have shown a lower toxicity, consisting in a dose-dependent apoptosis and a very limited or null necrosis. However, POG has also induced genotoxic damage close to that observed with sepiolite. BNB90 and DAPS have produced even lower toxic effects, consisting in the nearly complete absence of necrosis and limited apoptosis and DNA damage. Finally, bohemite has shown very limited toxicity, consisting of a slight but significant genotoxic damage at 100 µg/mL, while carbon nanotubes have induced no toxicity at all.
By using *D. discoideum* as a model organism, a similar toxicity ranking among the compounds has been obtained. However, data indicate that the selected endpoints are able to detect negative effects on replication rate and/or LMS at nanoparticle concentrations lower than 1 µg/ml, while DNA damage was evident only at higher concentrations.

NM02A-6
The aquatic toxicity and species sensitivity distributions of metal oxide nanoparticles
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The production of metal oxide nanoparticles has recently increased and in the future, nanoparticle-based products will become more and more commercially relevant. This increasing production will inevitably lead to a runoff to the aquatic environment. However, the knowledge of the potential risk and corresponding risk assessment procedures of metal oxide nanoparticles considering aquatic organisms are still limited. Therefore, our research focuses on the toxicity of these types of nanoparticles and more specifically on ZnO and SiO_2. Crustaceans (*Artemia franciscana* and *Daphnia magna*), algae (*Pseudokirchneriella subcapitata*) and fish (*Danio rerio*) were exposed in acute toxicity tests to the nanopowder, the nanodispersion as well as the bulk material of SiO_2 and ZnO. After 48 to 96 h (depending on the type of species), endpoints were determined (mortality for *A. franciscana* and *D. rerio*, immobilisation for *D. magna* and growth inhibition for *P. subcapitata*). Additionally, a setoff toward chronic toxicity testing has been done for ZnO nanoparticles and bulk material on *Daphnia magna*, with reproduction as a toxic endpoint. Our acute results showed that the exposure to the three different SiO_2 forms had no significant impact on any of the observed endpoints for *A. franciscana*, *D. magna* and *D. rerio*. However, the growth of *P. subcapitata* was significantly inhibited when exposed to the SiO_2 nanodispersion (EC_{50}: 23.1 mg/L), nanopowder (EC_{50}: 48.6 mg/L) and bulk material (EC_{50}: 28.0 mg/L). Moreover, ZnO nanoparticles and bulk materials appeared to be toxic to *D. magna* (EC_{50} dispersion: 2.40 mg/L, EC_{50} nanopowder: 3.10 mg/L, EC_{50} bulk: 4.22 mg/L) and *P. subcapitata* (EC_{50} nanodispersion: 0.03 mg/L, EC_{50} nanopowder: 0.05 mg/L, EC_{50} bulk: 0.04 mg/L). *A. franciscana* and *D. rerio* were not affected in the acute tests by any of the tested ZnO forms. The results indicate clear differences in species' sensitivities to the different nanoparticles. Therefore, an initiation towards a nanoparticle risk assessment was made by means of the construction of acute species sensitivity distributions. In the future, additional chronic toxicity tests will be performed to gain more insight in nanoparticle toxicity.

NM02B-1
Effects of natural water chemistry on nanosilver behavior and toxicity
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The success of nanotechnology will undoubtedly lead to the increased introduction of NPs to natural systems; and aquatic systems which integrate contaminant inputs from atmospheric deposition and terrestrial surface runoffs will likely act as sinks for these new pollutants. Differences in the chemical make up of these aquatic systems will control changes in surface properties of NPs, and could therefore impact their environmental behaviors, fate and interactions with both biotic and abiotic particles. A series of batch experiments were conducted to determine the effect of natural waters with different solution chemistries on nanosilver (nAg) particle dispersion, stability, and toxicity. A combination of physical, biogeochemical, and toxicological methods were used to characterize, track changes, and assess the biological impacts of nAg suspended in natural waters, both raw and manipulated. Briefly, the results show that: (1) nAg suspended in natural waters with high DOC/Ionic strength ratios have average particle sizes < 100 nm and behave as stable suspensions, while natural waters with high ionic strength/DOC ratios have average particle sizes >200 nm, resulting in less stable suspensions (2) the exposure to nAg using Ceriodaphnia dubia and Pseudokirchneriella subcapitata shows highest toxicity in culture media and natural waters with high ionic strength/DOC ratios. Overall our findings do not only help establish needed correlations between nAg particle properties and the potential for environmental exposure, but also the much needed information in support of the development of an overall risk assessment plan for nAg.

NM02B-2
The impact of silver nanowire length and surface coating on physicochemical properties in environmental media and on nanowire toxicity to Daphnia magna and Fathead minnow cells
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Nanowires are distinct class of high-aspect-ratio inorganic nanomaterials. AgNW represent a class of metal nanowires likely to have widespread applications in electronic devices. While there is extensive information on the environmental toxicity of many nanoparticles, there is very limited information on the potential toxicity of nanowires to aquatic organisms.
We have begun to address the potential impact of nanowires in the environment by assessing the physicochemical properties in relevant environmental media as well as the toxicity of silver nanowires (AgNW) to *Daphnia magn*a and Fathead minnow (*Pimephales promelas*) cell lines. We found that the solution behavior and toxicity of AgNW are affected by the nanowire dimensions (30 nm x 2 µm vs. 65 nm x 20 µm), surface coating (polyvinyl pyrrolidinone, PVP, vs. silica), solution chemistry in environmental media and interactions with media components. Future applications of nanowires must consider the potential environmental toxicity.

NM02B-3
Effects of environmental factors on the toxicity & bioavailability of silver and gold nanoparticles using a battery of marine species
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This project is a collaborative project between the Norwegian Institute of Water Research (NIVA), University of Oslo (UiO) and the Focas Research Institute and is funded by the Research Council of Norway and the Integrated NanoScience Platform of Ireland (INSPIRE).
The aim of the work was to investigate the interaction between nanoparticles (NPs) and marine species under realistic environmental conditions. Their toxicity under varying environmental conditions (e.g. salinity), potential uptake routes and their potential to associate with other marine contaminants (mixture toxicity) were all investigated. The objectives of this collaboration were to (1) investigate the toxicity and uptake of standard nanoparticles, to a battery of marine species, (2) understand and elucidate the effects of varying natural environmental conditions (e.g. salinity) on the toxicity of standard NPs to the previously tested battery of species, (3) include a thorough characterisation of the NPs under realistic environmental conditions for use in the interpretation of behaviour in the marine environment, (4) establish collaborative research links between the Focas Research Institute, NIVA and UiO for future research collaborations and exchange of expertise.
Two marine species, the harpacticoid copepod *Tisbe battagliai* and the Rhodophyte *Ceramium tenuicorne* were selected for testing as they represented different trophic levels and ecological niches.
To investigate the effects of salinity and to compare the sensitivity between species, Ag-PVP NPs were assayed at a range of salinities with the two test species. The *T. battagliai* assays were carried out in natural seawater of ca. 35"ð, while *C. tenuicorne* was assayed in 2A media at three different salinities 10, 20 and 30"ð. To ensure the performance of the test species, reference toxicants were run in parallel. Further assays with AgNO3 were carried out under all conditions to account for toxicity due to ionic silver and compared with the NP assays. All results are discussed in the context of sensitivity and varying salinities.
The uptake of Ag-PVP was also investigated in both species using TEM techniques. The potential for NPs to modify the toxicity of some common contaminants to *T. battagliai* is also discussed in relation to gold NPs. A thorough characterisation of all NPs in all media was conducted to support the toxicological findings.

NM02B-4
Effects of different n-oxides of similar size on bivalve immunocytes
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The aquatic environment represents the ultimate sink for manufactured nanomaterials and nanoparticles (NPs). Bivalve mollusks can represent a relevant group of test organisms for investigating the aquatic ecotoxicity of NPs. In bivalves circulating hemocytes, resembling the monocyte/macrophage lineage, are responsible for cell-mediated immunity. In Mytilus galloprovincialis, hemocytes have been shown to represent a sensitive target for the effects of various engineered NPs. The observed immunotoxic/inflammatory effects were mediated by activation of kinase-mediated cell signaling.
Data are presented on the in vitro effects and mechanisms of action of different n-oxides of the same nominal size (20-30 nm) (nTiO2, n-SiO2, n-ZnO, n-CeO2) on mussel hemocytes.
Physico-chemical characterization of NPs was performed before experiments by various tecniques such as Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), gas physical adsorption (BET), Dynamic Light Scattering (DLS), Inductively Coupled Plasma (ICP), etc.. DLS analysis revealed the formation of nano- and micro-sized agglomerates of NP suspensions in artificial sea water (ASW). Hemocytes were exposed to NPs (1, 5 and 10 _g/ml)

for different times (from 30 min to 4 hr) and functional parameters were evaluated: lysosomal membrane stability (LMS), ROS and NO production, lysozyme release. Moreover, cytosolic pH and apoptotic changes were measured by Flow Cytometry.
The effects on hemocyte LMS were dose dependent, with ZnO>TiO2@SiO2>CeO2 and unrelated to their actual size distributions. Differential effects were also observed, depending on the type and concentration of NP, with n-SiO2 showing the strongest effects on ROS and NO production, n-TiO2 inducing dose dependent lysozyme release, ZnO resulting in cytotoxic effects. The role of stress-activated MAPKs in mediating the responses were investigated. The results are discussed in relation to NP chemical composition and behaviour in ASW and mechanisms of action.
Overall, the utilization of immunotoxicity tests in mussel hemocytes represents an useful model that could provide rapid information when screening the potential impact of different NPs on aquatic organisms.

NM02B-5
Ecotoxicology of gold nanoparticles in endobenthic invertebrates, the tellinid clam Scrobicularia plana and the polychaete worm Nereis diversicolor
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Despite gold nanoparticles'expansive utilization and promising future in various fields, only a few studies deal with their behaviors or impacts on aquatic organisms in coastal environments. We explored the biochemical (biomarkers) and behavioral (burrowing kinetics and feeding rates) responses of two estuarine invertebrates Scrobicularia plana and Nereis diversicolor to gold nanoparticles (Au NPs) of three sizes (5, 15 and 40 nm) during a 16 d laboratory exposure at 100 µg L-1. In seawater the aggregation of Au NPs leads to a distribution with a maximum peak at 0.6 µm. After exposure, the Au body concentrations ranged from 6.0 to 29.2 µg g-1 wet wt for clams, and from 0.4 to 5.3 µg g-1 wet wt for ragworms. The bioaccumulation was higher for both clams and ragworms exposed to bigger NPs. Concerning results of defence (Metallothionein-like proteins: MTLPs, catalase: CAT, glutathione-S-transferase: GST and superoxide dismutase: SOD) and damage (thiobarbituric acid reactive substances: TBARS and acetylcholinesterase activity: AChE) biomarkers, Au NPs induced MTLPs in clams. Au NPs of 40 nm exposure also increased the activities of CAT (19%), GST (86%) and SOD (41%) in clams and those of 5 and 15 nm also increased GST activities by 60% and 82%, respectively. The Au NPs did not influence the lactate dehydrogenase (LDH) activities in clams, indicating no change of animal metabolic condition. In contrast, in ragworms, the Au NPs exhibited negligible alterations of CAT (except in 40 nm, increased by 46%), GST and SOD activities; however, a significant elevated LDH activity (41 -52%) was observed compared to control. For clams, no significant oxidative damage was observed referring to the comparable TBARS levels after exposure to all the three size Au NPs; while ragworms demonstrated 33 - 42% decrease in TBARS activities compared to control. Both species revealed no neurotoxicity effects following Au NP exposure compared to controls but in contrast increased AChE activities. These results could be explained by a stabilization effect of AChE by Au NPs leading to a minimal loss of enzyme function. Following the exposure to Au NPs, the burrowing kinetics were notably impaired for both clams and ragworms. Moreover, feeding rate also decreased markedly for ragworms. Because different species exhibit various mechanisms for fighting the nanotoxicity caused by nanomaterials, it is advised to use several aquatic organisms as biomornitoring models in aquatic environment.

NM02B-6
Effects of ionic-Cu, nano- and micro- CuO particles in the deposit-feeding snail, Potamopyrgus antipodarum
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Increasing use of nanoparticles (NPs) is likely to result in release of these particles to the aquatic environment where the NPs may eventually accumulate in the sediment compartment. However, little is known about the potential ecotoxicity of engineered NPs. We here consider the case of metal oxide NPs using CuO to understand if the effects of NPs deviate from micron-sized particles of CuO and ionic-Cu. To address this issue, we compared effects of sediment-associated ionic-Cu, nano- and micro- CuO particles to the deposit-feeding snail, Potamopyrgus antipodarum. Effects were assessed as mortality, specific growth rate, feeding rate, reproduction, and bioaccumulation after 8 weeks of exposure to nominal concentrations 0, 30, 60, 120 and 240 µg Cu/g dry weight sediment. Copper was administered in three forms: ionic Cu, 7nm CuO, and 5 µm CuO. The results demonstrate that nano-CuO had greater effects on growth, feeding rate, and reproduction of P. antipodarum than micro-CuO or ionic Cu. P. antipodarum accumulated more nano-CuO than ionic-Cu or micro-CuO, suggesting that consideration of metal form may be important when assessing risks of metals to the aquatic environment.

NM02C-1
Toxic effects and bioaccumulation of silver nanoparticles in the marine polychaete, Hediste (Nereis) diversicolor
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Silver nanoparticles (Ag NPs) are of concern due to their increasing use in consumer products which may pose risks for the aquatic environment. In this study, the toxicities of commercial PVP-coated Ag NPs (20 and 80 nm) on the marine sediment-dwelling polychaete, *Hediste (Nereis) diversicolor*, were compared with ionic-Ag ($AgNO_3$) after 10 d of sediment exposure, using burrowing behavior, lysosomal stability, DNA damage (comet assay) and bioaccumulation as endpoints. The nominal concentrations used in exposure scenarios were 0, 5, 10, 25, 50, 100 µg Ag/g dry weight (dw) sediment. Our results show that Ag is able to cause DNA damage and instability of lysosomal membranes in in *H. diversicolor* coelomocytes and that, this effect is related to both Ag concentration and form. Ag NPs tend to be more toxic than the ionic form. No toxicity of PVP coatings was observed. Ag body burden increased with increasing exposure concentration of both Ag forms, indicating that they are bioavailable to *H. diversicolor*. Further studies are currently being conducted to investigate if the bioaccumulation is also size-dependent, as well as to assess the effects of Ag NPs and Ag+ ions on burrowing behavior. Results from this work will also be presented.

NM02C-2
Chronic effects of nanosilver to *Daphnia magna*gt
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Nanoscale silver is one of the most promising nanomaterials for various future applications. Due to its antibacterial properties nanosilver is increasingly employed in a variety of consumer products including wound dressings, textiles, personal care products, paints, or food storage containers. Currently, no data exists for environmental concentrations, but the incremental production of nanosilver inevitably leads to an increased environmental release. Several studies have demonstrated the toxicity of nanosilver to different species of bacteria, suggesting that these effects may be detrimental to aquatic ecosystems. Potential routes of uptake by aquatic organisms include direct ingestion or entry across epithelial boundaries such as gills or body wall. Toxicity and exposure data for aquatic invertebrates, however, is currently lacking. There are only few studies on the acute toxicity of nanosilver. Long dose and long-term invertebrate exposures giving priority to sublethal effects provide useful completion.
The aim of this study is to evaluate the chronic toxicity of nanoscale silver on *Daphnia magna*. The effects on growth, reproduction, and viability were assessed in a multi-generational study. Particle size and shape were characterised by dynamic light scattering using a zeta sizer and transmission electron microscopy.
The results show that with increased exposure duration the toxicity of nanosilver to *D. magna* increases. The exposure of consecutive generations of *D. magna* results in an effective concentration (LOEC/2=0.75 µg L-1) about ten times lower compared to the 21-d reproduction test (NOEC=6 µg L-1). Therefore, long-term studies should be given a high priority to make an adequate evaluation of the potential environmental risks of nanosilver and nanomaterials in general.

NM02C-3
Do TiO2 nanoparticles affect earthworm reproduction?
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The toxicity of three different TiO2 nanoparticles was investigated in the earthworm reproduction test according to the OECD test guideline No. 222. All applied nanoparticles were derived from the OECD-Sponsorship Programme and differed in parameters such as primary particle size, crystalline structure, BET surface and zeta potential. The tests were performed in a natural sandy soil. TiO2 was applied via powder in the soil simulating an atmospheric immission. In preliminary experiments the homogenous distribution of the particles via this procedure was demonstrated. Suitable amounts of TiO2 powder to achieve the desired final soil content were mixed homogenously with dry soil. Care was taken to avoid the modification of the TiO2 crystalline structure. Uncontaminated sandy soil was spread on a plate, the carrier material with the TiO2 powder distributed on the soil, and the whole material was mixed carefully. The water content was adjusted to 55% of the maximum water-holding capacity. Test concentrations were: 50, 100 and 200 mg/kg soil dry matter.
No influence on the biomass of adults was observed, but the reproduction rate was significantly increased. A maximum stimulation of about 50% was observed. The extent of stimulation differed between the three nanoparticles. Unexpectedly, the nanomaterial with the largest primary particle size and a small surface resulted in the maximum effect. Experience so far indicates that a small primary particle size and a large surface result in increased toxicity. Looking at the total number of the offspring and not at the percent effect leads to an interesting observation. The number of offspring in the control was lower in winter, increasing in summer. Such an increase in the number of offspring was not observed in the containers with TiO2. Over the testing period of several months the number of offspring of earthworms exposed to TiO2 contaminated soil varied to a much lower extent than the number of offspring of earthworms incubated in control soil. Usually the reproduction rate of Eisenia andrei - even of those earthworms cultured in the laboratory since many years - shows a seasonal variation. A nearly constant reproduction rate throughout a year could be the consequence of a disturbance of the circannual biological rhythm by TiO2. Further experiments have to verify the hypothesis.

NM02C-4
Comparing the reproductive toxicity of ZnO nanoparticles, bulk ZnO, and ZnCl2 to the earthworm Eisenia andrei
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Nanotechnology is developing very fast and industrial applications are many. Zinc oxide (ZnO) nanoparticles (NPs) are widely used in consumer products e.g. as UV protecting agent and can be expected to enter the environment via sewage sludge applied to soils. ZnO NPs are some of the most toxic in aquatic exposures, but investigations of their effects in terrestrial systems are lacking. These circumstances make the study of NP zinc oxide in terrestrial ecosystems very relevant. It is known that zinc can have negative effects on life-cycle responses in the earthworm E. fetida, but the comparative effects of nano and non-nano or dissolved zinc in soil have not yet been investigated in detail. In this study we compared the reproductive toxicities of NP, bulk (non-nano) and dissolved Zn to the earthworm Eisenia andrei. The main results of the experiment were: 1) ZnCl2 toxicity was greater than NP and bulk Zn forms and 2) the reproduction rate was the most sensitive endpoint in the experiment, since it was affected by all forms of Zn. The estimated LC50 values for ZnCl2 (1436 mg/kg week two, 1235 mg/kg week four) and the reproduction rates recorded in this experiment are comparable with other studies. The results supported the general consensus that toxicity of ZnO NPs to earthworms is determined by the amount of free zinc ions and they also suggested that the nanoparticle agglomeration and adsorption behaviour in the soil affected the bioavailability to the worms. In light of the present results it is likely that the risk assessment of ZnO NP in the environment does not need to go beyond the one of dissolved zinc.

NM02C-5
Ecotoxicity of ZnO-NP, bulk ZnO and ZnCl2 to Folsomia candida in relation to bioavailability in soil
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Only nine ecotoxicological studies are available to assess the effects of metal nanoparticles on soil organisms. Most inorganic nanoparticles are tested in aquatic systems, while the ecotoxicity of

the majority is not assessed in soil. Due to adsorption and dissolution of nanoparticles the toxicity of metal nanoparticles in soil is difficult to establish. The present study aims to determine the chronic toxicity of zinc oxide nanoparticles (ZnO-NP) to Folsomia candida, by studying survival and reproduction in freshly spiked soil. To unravel the contribution of zinc oxide particle size and free zinc to nanoparticle toxicity, bulk ZnO (i.e. non-nano) and ZnCl2 are tested for comparison. Toxicity tests were performed in natural Lufa 2.2 soil using standard test procedures as described in ISO guideline 11267.
For all three compounds, zinc concentrations in the porewater increased with increasing soil concentrations. This is reflected by the Freundlich isotherm yielding sorption constants Kf of 61.7, 106 and 96.4 l/kg (with corresponding n values of 1.50, 1.34 and 0.42) for ZnO-NP, bulk ZnO and ZnCl2 respectively.
Survival of F. candida in soil spiked at concentrations up to 6400 mg Zn/kg d.w. with ZnO-NP and bulk ZnO was not affected. Reproduction was reduced in a dose-dependent manner and 28-d EC50 values of 1964, 1591 and 298 mg Zn/kg d.w. were estimated for ZnO-NP, bulk ZnO and ZnCl2, respectively. It seems that the size of zinc oxide particles does not contribute to a significant difference in the effect observed on springtail reproduction. Compared to ZnCl2, the EC50 of ZnO-NP based on actual zinc concentration in soil was almost 7-fold higher, but the difference in EC50 values is small when based on porewater concentrations. EC50 values of 10.1, 7.94 and 16.8 mg Zn/l were calculated for ZnO-NP, bulk ZnO and ZnCl2, respectively based on measured concentrations in the soil pore water.
We conclude that ZnO particle size does not affect the reproduction of the soil organism F. candida. It is very likely that the zinc ions released from the nanoparticles are responsible for the observed toxic effects rather than the nanoparticles as such.

NM02C-6

In vitro nanotoxicology bridges earthworms and humans: comparative analyses of the molecular and cellular toxicity

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Silver nanoparticles (AgNPs) are among the most widely used materials in commercial nanoproducts and as a potential tool for biomedical application. Still, little is known about their immunological consequences. Invertebrate models have long been exploited to study the evolutionary development of innate immunity by cross-referencing to more complex human immune systems. Here we illustrate our initial approach to compare the gene and cellular responses of earthworm coelomocytes and human immunocytes to silver ions (Ag^+) and AgNPs *in vitro*. One-step conjugation of AgNPs with serum albumin provided extensive colloidal stability, and the AgNP suspension was sterically stable over time under the test condition, with a free Ag^+ fraction of <1%. Cell viability, oxidative stress and gene expressions of coelomocytes and human cells were assessed following exposure to Ag^+ (0 - 1.35 µg/ml) or AgNPs (0 - 5.91 µg/ml). Earthworm coelomocytes were more sensitive to Ag^+ than the human immunocytes (THP-1 monocytes, THP-1 macrophages and human peripheral blood mononuclear cells). Coelomocytes were also most susceptible to AgNPs while EC_{50} values for THP-1 monocytes and macrophages could not be estimated within the concentration range tested. Remarkably, though, the dose-response curve of THP-1 macrophages for the AgNP treatment was similar to that of coelomocytes. Furthermore, we observed a strikingly similar time-course pattern for the increase in intracellular generation of reactive oxygen species (ROS) between coelomocytes and THP-1 monocytes exposed to Ag^+ or AgNPs. Following AgNP exposure, genes involved in innate immune systems, metal detoxification and anti-oxidising mechanisms showed similar responses between coelomocytes and THP-1 monocytes suggesting that these molecular pathways are potentially affected by the interference of AgNPs with the cellular machinery. Progressive induction of some of the genes observed in our in vitro assays is also reported in a separate study on temporal molecular toxicity of AgNPs to whole worms in soil (Heckmann et al. in prep), supporting the importance of those genes for the defence mechanisms against AgNPs. With the aid of *in vitro* techniques, our comparative approach begins to provide exciting insights into the conserved molecular and cellular mechanisms of NP toxicity between invertebrates and vertebrates.

NM02D-1

Interaction of nanosized Poly (amido) amine dendrimers with fresh water ecological organisms and fish cells

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Nanotechnology is a highly promising and exciting technology that spans many areas of science and technological applications. Polymeric nanomaterials are widely used in different aspects of the medical field in terms of diagnosis, tissue engineering and as drug delivery devices. As nanomaterials are currently being widely used in modern technology, there is an increasing need for information regarding their human health and environment. This study focussed on the eco and mammalian toxicological impact of three generation of poly-amidoamine (PAMAM) dendrimers (G-4, G-5 and G-6) on a battery of bioassays representing different trophic levels for the ecotoxicological study. The acute eco and cytotoxicological effects of poly-amidoamine (PAMAM) dendrimers (G-4, G-5 and G-6) were evaluated in a bacterial species (Vibrio fischeri), unicellular algae (Pseudokirchneriella subcapitata), two crustaceans (Thamnocephalus platyurus and Daphnia magna) followed by cytotoxicity assessment with two different fish cells (RTG-2 and PLHC-1) to represent vertebrate species. The ecotoxicity results demonstrate that there is systematic generation dependent ecotoxicological [1]. The origin and molecular mechanism of toxicological response due to the exposure of PAMAM dendrimers was explored with fish cell (PLHC-1). Toxicity of the PAMAM dendrimers starts with generation of reactive oxygen species [2], and then lead to DNA damage and apoptosis will be discussed.
Key Words: PAMAM dendrimers; Ecotoxicology; Cytotoxicity; Oxidative stress; DNA damage; apoptosis.
Reference:
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NM02D-2

Developmental toxicity of metal oxide nanoparticles on Xenopus laevis: focus on the disruption of the intestinal barrier

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Despite the huge amount and rapidly increasing data on the toxicological properties of nanomaterials, the ecotoxicological threatening of such contaminants is far to be characterized. In particular aquatic ecosystems, representing a terminal receptor of nano-contaminants and a realistic accumulation site, merit more attention.
This work contributes to the knowledge of the potential adverse developmental effects inducible by metal oxide nanoparticles (NPs) on amphibian embryos. Lethal and teratogenic potentials of commercially available ZnO, CuO, TiO2 NPs (nZnO, nCuO, nTiO2) were characterized for their hydrodynamic behaviour and tested on standardized FETAX procedure. Embryos were histologically screened to detect lesions in primary organs. No mortality was observed after NPs' exposure, while a significant concentration-dependent growth retardation was induced especially by nCuO. Moreover nCuO resulted to be a powerful teratogen, with survived embryos characterized by diffuse severe malformations. nZnO did not induce severe teratogenic effects, while at histological and ultrastructural levels intestinal mucosa showed diffuse lesions.
At comparable nominal dimensions and mass concentration, metal oxide NPs produced different effects on Xenopus development, confirming that toxicity depends on both ion dissociation and particles themselves reactivity. nCuO embryotoxicity mainly derived from dissolved copper ions, while that of nZnO was apparently associated to the NP reactivity.
The NPs tested entered the gut epithelium, producing additional teratogenic potentials in late developmental stages, by an alteration of the intestinal physiology or a NP translocation through other organs. The imaging techniques evidenced the presence of NPs beyond the intestinal lumen, in the surrounding connective tissue and even in many non-target organs.
While for nCuO and nTiO2 no conclusive indications about their teratogenic mechanisms of action have yet emerged, our experimental data support the hypothesis that nZnO can sneak into the paracellular space, reaching the basement membrane. This mechanism probably involves intestinal cell injuries leading to cytoskeletal rearrangements, responsible of the possible disruption of the tight junctions.
In conclusion, the comparative study of the embryotoxic potential of three widely used metal oxide NPs, suggests that different mechanisms drive the toxic effects and the translocation pathways.

NM02D-3

Aquatic toxicity and characterisation of carbon nanotubes in amphibian larvae Xenopus laevis

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Carbon Nanotubes (CNTs) are already used in some commercial applications. The question of their release in the environment occurring during life cycle of manufactured product containing CNTs may soon become relevant. Nevertheless, little is known about their potential ecotoxicity. Investigations of their environmental hazard were conducted in the framework of the Joint Research Laboratory NAUTILE (Nanotubes & Ecotoxicology). Two kinds of CNTs, synthesized using similar processes (Catalytic Chemical Vapour Deposition), but very different in nature, were studied: double-walled nanotubes (DWNT, CIRIMAT) and multi-walled nanotubes (MWNT, Graphistrength C100, Arkema France).
The present study examines the potential impacts of CNT in the aquatic environment, since this compartment could be the ultimate receptacle of contamination, depending on their emission scenarios, thus making the aquatic assessment of nanoparticles of particular importance.
The tools which were carried out have helped us to push the investigation towards sensitive biological models such as amphibian larvae (Xenopus laevis), using international standardized bioassays (IS0, 2006). For a given type of CNT, raw and chemically dispersed CNTs have been added to the exposure media, using surfactants, to assess biological variations in relation with their physical dispersion state. Two different endpoints were assessed: (i) toxicity (mortality and growth inhibition) and (ii) genotoxicity (induction of micronucleated erythrocytes). In addition, biological preparations of amphibian organs from larvae exposed to CNTs were analyzed using optic microscopy, Transmission electron microscopy and Raman spectroscopy.
The results show growth inhibition in larvae exposed to 10 and 50 mg.L-1 of raw CNT. Modulated effects on growth parameter were obtained in organisms exposed in presence of surfactant. No genotoxicity was evidenced in erythrocytes of larvae exposed to CNT, except with one of test conditions in presence of surfactant. The Raman analysis confirmed the presence of raw CNT into the lumen of gut larvae but not in intestinal tissues and cells, nor in the circulating blood of exposed larvae.
These data strongly support that the chronic toxicity observed in larvae exposed to high concentrations of CNT could be due to physical effects (gill clogging and/or abrasive effects) which are not necessarily related to intrinsic effects of individual CNT.

NM02D-4

Rusting of the Trojan horse - Kinetics and mechanisms of ion release from nano-silver surfaces

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Nano-silver (nAg) is being manufactured at large scale and incorporated into various consumer and medical products as a broad-spectrum antimicrobial agent. The high production volume raises concern about silver release to the environment and potential adverse impacts on human health and environment. The major pathways in the antibacterial activity, eukaryotic toxicity and aquatic toxicity of nAg involve the silver cation - Ag+, which is an established thiol toxicant. In these ion-based toxicity pathways, nAg particles act as a "Trojan Horse", a reservoir of metallic silver that can be delivered to biological targets and released as active Ag+ ions. This talk focuses on the origin of Ag+ in nAg particle suspensions. The ion release is shown to be a cooperative oxidation process requiring both dissolved O2 and H+, and to be inhibited by natural organic matter. Both data and thermodynamic modeling indicate that metallic nAg will not be a persistent toxicant in particle form in environmental compartments containing O2. Understanding ion release mechanisms allow a drug delivery paradigm to be applied to the nAg system, in which various chemical approaches are used to achieve controlled release formulations. After presenting thermodynamic calculations of silver species partitioning in biological media, the rates of oxidative silver dissolution are measured for nanoparticles and macroscopic foils and used to derive unified area-based release kinetics. A variety of competing chemical approaches are demonstrated

for controlling the ion release rate over 4 orders of magnitude. Release can be systematically slowed by thiol and citrate ligand binding, formation of sulfidic coatings, or the scavenging of peroxy-intermediates. Release can be accelerated by pre-oxidation or particle size reduction, while polymer coatings with complexation sites alter the release profile by storing and releasing inventories of surface-bound silver. The ability to tune biological activity is demonstrated through a bacterial inhibition zone assay carried out on selected formulations of controlled release nAg. Our results have important implications for the fate and transport of nAg in the environment and in biological systems. Ion release kinetics is proposed that can be incorporated into quantitative environmental fate and transport models. Finally, the ability to control ion release rate will allow optimization of nAg-based antibacterial products for performance, lifetime, and safety.

NM02D-5

Engineered nanoparticles as toxic metal carriers in the aquatic systems: quantum dots like Cu and Pb nanovectors

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Despite of the expected enhanced surface properties, there is a paucity of data concerning the role of the engineered nanoparticles as trace elements carriers. By exploring the capabilities of the multidetection instrumental platform consisting of asymmetrical flow field-flow fractionation coupled in-line to UV spectrometry and inductive coupled plasma mass spectrometry, the present study examines the role of the carboxyl-terminal group polymer coated CdSe/ZnS quantum dots (QDs) as a potential nanovectors of toxic trace elements in the aquatic systems. More specifically the size and elemental distributions was probed by quantifying the association of Cu and Pb as a function of the size distribution, as well as the effect of the Suwannee River fulvic (SRFA) or humic (SRHA) acids, representing the dissolved organic matter in waters.
Obtained results showed a monodisperse distribution of quantum dots with average hydrodynamic diameter of 12.8 ± 0.5 nm. Addition of both 20 mg L^{-1} SRFA, or SRHA, and 100 µg L^{-1} of Cu and Pb did not significantly influence the QDs hydrodynamic size distributions. The amount of Cu and Pb bound to the Qdots was three times larger than that associated to fulvic and humic acids In the ternary system containing metals, humic acids and QDs, the amount of Cu or Pb bound to the QDs was 12 and 5 times higher than that associated to the SRFA or SRHA, respectively. These observations suggest that QDs bind Cu and Pb to a larger extent than SRFA and SRFA and have the extended potential to play a role of metal vector in the environment.
The environmental implications of the results are discussed with respect to the transport of trace metals in the environment as well as their consequence for bioavailability and potential biological effects.

NM02D-6

Heterocoagulation of silver nanoparticles

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Increasing exposure of the enviroment to silver nanoparticles (AgNP) has raised concern because of possible ecotoxicity. However, the transport mechnanisms of AgNP are not clear, but there are indications that heterocoagulation of AgNP with naturally occuring colloids may be important. This study investigates, for the first time, heterocoagulation of PVP-coated AgNP (10 nm nominal diameter) with maghemite (Fe2O3, < 50 nm nominal diamter) and montmorillonite (135 nm hydrodynamic diameter) at pH 4 and pH 8 as a function of increasing NaClO4 concentration. Coagulation rates of AgNP in individual suspensions were relatively low, with critical coagulation concentrations of 21 and 45 mmol L-1 at pH 4 and 8, respectively. However, aggregation rates increased with several orders of magnitude in AgNP - maghemite mixtures at pH 4, regardless of NaClO4 concentration. In the case of AgNP - montmorillonite mixures, the rate increase relative to indiviudal suspensions was significant, but less compared to mixtures with maghemite. Simialrly, rate increases were much less in all cases at pH 8. This study suggests that heterocoagulation with natural colloids may be an important mechanism that controls the fate of AgNP and other engineered nanoparticles in the environment, particularly in relatively acidic soils and natural waters with a low ionic strength.

NM03 - Risk assessment and risk management of nanomaterials

NM03-1

Engineered nanomaterials in rivers - exposure scenarios for Switzerland at high spatial resolution for nano-TiO2, nano-ZnO and nano-Ag

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Two models, one based on probabilistic material flow analysis and one based on graph theory, were combined to calculate predicted environmental concentrations (PECs) of engineered nanomaterials (ENMs) in Swiss rivers at high spatial resolution. PECs for nano-TiO_2, nano-ZnO and nano-Ag were calculated for 543 river sections downstream from 543 sewage treatment plants at base flow conditions. Additionally, flow measurements at 20 selected locations over a 20-year period (1988-2007) were used to assess temporal variations. Two scenarios were modeled - a reactive scenario considering ENM agglomeration/ degradation/sedimentation in aqueous systems and a conservative scenario assuming no ENM transformation. At base flow the highest PECs were found for nano-TiO_2: The PECs ranged, depending on the location, from 0.1 ng L^{-1} to 7,800 ng L^{-1}modal values), followed by nano-ZnO (0.004 ng L^{-1} to 500 ng L^{-1}) and nano-Ag (0.2 pg L^{-1} to 32,500 pg L^{-1}). This study shows that linking a probabilistic material flow analysis to a geo-referenced model fills two gaps: (i) considering the geographical distribution of the ENM emissions and local river flow rates allows for a prediction of a more realistic range of possible environmental concentrations than at a regional level; (ii) a clear distinction between input uncertainty and river flow variations can be achieved. Temporal river flow variations influenced the ENM concentrations up to a factor of 10 - when considering the 15%-85% quantiles of flow - the uncertainty in the ENM loads caused a difference in the PEC calculations up to a factor of 5 - considering the same quantiles for ENM emissions.

NM03-2

A way forward in exposure assessment of nanomaterials in the aquatic environment

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The current approach to ecological risk assessment of chemicals is based on the quotient of a predicted no effect concentration and a predicted exposure concentration. We have gathered knowledge supporting the prediction of the exposure concentration of nanomaterials (NMs) in the aquatic environment and have evaluated the adequacy of the current guidance documents for use with NMs and therefore have also conducted a literature review on two important environmental fate processes for NMs, sedimentation and dissolution. This resulted in an overview of the available quantitative data for sedimentation and dissolution of NMs. We have used this overview to propose a way forward in modeling the exposure concentration of NMs in the water phase. Transport to sediment seems to be of greater relative importance than advection or dissolution of NMs. Both the transport of nanomaterials from water to sediment and the dissolution of nanomaterials can be incorporated into current exposure models simply by adding first-order rate constants. Our proposed exposure model for nanomaterials can be used to improve current risk assessment for nanomaterials.

NM03-3

Developing exposure scenarios for manufactured nanomaterials: knowledge gaps and research needs

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The introduction of manufactured nanomaterials (MNMs) and products enhanced by MNMs onto the global market is developing at a rapid pace and this means that the potential for human and environmental exposure is also increasing. Exposure assessment for MNMs is a specialised field that requires the use of advanced instruments and specialised expertise and it will often not be feasible for companies to measure exposures. For risk assessments for MNMs it is essential that information on exposure is shared between scientists and organisations. The NANEX project was funded by the European Commission to try to develop a catalogue of exposure scenarios (ESs) for MNMs based on publicly available information as well as information collected during measurement surveys. The results were used to carry out a gap analysis and determine research priorities. The focus was on exposure to carbon nanotubes (CNTs), nano-silver (nano-Ag) and nano titanium dioxide (nano-TiO2).
In total, 57 occupational ESs were developed; including 28 for CNT, 13 for nano-TiO2 and 4 for nano-Ag. Most of the scenarios were related to production/synthesis of the nanoparticles, although some were also developed for downstream use. For consumer exposure, there was a paucity of information and consumer ESs developed were based on very limited information.
There is a critical lack of exposure information available in the public domain, and any available exposure data are generally presented in a way that they are difficult to interpret and use for 'read-across' to similar ESs. Some level of harmonization of reporting of results of exposure measurements and contextual information is urgently needed. The NANEX project suggested a minimum dataset for reporting exposure studies for MNMs, which includes both nano-specific and more general items. One important realisastion is that exposure to MNMs is multi-factorial, and it cannot currently be described satisfactory by a single exposure metric. A white paper on research priorities for exposure assessment to MNMs is under development. The shortterm priorities include development of efficient risk management strategies, development of sampling instruments and measurement methods and harmonization of characterisation and quantification of exposures. The medium- and long-term research priroities include the study of determinants and modifying factors for the various relevant exposure metrics and development of quantitative exposure models.

NM03-4

A weight of evidence methodology for risk assessment of engineered nanomaterials

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The production and use of engineered nanomaterials (ENMs) are addressed by the European REACH regulation ð'- 1907/2006, which gives industries the responsibility of the Chemical Safety Assessment (CSA) for any produced or imported industrial substance. The required CSA follows the traditional risk assessment (RA) framework, including hazard assessment, exposure assessment and risk characterisation steps. It has been recognized, however, that substantial limitations and uncertainties make the conventional RA infeasible to apply to ENMs today, which leaves regulators with little support in the near term. Knowledge gaps have been gradually filled by new research results and uncertainties have been reduced, but this process advances slowly and it will take decades [1], while quantitative risk assessment results are urgently needed to support timely regulatory decisions and risk management actions [2]. In response to this need, a number of methodologies and tools to assess the risks from ENMs, in spite of the limitations, have been proposed. Most of them, however, are not intended to facilitate regulatory decision making, but instead to serve as preliminary hazard/risk screening and/or research prioritization tools. The present deficit of quantitative data and scientifically sound approaches will lead in the near and in the medium terms to uncertain and ambiguous, largely qualitative risk estimations based on expert judgments, which may fail to support proper risk management actions. Therefore it is important to study the possibilities to aid the traditional RA framework with complementary/ alternative tools in an attempt to achieve quantitative RA of ENMs. In this context a novel approach for RA and prioritization of ENMs, including uncertainty evaluation, is being developed within the FP7-funded ENPRA project. The main goal of the proposed methodology is to quantitatively assess and rank human health risks due to exposure to ENMs in occupational and/or consumer settings. This presentation will illustrate the novel ENPRA approach in the context of the feasibility of available methodologies and tools for risk assessment of ENMs and will discuss its implications for ENM risk management and regulation.

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NM03-5

A comprehensive environmental assessment approach to making informed decisions about engineered nanoparticles

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Metal-based nanoparticles are being developed in numerous shapes and sizes to fit a wide array of consumer, industrial, and military applications. Material science research into the development of new nanoparticles is far outpacing environmental and human health and safety research, yet the health and safety data are critical for acquisition decisions, regulatory decisions, worker safety, product use and disposal, and public acceptance of nanoparticle-containing products. Traditional life cycle analyses address key steps in nanoparticle synthesis, use, and disposal, but lacks specific information regarding fate and effects in the environment. Conversely, traditional environmental risk assessment addresses fate and effects of chemical stressors in the environment, but only considers chemical that goes into the environment and does not take into consideration the entire chemical stock from the manufacturer. Therefore, we propose using a comprehensive environmental assessment (CEA) approach, detailed by Davis (2007) to evaluating nanoparticles from "cradle to grave". CEA combines life cycle analysis parameters (e.g., manufacture, storage, use, disposal) with traditional risk assessment parameters (e.g., characterization, exposure, effects, assessment) to give a more comprehensive understanding of nanoparticle exposure and effects in different environmental settings. We will use aluminum nanoparticles as a case study. By applying the CEA approach, we can better assess for a manufacturer or acquisition authority the key aspects of the Al nanoparticle life cycle that will pose a potential impact on environmental processes and health effects. It will also help identify data gaps that need to be addressed prior to acquisition or risk management decisions. In conclusion, the use for CEA for nanoparticles will improve acquisition, risk, and regulatory decision making and management prior to any unforeseen adverse environment, health, and safety (EHS) events that could dramatically impact the use of these revolutionary new materials.

NM03-6

Decision-directed approach to EHS strategy for nanomaterials

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The recent emergence of nanotechnology in both the marketplace and the public consciousness coincides with increased recognition of the importance of an integrated, systems approach to environmental, health and safety that includes life-cycle thinking, public participation, and adaptive management of risks associated with emerging technologies and chemicals. As a result, environmental research strategy documents like those published by the US Environmental Protection Agency (EPA) and the National Nanotechnology Initiative (NNI) emphasize a broad, comprehensive approach that includes identifying nanomaterial source terms at all stages of the nanomaterial life-cycle, characterization of environmental fate and transport properties, identification of toxic and abiotic effects, and pre-emptive engineering of novel materials to overcome anticipated public or stakeholder objections. Nevertheless, the same federal agencies that have advocated for such forward-thinking approaches have been criticized for failing to articulate a strategy and framework for effectively linking environmental risk research to the decision-making needs of product developers, regulators, consumers or other stakeholder groups. Two analytic approaches that have yet to be sufficiently integrated can provide a powerful approach to better connecting the nanotechnology research agenda with product developer, policy-maker, or consumer decision needs: value of information (VoI) and multi-criteria decision analysis (MCDA). VoI explores the sensitivity of a decision under uncertainty to new information before making a decision, while MCDA allows comparison of decision alternatives that are not reducible to a single criterion (such as cost-benefit analysis). Both MCDA and VoI have been used successfully in industry and have attracted increasing attention in government and regulatory applications. However, actual implementation in public sector is still rare. This presentation will provide methodology and illustrate applications of VOI and MCDA to enhance risk assessment for nanomaterials. Implications for nanomaterial EHS risk governance will be discussed.

PE01 - Data-driven, knowledge-based, and QSAR modelling in ecotoxicological assessment

PE01-1

Who needs lab work? An investigation into predictive ecotoxicological computer models and naphthenic acids.

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Computer based predictive ecotoxicology models are increasingly used to assess adverse effects of chemicals that are released into the environment. Ease of use and cost effectiveness makes these models attractive alternatives to field and laboratory testing.
In the present study the toxic effects of individual carboxylic ('naphthenic') acids were determined by two predictive quantitative structure activity relationship computer models (ECOSAR v.1.00a and ADMET v. 5.0). In the case of ECOSAR some physiochemical parameters, and toxicity to fish, algae and crustaceans were predicted. ADMET is a more detailed model based on human toxicity and provides physiochemical parameters, enzyme metabolism, human liver effects, mutagenicity as well as some ecotoxicological predictions.
The so called 'naphthenic' acids are a group of compounds that are of increasing concern due to their presence within oil industry waste, including oil sands process waters. Few of these compounds are commercially available and have to specially synthesised so predictive models may be helpful for targeting specific structures for synthesis.
Toxicity predictions for the effects of the acids on *Tetrahymena pyriformis* from the ADMET model and on *Daphnia magna* from ECOSAR were compared to measured Microtox[TRADEMARK] bacterial assay results for *Vibrio fisherii*. For an investigation of possible quantitative structure activity relationships acids were grouped into classes (e.g. aliphatic, aromatic).
Both models produced statistically significant trends ($p = <0.01$ and $<0.001x$ to y; $R^2 = 0.2292$ and 0.4323) against the Microtox[TRADEMARK] tested data for some series of acids but neither distinguished between the effects of isomeric acids (e.g. iso, tertiary and secondary alkyl substituted acids).
Predictive models may be used to target work on potentially toxic chemicals that are released into the environment but it is recommended that any data obtained from them are verified by thorough laboratory based testing.

PE01-2

A combined expert knowledge and data-driven approach to predict biotransformation pathways of organic contaminants

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In silico systems that predict plausible structures of transformation products (TP) formed through microbial transformation of xenobiotics could play an important role in obtaining a more comprehensive picture of environmental exposure to TPs. Lists of predicted TP structures can be used to screen environmental samples for TPs or for model-based prioritization of plausible TPs at the first tiers of chemical risk assessment. Existing rule-based expert systems that predict biotransformation pathways typically suffer from low selectivity, i.e., many more products are predicted than are experimentally observed. This often prevents their application, e.g., to support inclusion of TPs into chemical risk assessment. Here, we present two data-driven approaches to increase the selectivity of rule-based biotransformation pathway prediction and demonstrate how their implementation increased the selectivity in one specific rule-based system, the University of Minnesota Pathway Prediction System (UM-PPS) (http://umbbd.msi.umn.edu/predict/). The approaches include the data-driven extraction of relative reasoning rules from data contained in the University on Minnesota Biocatalysis/Biodegradation Database (UM-BBD), a manually maintained collection of literature-reported biotransformation pathways, and the targeted generation and analysis of experimental biodegradation data for pertinent transformation rules. Implementation of relative reasoning rules reduced the number of predicted first generation TPs by about 50%, increasing selectivity from 10 to 20%. At the same time, the percentage of correctly predicted, experimentally observed products slightly decreased for external validation, demonstrating that uncertainty and heterogeneity in the training and validation data limit optimization in rule-based systems. For the investigation of pertinent transformation rules, a combined experimental, analytical, and data processing procedure was developed for the high-throughput identification of TPs formed in sludge-seeded bioreactors. The approach was applied to refine transformation rules for the amide functional group by investigating transformation pathways of 30 diverse amides. Altogether 53 TPs were identified and the data were analyzed in terms of electronic and steric features dictating the observed transformation pathways. A metabolic logic for the preferred biotransformation pathways of amides was derived that has been implemented into UM-PPS.

PE01-3

Quantitative read-across for predicting toxicity and environmental fate related properties of organic compounds

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Read-across provides predictions of compound properties through interpolation from known values of structurally similar substances. As such, it does not rely on molecular descriptors and their regression relationship to some target property, but on an approach to define structural similarity. Because chemical similarity itself cannot uniquely be defined, it requires reference to a concept and an associated operational procedure. To this end, we employ the methodology of atom-centered fragments (ACFs) that has recently been proven useful as tool to rank, in a predictive manner, the reliability of QSAR applications.[1]
The ACF method decomposes molecules into structural fragments consisting of a central atom and bonding neighbours. An ACF is then defined through the atom type and the number and type of bonding neighbours and associated bond types. Each non-hydrogen atom serves as central atom, while both non-hydrogen atoms and hydrogens may be used as neighbour atoms to define a particular ACF. First-order ACFs take into account only the directly bonded neigh¬bour atoms, second-order ACFs consider all atoms up to path length two in each bonding direction, and higher-order ACFs are defined accordingly.
In the present communication, we show the application of this ACF approach for quantitative predictions of compound properties, thus yielding an approach complementary to traditional QSARs. Read-across models for predicting acute toxicity towards fish and daphnids, the bioconcentration factor, and several partition coefficients with relevance for the environmental fate are presented. They demonstrate the power of the ACF methodology to identify property-relevant structural analogues. With regard to REACH, the results suggest the ACF-based read-across methodology as general non-animal approach for predicting aquatic toxicity, and thus to support the reduction and replacement of animal testing for chemical safety assessment.
Financial support by the European Commission through the project OSIRIS (Contract No. 037017) is gratefully acknowledged.
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PE01-4

Growth inhibition of 20 selected antibiotics and evaluation of non-testing support tools in environmental risk assessments

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Pharmaceuticals have been detected in sewage treatment plant effluents, in surface-, sea-, ground-, and drinking water. Concerns have thereby been raised regarding the fate and effect of pharmaceuticals in the environment. In this project antibiotics, including antimycotics and antivirals, were investigated. Risks associated with antibiotics concern bacterial resistance as well as their toxic effects. From a data base of 97 antibiotics prescribed for human use, a training set of 20 antibiotics was carefully selected to represent the chemical variation of the target group. The toxic activity of the 20 antibiotics was measured in a bacterial growth inhibition assay. Five of the tested substances showed a rather high activity with EC50 values below 1µM. Seven antibiotics showed very low, or no activity with EC50 values above 150 µM. The remaining eight tested substances had a rather even distribution of EC50 values from 3 to 90 µM. Classical univariate and multivariate QSAR approaches covering all compounds of the training set showed to fail. Hence, the dataset with 97 antibiotics was split into four groups using hierarchical cluster analysis

aiming to develop cluster specific models. The clustering was based on the compounds variation in calculated chemical descriptors and the groups were separated largely based on molecular size and hydrophobicity of the substances. QSARs based on partial least squares projections to latent structures (PLS) were created and five non-tested antibiotics were suggested with potential toxic effect based on structural similarities with the most potent of the training set. Derived data was in addition compared with data provided by the risk assessment initiative in Swedish FASS and by data estimated using ECOOSAR. Initial comparisons indicated some incoherence between available ERA tools and the toxic potentials of the drugs as determined in this study.

PE01-5

Biocides in paints in urban areas: modelling an underestimated source of environmental contamination

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Biocide contamination of receiving waters is generally linked with agriculture. However, recent studies have shown that urban contributions should be also considered. One of the suspected biocide sources in the urban environment is building paint. Biocides like diuron, irgarol, terbutryn, carbendazim, etc., are conventionally used in paint to control fungi, algae, bacteria and other microorganisms that can colonize building façades.

The problem of biocides in urban areas is closely linked to meteorological conditions and in particular to rain events. As a consequence, it is important to understand how rainwater collects and transports biocides from façades and how these biocides are transported in sewer systems to receiving waters. In this study, we present a conceptual model describing façade leaching and couple it with a Wind Driven Rain model and a classical hydrological model to compute the contribution of a city to the biocide load from building paint.

For the entire city of Lausanne (Switzerland, 200'000 inhabitants), a global production of 2200 kg/year of terbutryn leached by rain was estimated considering local building characteristics and meteorological information. The leaching model fitted well the peak in concentration measured at the bottom of the wall at the initial stage of the rain event. However, concentrations measured in an urban river in the watershed leads to the conclusion that most of this leachate does not reach directly receiving waters, but is infiltrated into soil or reaches the sewers after some delays in drainage pipes.

Release of biocides from façade leaching in the environment is systematic during rain event. It is of greater importance to estimate the dynamic of biocides during rain events and to compare these values with dedicated time varying environmental quality criteria.

PE01-6

Treatment of volatile substances in the Activated Sludge Respiration Inhibition Test (OECD 209)

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Background and goals

The OECD 209 test is used to assess toxic effects of chemicals towards microorganisms in sewage treatment plants. In the revised guideline from 2010 volatile substances are for the first time explicitly addressed: i) the volatility of the substance should be known, ii) >80% of the substance has to remain in the reaction mixture, iii) a modified experimental set-up is needed for volatile substances but not sufficiently defined.

The goals of this work were i) to predict if substance volatility is an issue (i. e. the >80% target is missed), ii) to suggest experimental modifications for these cases, iii) to deduce an approach to recalculate ECX-values from old studies with volatile substances in which evaporation losses had been disregarded.

Approach

The Henry's law constant H of a substance was used as measure of its volatility.

Evaporation losses L of substances with different H-values were measured in a sludge-free set-up according to OECD 209. L-values after 30 and 180 min of exposure were plotted as a function of H, curve-fitting was performed and critical values Hcrit resulting in losses L of 20% were calculated.

The scientific literature was screened for possible modifications of OECD 209 for volatile substances resulting in reduced evaporation losses.

The L=f(H)-functions determined and mathematical description of air-stripping as first order-process were used to deduce an approach to recalculate ECX-values from old studies with volatile substances.

Results and discussion

i) L=f(H)-plots obtained showed the characteristics of saturation curves. Nonlinear fitting was performed with Monod-type equations.

ii) Critical values Hcrit of 0.6 and 4.8 (Pa m3)/mol were determined for 180 and 30 min of exposure under the test conditions. If H of a substance exceeds these values modifications of the set-up are required.

iii) The following modifications are options to reduce evaporation losses:

- Reduction of exposure time to 30 min or further
- Reduction of aeration rate
- Reduction of stirrer speed
- Reduction of incubation temperature

iv) The following steps for mathematical ECX-correction are suggested:

- Determination of H for test substance
- Calculation of L with L=f(H)-function
- Calculation of substance concentrations at the end of exposure c(t) from initial concentrations c(0)
- Calculation of the linear means clin from c(0) and c(t) using first order-kinetics
- Recalculation of ECX-values using the clin-values as corrected substance concentrations.

PE02 - PBPK modelling in ecological risk assessment

PE02-1

PBPK models in risk assessment - principles and application

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Regulatory agencies within Pharma have embraced advances in PBPK modelling and incorporated them into regulatory requirements, approval pathways and regulatory decisions. Within the Ag Chem industry risk assessments are predominantly conducted on the basis of dose-response assessment and the application of generic uncertainty factors. The use of chemical-specific adjustment factors through the use of PBPK modelling would enable the risk assessor to consider and incorporate data on the mechanism of action together with toxicokinetics and toxicodynamics to enable more realistic human and mammalian risk assessments to be carried out. PBPK models are part of a continuum of increasingly data-informed approaches ranging from the default based on external dose to more biologically mechanism based dose response models. The physiologically based pharmacokinetic (PBPK) model is a compartmental model, but differs from classical pharmacokinetic models in that the compartments represent actual tissue and organ spaces and their volumes are the physical volumes of those organs and tissues. This talk will use examples to explain what studies are used to generate toxicokinetic data on which PBPK models can be based and how they can be validated through simulation and comparison with in vivo data and utilised to scale across species to indicate actual safety margins ready for the application of appropriate chemical specific safety factors.

PE02-2

Developments in blood sampling and chemical analysis for PBTK modelling

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Analysis of biological samples has undergone an evolution in the last 20 years leading to huge advances in the type and sensitivity of measurements that are now possible.

Developments in sampling, extraction, separation and detection techniques have moved the analytical aspects of studies from the peripheral to the mainstream. Developments such as DBS, UPLC, nano-LC and LC-MS/MS have combined to allow detection levels only dreamed about just a few years ago.

PE02-3

Generic approach for developing PBTK models for different species

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Physiologically based toxicokinetik (PBTK) modeling is more and more recognised as an appropriate tool for refinements of risk assessments which enables extrapolation to different exposure routes and patterns as well as extrapolation between different species. However, it is still a bit stigmatised as being too demanding in effort and data requiremtns and also too complex to be used in a regulatory environment. Efforts for developing and applying PBTK models can significantly be reduced by using a generic model structure and employing quantitative property-property relationships for estimating a large fraction of the chemical specific model parameters.

Methods for estimating steady state tissue:plasma partition coefficients and permeabilites of organic chemicals from their physicochemical poperties are discussed. For the such calculated partition coefficients a comparison to experimentally determined values shows a good agreement. Parameterising a generic PBTK model with the estimated parameter values together with in-vitro determined clearance values in many cases leads to results that compare well with in-vivo toxicokinetic data. Remaining discrepancies can often be resolved by limited refinements or inclusion of additional processes not considered in the generic structure, as e.g. active membane transport processes.

The above mentioned workflow demonstrated to work well and efficiently for developing PBTK models for several pesticides and examples of this will be shown. The such derived models are largely based on prior information independent of results from a particular toxicokinetic experiment. Thus they can be seen as predictive models which are only verified with and not fitted to oberved data and are thus well suited for being used in extrapolations to un-investigated scenarios by respective knowledge guided re-parameterisation.

PE02-4

Application of physiologically based toxicokinetic (PBTK) modelling in bird and wild mammal risk assessment

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The current risk assessment paradigm for birds and mammals estimates risk as the ratio between toxicity and exposure using as inputs effects from an instantaneous gavage dose (acute) or the dietary toxicity over several days or weeks (chronic) with a single day or multiple repeats of a daily exposure, respectively. The acute risk assessment does not take account of toxicokinetic (TK) processes such as absorption and elimination or any associated behavioural effects limiting further exposure (avoidance). One option to refine risk is to explore the rates of absorption and elimination and behavioural responses through PBTK modelling, optionally in combination with dynamic process in fate and dissipation in the environment. The use of PBTK modelling is recommended as a refinement option by the European Food Safety Authority in their Birds and Mammals Guidance document (EFSA Journal 2009). A condition of PBTK modelling is that toxicity is the result of systemic exposure and is reversible as the systemic dose declines. The toxicokinetics may be evaluated by blood sampling and analysis following oral gavage dosing. Analytical approaches like liquid chromatography and mass spectroscopy (LCMS) allows very small blood volumes to be used with satisfactory limits of determination. Model simulations show that the systemic dose is sensitive to feeding and absorption rates, the avoidance threshold and elimination rate. The combination of feeding and absorption rates can lead to very different and counter intuitive outcomes for risk assessments. For acutely toxic seed treatments, high feeding and slow absorption rates present high risk, while high absorption rates do not and allow animals to regulate their feeding rate to below a harmful systemic dose by avoidance. Furthermore, applications of PBTK modelling may compare the contribution to the systemic dose made by oral, dermal and inhalation exposure and evaluate the risks from food chain bioaccumulation. The acceptability of PBTK models in regulatory risk assessment will depend on good validation of the PBTK models. This may be achieved by analysis of blood concentration in dietary studies. Examples of different scenarios for all these applications will be explored in this presentation together with the conditions and assumptions for PBTK modelling.

PE02-5

Bridging insight from toxicokinetics and foraging ecology: case studies of acute risk assessment for insectivorous birds

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The recently enacted EFSA guidance for risk assessment of birds and mammals (EFSA Journal 2009; 7(12):1438) explicitly mentions consideration of toxicokinetics for conducting higher tier risk assessments of acute exposure. In the case of risks from acute exposure to compounds that have high rates of elimination (ke); dietary intake (feeding rate per minute, FPM of contaminated food items) may be too slow to approach the internal exposures that result from single,

bolus administration by gavage of the pesticide as performed during testing. In 2005 the EFSA PPR laid out a basic framework for considering ADME processes during higher tier evaluations (EFSA Journal 2005; 240:1-21). As laid out, the approach rests on estimating two main inputs: FPM and ke. The opinion derived an estimate of FPM from a very limited number of studies and proposed a rather cumbersome approach for estimating ke. I have collected information from field and experimental studies to validate assumptions about FPM that may be used in these assessments. Data from small passerines demonstrate that birds precisely control the rate and timing of food consumption during the day to balance the need to secure adequate reserves for surviving during the night, and avoiding excess body mass during the day to facilitate take off and reduce predation risk. Field and laboratory studies have consistently reported that birds feed preferentially in the early morning and before dusk, with over 80% of mass gain occurring before noon. In addition, insectivorous birds experimentally forced to increase food intake through exposure to low temperatures and forced flying, showed an increase in meal size, but did not reduce time between feeding bouts (11-12 min). This demonstrates that birds are constrained by a digestive bottleneck, where feeding bouts are dictated by a fixed stomach and gizzard capacity. The increased food ingestion that occurs during periods of increased demand happens as a result of feeding over a larger proportion of available light hours. I also demonstrate, through nonlinear equation modeling, that ke should preferentially be derived from hen metabolism studies rather than as proposed, from the long-term NOEL and acute oral LOEL in the same species. The approach will be demonstrated with case studies using different birds and pesticides.

PE02-6

Measurement of pesticide intake rates and avoidance thresholds for small mammals

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Behaviour e.g. food avoidance, can influence risk by preventing body burdens from exceeding harmful thresholds and is a measurable response that may be included in PBPK models. Standard avoidance tests only measure the response of wildlife to treated food items over a fixed period, e.g. how much does an animal consume over a 4 hr period compared with untreated food. Far more information can be generated from studies if the response of the animal to treated food is monitored continuously over the exposure period. Such data can be used to measure not just the intake rate but also the avoidance threshold and recovery period. The objective of this work was to develop a laboratory based method which could be used with small mammals to determine the highest ingestion rate of different pesticide active ingredients and formulations in food that may be tolerated and to determine if avoidance influenced the response. Two species of small mammal were used, the laboratory rat (Rattus norvegicus) and the woodmouse (Apodemus sylvaticus). The laboratory rat was chosen as LD50 data are routinely available and the wood mouse was chosen as a focal species of the small mammal likely to feed on treated seeds. Specific measures included an avoidance response time (feeding time before animals stop feeding) and the avoidance threshold (AVT) expressed as both the dose ingested and dose rate to the point at which feeding stopped. Dietary concentrations were prepared as a block made from ground diet and a binding agent into which the pesticide was incorporated. As data were required on the uptake of the treated diet over time, a remote method of monitoring uptake was used by videoing the output from a balance on which the diet was placed on the first day of exposure to treated food. Food consumption was measured to estimate the ingested dose and the avoidance threshold (AVT). The time from when individuals started to feed to when they first avoided food (avoidance response time) was determined with reference to controls.

RA01 - (Non-)Extractability and bioavailability of organic chemicals in relation to analytical and regulatory issues

RA01-1

Unified model for sorption, sequestration and degradation in soils and sediments

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The objective of this study is to combine ad/desorption models for organic compounds with the growth and degradation kinetics of microbes in a mathematical simulation model. The goal is to interpret and predict observed effects, such as increasing persistence with time, decreasing degradation rates with concentration, and effects of amendments on sorption and degradation. A second objective is the mathematical definition of the terms "persistence", "bioavailability"and "bioaccessibility".

A numerical model was set up that combines ad/desorption, microbial metabolism and the formation of non-extractable residues (NER). It contains the compartments non-aqueous phase liquids or solids (N), dissolved compound (D), adsorbed (A) and sequestered (S) compound, bacterial mass (X) and biotic as well as abiotic NER. The exchange between these compartments is expressed by rates. Bacterial growth follows Monod kinetics minus decay (maintenance) rate, degradation is due to bacterial maintenance or growth. The evolving non-linear differential equations are solved numerically. The model is formulated in activity notation and implemented in Matlab. Comparison to the analytical Best equation gave (for suitable scenarios) full agreement, which is a verification of the model structure, mathematics and implementation of the numerical model. Validation by comparison to experimental studies is underway (see Rein et al., this session).

The unified model allows the simulation of sorption, sequestration, bacterial growth and degradation processes simultaneously and coupled together. By this, we hope to get a better understanding of aging and persistence in soil and of the formation of bound residues (better: non-extractable residues), but the goal is also the optimization of amendments, such as DOC, compost or charcoal.

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RA01-2

Microbial contribution to the bound residue formation in soils

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During degradation of organic pollutants in soil, a significant amount of the carbon remaining in soil is transformed to so-called bound or non-extractable residues. These residues can only be detected by use of isotope labeled compounds and are considered to consist of the parent compound or metabolites sorbed to the soil matrix. However, biomolecules such as fatty acids and amino acids were also found in the residue fraction indicating that C from the pollutant has been assimilated by microbial biomass. In order to estimate the extent of biogenic residue formation after incorporation of the carbon into microbial biomass, we investigated the fate of microbial biomass in soil using 13C-labeled bacteria. Even after one year of incubation, the remaining C was about equally distributed between microbial biomass different from E. coli and non-living soil organic matter. Hence, the latter fraction contributes to the 'bound residue' formation from pollutants in soil. The significant contribution of biogenic residue formation was confirmed for the microbial degradation of TNT, PAH´s, some pesticides and pharmaceuticals.

RA01-3

Covalent binding of sulfonamide antimicrobials to organic matter and soil: the role of oxidative enzymes and metal oxides

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Rapid formation of nonextractable resiudes (NERs) of sulfonamides in soils indicates that covalent bonding is initially the dominant process of sulfonamide dissipation. As electrophilic quinone groups of organic matter, which are sufficiently reactive to undergo nucleophilic attack by sulfonamides are probably not stable for longer periods, we hypothesize that a continuous formation of these quinones by oxidative enzymes or Mn/Fe oxides is a prerequisite for NER formation. Understanding the nature and stability of the formed bonds is essential for an appropriate environmental risk assessment for these veterinary antimicrobials. Therefore, we studied the NER formation mechanisms of the radio-labelled sulfonamide sulfamethazine (SMZ) and the stronger nucleophile para-ethoxyaniline (EXA) with natural Leonardite humic acid (LHA) in solution and agricultural soil samples after selective removal or addition of oxidants. Additionally, we tested the stability of the obtained NERs against desorption and pressurized liquid extraction. Studies using dissolved LHA show that 35% of the sorbed SMZ could be desorbed in single solute SMZ system and 45% in the competition experiment with EXA. Strong covalent bonding of SMZ occurs in the presence of laccase in both single solute and competition experiment. In the control soil samples, 60% of SMZ formed NER in the presence of oxygen and only 30% under anaerobic conditions. Slower kinetics were shown for the reaction of SMZ when EXA was added few days prior to SMZ application. Addition of Mn oxide and model hydroquinones significantly decreased the extractability of SMZ and thus increased NER formation to > 85%. The study clearly shows that the action of oxidative enzymes or oxidants is involved in the formation of NER of sulfonamides in soils. But also quinone groups readily adding sulfonamides upon their entry in the soil are to certain extent present in soil organic matter which explains the NER fromation also under anaerobic conditions. After this initial phase the subsequent, slow NER formation phase might be either controlled by (i) the desorption of sulfonamides from the solid phase, which subsequently react with quinones continuously or (ii) a slow conversion of "unstable"covalent bonds into "stable"ones more resistant during the extraction procedure.

RA01-4

Instantaneously decreased extractability of sulfadiazine in soil batch experiments

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Effects of antibiotics in soil on microbial end points, leaching potential and biodegradation depend on their (bio)availability, which is generally approximated by extraction methods with mild solvents. The sulfonamide antibiotic sulfadiazine (SDZ) shows typical 'aging' in soil, i.e. decreasing extractability over time. A sequential extraction method proved to be appropriate to investigate sequestration of SDZ in batch experiments (Förster et al., 2009, ES&T). Concentration dynamics over 200 days in these extracts could be described well by a conceptual kinetic model which included a reversible sequestration process as well as an irreversible sequestration resulting in 'non-extractable residues' (Zarfl et al., 2009, Chemosphere). The experimental results along with the model simulations indicated reduced extractability of SDZ already shortly after the start of incubation. We assumed this finding to be attributed to very fast processes, being possibly of relevance for risk assessment. Therefore, we performed a set of soil batch experiments focussing on the first 24 hours of incubation and tested two hypotheses: (i) manure leads to formation of micro-aggregates or provides additional sorption places, (ii) water content of the soil before spiking SDZ and/or manure influences the observed effect. SDZ was added with water or manure to soils of different water content (dry vs. wet). We used the proved sequential extraction method (1: $CaCl_2$, 2: methanol, 3: acetonitrile/water with microwave); analysis of SDZ was performed by LC-MS/MS.

Immediately after spiking SDZ solution to soil, extractability was reduced in all setups confirming processes on a time scale of minutes. No considerable differences in the initial SDZ distribution between the different experimental treatments were observed, so none of our two hypotheses could be confirmed. However, during the following 24 hours of incubation, sequestration proceeds further, reducing extractability of SDZ with $CaCl_2$ and methanol (EAS fraction), while simultaneously increasing extractability with the high-temperature method (RES fraction). Rate constants for a sequestration process from EAS into RES appeared fairly constant in all experiments. In contrast, rate constants for an additional sink process out of EAS were clearly lower in the water compared to the manure treatments, indicating a linkage to microbial activity.

RA01-5

The influence of alternating dry-wet cycles on the water-extractability of aged 14C-pesticide residues in soils

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Due to future climate predictions, an increase in droughts, followed by heavy rain events can be estimated. Soil drying and rewetting may have a considerable impact on an increased release of pesticides present in agricultural soils, representing a potential risk by pulse inputs to deeper soil layers or aquifers after rain events. Laboratory studies using soil containing environmentally long-term aged (9-17 years) 14C-labeled residues of the herbicide ethidimuron (ETD), methabenthiazuron (MBT) and the fungicide anilazine (ANI) showed a significant increase of 14C-activity in the water-extract after soil drying. The total water-extracted 14C-activity (the amount of residual 14C-activity in the sample equals 100%) accounted for 44% (ETD), 15% (MBT), and 20% (ANI) after 20 alternating dry-wet cycles. The amount of water-extracted 14C-activity from the constantly moistened soil remained significantly lower at 16% (ETD), 5% (MBT), and 6% (ANI) after 20 cycles, respectively. LC-MS/MS analyses of the raw water extracts of the dried

and rewetted soils revealed the parent compound ETD and MBT in detectable amounts (15.0 µg ETD kg-1 and 0.23 µg MBT kg-1 in total, in 0-10 cm ETD-soil / 0-30 cm MBT-soil), accounting for 1.83% and 0.01% of total applied parent compound per soil layer, respectively), but neither ANI nor the main ANI metabolite dihydroxy-anilazine could be detected. In comparison, the constantly moistened soil released significantly smaller amounts of residual pesticide fractions (2.76 µg ETD kg-1 in total, in 0-10 cm ETD-soil), accounting for 0.34% of total applied parent compound, respectively, but no MBT or ANI residues were detected).
For all soils the water-extracted dissolved organic carbon (DOC) was significantly higher in the previously dried soils, compared to the constantly moistened soils (ETD-soil: 10.8 vs 4.8%; MBT-soil: 8.4% vs 3.7%; ANI-soil: 9.8% vs 4.6% of total organic carbon in the soil). In case of the previously dried soils, the DOC content correlated with the measured 14C-activity in the aqueous liquids (ETD-soil: r=0.80; MBT-soil: r=0.81; ANI-soil: r=0.91).
The overall finding demonstrates a readily water-extractable pesticide residue fraction which can easily be removed from the soil, representing a potential risk for leaching. The data suggest that an increase in environmentally relevant dry-wet cycles may result in an increased remobilisation and release of aged pesticide residues in soils.

RA01-6
Hidden hazard or safe sink? Approaches to consider non-extractable residues in the regulatory assessment of chemicals.
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Formation of non-extractable residues (NER) is regularly observed in studies on the fate of organic chemicals in soil. NER formation may be interpreted as a specific form of compound persistency ('hidden hazard') or as a detoxification step ('safe sink'). Despite the considerable scientific progress made in analysing NER and identifying their binding types, these insights have not yet been utilised in regulatory risk assessment.
In a workshop held at the German Federal Environment Agency (UBA), it was agreed that three main types of NER should be considered in regulatory schemes: Fixation of substance molecules by physical entrapment in the soil matrix can be reversed under certain environmental conditions. Those 'Type 1' NER must be considered as a reservoir for remobilisation of a chemical over prolonged times. In contrast, formation of strong chemical bonds between substance molecules and soil matrix will produce 'Type 2' NER, which are unlikely to be released in their original structure under environmental conditions. Finally, NER can also be formed via incorporation of single labelled atoms or small fragments from the original substance into biomass. These 'biogenic' NER are no longer structurally related to the original substance. While the formation of Type 2 and biogenic NER can be considered a 'safe sink', Type 1 NER would constitute a 'hidden hazard'.
A generic extraction scheme was suggested for residue analysis in the standard studies on the fate of organic chemicals in soil. Specific methods are required to determine the amount of biogenic NER. Extraction with non-destructive methods allows concluding on the available residue fractions. To differentiate between Type 1 and Type 2 NERs, a set of destructive extraction methods differing in strength is available, which may be complemented with sophisticated spectroscopic techniques. Where no information on their nature is available, NER should in principle be assumed to belong to Type 1.
Formation of Type 1 NER will have different implications on the environmental risk and hazard assessment. In particular, their potential for substance remobilisation will significantly impact groundwater risk assessment and persistence assessment. Existing trigger values and decision criteria for NER formation were deemed inappropriate for addressing those concerns; hence, a need for developing new criteria was identified.

RA02 - Aquatic and terrestrial mesocosm and field studies - Messages from complex systems to academia, regulators, and industry

RA02-1
Nutrient masking of macroinvertebrate community responses to ternary mixtures of insecticides
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To determine the effect and effectiveness of mixtures of three insecticides (chlorpyrifos, dimethoate and imidacloprid) in nutrient limited and enriched streams, benthos were field collected and exposed to nutrients and insecticides over a 3 week period using artificial streams in a 2 x 5 factorial design: two nutrient levels and five concentrations of the ternary insecticide mixture. The two nutrient levels were nutrient limited (oligotrophic) and nutrient amended (mesotrophic). Equivalent toxic unit doses were summed to create a ternary insecticide dose (e.g., 0.1 + 0.1 + 0.1 = 0.3 TU). The five concentrations of the ternary insecticide mixture were control groundwater, 0.3, 0.6, 0.9 and 1.2 TU. A key result was that the presence of moderate nutrients masked the effects of insecticide mixtures on a suite of macroinvertebrate community metrics, particularly at low toxic unit doses (< 0.6 TU) which are ubiquitous in the environment. Thus, nutrient status is likely an important modifying factor that is underrepresented in the toxicological literature and yet greatly contributes to the difficulty of assessing community level responses in real systems. Further study of nutrient masking in more complex pesticide mixtures is warranted.

RA02-2
Detectability of fifeteen 12,000L outdoor control aquatic mesocosms over one year
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Zooplankton abundance and species richness in 15 untreated 12,000 L outdoor micro/mesocosms (n = 15) were monitored over the course of 1 year to document the inherent variability and statistical detectability between replicates. Hence, the first aim of the paper is to document the normal operation ranges of abiotic and biotic parameters. The second aim is a calibration study of the cosms, statistical power analysis were applied to derive the statistically minimal detectable difference (MDD) between replicates with default values set at; alpha = 0.1 and beta = 0.2. Copepod abundance and species richness generally demonstrated the best detectability at 0.31 and 0.16, respectively, (n = 15); 0.59 and 0.33 (n = 3). Total zooplankton abundance and species richness had the lowest detectabilities at 0.19 and 0.14, respectively, (n = 15); 0.35 and 0.3 (n = 3). Rotifers, due to their opportunistic and rapid life traits, had the lowest single-species. abundance detectabilities at 0.54 (n = 15); 0.8 (n = 3),whereas macroinvertebrate species richness had the lowest detectability at 0.43 (n = 15); 0.7 (n = 3) over 1 year. We recommend *a priori* calibration of the study design relative to relevant MDDs. Moreover, it is suggested to consider alternatives to statistical null hypothesis testing.

RA02-3
Examples of the novel design of mesocosm studies for compounds with differing properties
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A great deal of advance thought and planning is required for many substances before initiating a mesocosm study. Otherwise there is a risk of conducting a study that is of limited regulatory use. Here we will give advice and considerations for mesocosm studies with compounds of varying properties and with different use patterns. This will be illustrated using two example state-of-the-art mesocosm studies conducted in 2009. In one example the compound has a short aquatic half life and is toxic to invertebrates; in the other example the compound has a long aquatic half-life and is toxic to algae. One study required special consideration of the exposure profile in the static mesocosm system versus the reality of flowing water, and a novel design following the principles of E-Link was used. The other study required special consideration of the analytical method to prove correct dosing. Both novel designs had different challenges, and both were technically successful. The thought and planning required for these studies will be described and discussed; and other design (and cost) considerations will be mentioned. It is hoped that this presentation will advance mesocosm study design and offer advice to those considering a mesocosm study. Due to the increasing complexity of exposure calculation and risk assessment, mesocosm studies must also increase in their design complexity in order to stay relevant to the regulatory risk assessment. It will therefore be necessary for both laboratories and regulators to be open to novel designs which do not necessarily fit with recent standards for mesocosm assessment.

RA02-4
Ecological impacts of time-variable exposure regimes of the fungicide Azoxystrobine on the zooplankton community of outdoor microcosms
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The risks of pesticides to aquatic ecosystems are often assessed by performing cosm experiments evaluating a particular exposure regime (e.g. 1 pulse Applikation), Not necessarily corresponding with the exposure part of risk assessment (e.g. multiple applications).This mismatch is one of the biggest challenges in contemporary ecological risk assessment. The aim of the present study is to compare the effects of a chronic exposure with the effects of a peak exposure and a multiple peak scenario with an identical Area Under the Curve. For this, microcosms (water volume approx. 1270 L) were established using sediment of fine clay having communities typical of macrophyte-dominated freshwater ecosystems. Azoxysrobine is a systemic and strobilurin fungicide that currently is used on a number of agronomic and horticultural crops worldwide. Azoxystrobine was introduced into outdoor- microcosms using four different regimes; (1) A single Applikation of 31 µg a.s./L, (2) four applications of 16 µg a.s./L, with a time interval of 10 days and (3 and 4) a continuous exposure of 10 and 33 µg a.s./L for 42 days. Treatment levels of 1 and 4-pulse were based on 42d-Time Weighted Average (TWA) of 15 µg a.s./L which fall between the chronic 10 & 33 µg/L treatments.The Multivariate analyses of the zooplankton data set, reveals small variation in the pre-treatment period and large concentration-dependent differences with the control after start of the treatment. Effects are first observed for the single Applikation and the chronic 33 µg/L treatment followed by the 4 Applikation and 10 µg/L treatment. Taxa belonging to Copepoda (Nauplii, Cyclopoida and Calanoida) and Cladocera (Daphnia longispina) are the most responsive zooplankton species observed. Rotifera are indicated to have increased due to azoxystrobin exposure as a result of indirect effects. By the end of the experimental period, PRC shows the same effects magnitude for the pulsed treatment regimes, which are placed in between the chronic treatment regimes. This indicates that for long-term effects the TWA could be more important for most zooplankton species than the peak concentration.

RA02-5
Nematode species at risk - a metric to assess pollution in soft sediments of freshwaters
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RA02-6
Comparison of arthropod community responses to an insecticidal active in different geographic regions
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At present the hypothesis that arthropod communities in different geographical c.q. climatological regions respond differently to exposure to plant protection products has not been tested empirically.
In total ten GLP field studies at nine different locations with different cropping systems were used to test hypotheses on effects of geographical gradient on ecotoxicological response at population and community level. All trials had a randomized block design with 4 replicate plots (n=4) per treatment (active substance OP1 and a water-treated control). At the population level direct impact and duration of effects were calculated. Community level analysis was performed using Principal Response Curves analyses (PRC).
Arable arthropod communities were least similar (23-27% species overlap), and orchard communities were most similar (54-69% species overlap). Species overlap in the different cropping systems between and within geographical regions was similar. Species abundance (evenness) differed considerably in arable studies, but less so in orchard studies.
The two grassland studies had a similar distribution of initial effect classes, but the proportion of unaffected taxa was higher in the South. The arable studies, which tested higher application rates, had effect size distributions that were more skewed to the larger effect classes. In general all sites had a qualitatively similar response pattern. For the two apple studies involving relatively low application rates in North and South France, effect-class distributions were similar, with most taxa occurring in the 60-100% effect classes. Deviant results were obtained for two Spanish orchard

studies. More than 50% of the taxa was not affected after treatment in these trials.
PRC responses of North and South studies expressed by the first ordination axis were similar in all crop types. Both magnitude and duration of responses were similar, except the magnitude of the two studies performed in Spain were lower.
It is concluded that OP1 treatment effects were similar in studies performed in N- and S-Europe. Only a slight trend was observed that more and longer lasting adverse effects were detected in studies performed in the North.
In exception to the conclusion above, adverse OP1 treatment effects were clearly lower in orchard studies performed in Spain. Analysis of more Mediterranean studies is needed to examine whether this was related to geographical location or to other factors.

RA04 - Environmental risk assessment and management of Plant Protection Products (PPPs) and biocides

RA04-1

Assessing the mixture ecotoxicity of biocidal products- the challenge at product authorisation

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Biocidal products are typically mixtures of one or more active substances as well as further ingredients. With the inclusion of the first substances in Annex I of the Biocidal Products Directive (BPD) 98/8/EC the authorisation of the corresponding biocidal products at national level is underway and European member states (MS) are facing mutual recognitions.
For the authorisation of biocidal products an extensive environmental risk assessment (ERA) is required in accordance with the BPD: not only every active substance in the product has to be subject of an environmental risk assessment, but also substances of concern, i.e., substances leading to classification of the product or having PBT, endocrine or CMR properties, have to be evaluated separately. In addition, estimation of mixture toxicity of the ingredients is required. The latter can be derived from ecotoxicological tests with the product itself, if a direct release of the product in the environment is possible. However, the product itself often does not represent the ecotoxicological relevant mixture since leaching and other paths of exposure will change the composition of the mixture. In that case, the ecologically relevant mixture, e.g. leachate water, has to be tested.
It is well accepted that mixtures of substances usually elicit a different toxicity than the isolated substances itself and additive effects up to synergistic effects are possible. Tests with the product or the ecologically relevant mixture therefore provide a meaningful method to demonstrate the degree of mixture toxicity by the joint action of the active substance(s) and further components of the product.
The German Federal Environment Agency is responsible for the environmental risk assessment of substances within the review programme of the BPD as well as for national product authorisation and proposes a tiered approach for the assessment of biocidal products based on the expected exposure pathways which also considers synergistic effects. The aim of the approach presented is to assess the mixture ecotoxicity of products and, where relevant, of ecologically relevant mixtures, and at the same time relieve the data requirements for the applicants as well as additional animal experiments.
The presentation gives an overview over the existing concepts for the assessment of mixture toxicty as well as on the proposed approach for product authorisation.

RA04-2

Challenges to determine persistence of plant protection products in the PBT and vPvB classification for the new EU regulation 1107/2009

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The new regulation for plant protection products (PPPs) 1107/2009 foresees regulation hazard based POP , PBT and vPvB cut -off criteria. The persistence (P) in the environment as a substances intrinsic cut-off criterion is utilized in conjunction with bioaccumulation (B), environmental toxicity (T) and long-range environmental transport. Considerable challenges are associated with the practical interpretation of persistence (P) as a hazard based cut-off criterion for the data rich cases of pesticides which may result in unjustified P classifications.
The vast amount of available information for pesticides in different environmental media and under different boundary conditions (laboratory and field conditions) allows a detailed and differentiated assessment of the behaviour. Detailed process based models in combination with predefined environmental scenarios (both agreed and established on EU level) and fate parameters obtained from low and higher tier studies are part of the pesticide regulation. These approaches allow to quantify with a significant level of certainty the persistence of pesticides within environmental compartments and the transfer and transport processes between them.
Approaches to determine pesticide fate parameters in different compartments are outlined and the exposure assessment for environment are presented with link to the P-classification.
Different proposals from academia, regulators and industry to address the P classification are compared with the results of the exposure assessments for pesticides in the EU.
Examples are provided that a hazard based assessment can lead to false identification of a potential concern whereas appropriate scientific based risk assessment shows an acceptable risk.

RA04-3

Regulation 1107/2009/EC and upcoming challenges for exposure assessment of PPP - Harmonisation or national modelling approaches?

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In the new European Pesticide Regulation 1107/2009/EC which will replace the existing EU Directive 91/414/EC in 2011, the harmonisation of risk-minimizing measures for the protection of aquatic organisms in the Member States is considered as important task. However, in many EU countries different models are currently used for the calculation of predicted environmental concentrations (PEC) in surface water and sediment and also the considered risk mitigation measures, such as spray drift reducing nozzles or buffer zones, differ between countries due to different geographical, climatic or agricultural conditions. This makes risk evaluations rather time-consuming for both the notifiers and the evaluating authorities, in particular concerning mutual recognitions of approvals. In the present study we compare EU and current country specific exposure modelling approaches and mitigation measures in order to evaluate if and to which extent harmonisation of exposure assessments in the EU is feasible.
The environmental exposure in surface water of 19 active substances was evaluated systematically according to EU and country specific modelling approaches. Calculations were conducted following EU requirements (FOCUS models) and country specific models for the UK, Germany and the Netherlands.
In the present analysis we show that EU and country specific models differ considerably in basic model assumptions, complexity and relevant parameters selected. Additionally, an important characteristic of the country-specific models is the prioritisation of specific entry pathways of pesticides (spray drift, drainage and runoff) over substance specific properties. The acceptance of available risk mitigation methods differs significantly in each of the investigated Member States.
The comparison with the EU modelling procedure shows that the national models do not necessarily comprise worst-case calculations. This fact particularly became apparant by comparing the PEC calculated following German modelling recommendations with the outcome of FOCUS calculations.
The results of this study highlight the differences of national exposure assessments for the protection of aquatic organisms. In spite of the possibilities of risk reduction, it is doubtful if any of these measures can be harmonised e.g. set as a standard all over Europe or at least in the countries of one zone according to the zonal approach of the new regulation 1107/2009/EC.

RA04-4

Improving definition and selection of focal species used in risk assessment

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Risk assessment investigates the potential impact induced by active ingredients on non-target organisms. In the higher tier of this assessment, focal species are chosen among a pool of real species likely to be exposed to treatment. According to Guidance Document, risk assessment must focus on occurrence, dominance, ecological and biological parameters of focal species, but it doesn't mention how to take into account each parameter in a single definition of focal species. Species occurrence within a given habitat is often used as the main criteria. However, it doesn't reflect the specificity of a species to a targeted habitat because very frequent species may also occur in many other types of habitat. In this presentation, we combined occurrence of species within a given crop (i.e. fidelity) with species specificity to identify focal species using the Indicator Value (IndVal) method and data from the French Breeding Bird Survey. We calculate the specificity and the fidelity of 94 common bird species, counted on 3 000 sampling points located in arable lands covering all French regions. The IndVal of a species increases with both its occurrence and its relative abundance in crops. Thus, top ranked species with a high indicator value are both frequent and abundant in selected fields compared to other types of habitats. Because the more frequent species are often generalist, frequent in a wide range of habitats, IndVal method enables to remove down generalist species and to take up specialist species into the ranking. Then, knowledge on their biology and ecology (diet, feeding layer...) contribute to select those that are more representative for birds exposure. Although the concept of specificity has a strong ecological meaning, it is largely overlooked in eco-toxicological risk assessment. Because specialists species occupy narrow ecological niche, they are good indicators of various environmental pressures and seem more vulnerable to environmental changes than generalists. Integrating specificity in the definition of focal species is a new emphasis in the area of ecological risk assessment and is likely to integrate species characteristics like geographical distribution, biological and ecological traits that make it dependent to this habitat. We propose to unify focal species definition by using the IndVal method. It could be extensively used at several scales and could contribute to the current issue on risk assessment using trait-based approach.

RA04-5

Defining soil ecological exposure scenarios for pesticide risk assessment in the EU: an ecoregion approach

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The current terrestrial risk assessment of pesticides is performed assuming a common exposure scenario for the entire EU based on the total concentration in the top 5 cm of soil. Aiming at modelling ecotoxicologically relevant concentrations in soil (ERCsoil), an alternative to this single exposure scenario is needed. Ecologically relevant scenarios should take into account the biogeographical differences within the EU in terms of soil, climate and the vertical distribution of soil organism communities. Here we present the progress achieved by EFSA on the development of an EU-27 Ecoregion map and associated exposure scenarios.
To test this approach, a compilation of biogeographical information on selected key soil fauna groups representing different morphological and ecological characteristics influencing exposure was performed. Data on presence of earthworms, enchytraeids, collembolans and isopods were collected from the literature for three model countries covering a maximum biogeographical gradient in Europe: Finland, Germany and Portugal.
Results obtained proved that the concept works. Ecoregion maps were produced for earthworms and enchytraeids and revealed marked differences between countries. For isopods, models revealed a dominance of litter dwellers in all countries, showing no differentiation. For collembolans, the lower resolution scale of some explanatory variables, allied to a bias in data towards forest sites, did not allow a discrimination between countries.
Based on the distribution maps for earthworms and enchytraeids, depth profile maps where to model ERC could be constructed. The results pointed out that for most of the situations in these three countries, the "worst case"soil depth profile for short-term risk assessment would be litter (if present) or 0 to 1 cm depth instead of the currently used 0 to 5 cm depth. This occurs since a large area in these countries is dominated either by epigeic species or by species that feed on litter (e.g., anecic earthworms). For other life-forms or for long term exposure, other depth profiles may represent the realistic worst case situation. For refined risk assessments, the geographical variation in depth profiles, crop and soil management information, as well as data about the ecology of soil organisms (e.g. different dominance distribution of soil communities) could be considered. Provided that information from other countries is available, the approach could be extended to the entire EU territory.

RA04-6

When the active substance/PPP does not fit the legislation

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Directive 91/414/EEC, Regulation 1107/2009 and Directive 2009/128/EC are all related to the regulation of pesticides. Each of them intends to cover all different types of pesticides. CLP Regulation 1272/2008 concerns nearly all chemical substances and mixtures. It is by no means easy to make this type of generalised legislation, and it is by no means easy to cope with them if your active substance or plant protection product (PPP) does not fit the legislation. Authorities have to find ways to fit the legislation. Unfortunately this is not always done properly, resulting in non scientifically justified decisions, confusion and unnecessary effort to find solutions to problems. Equivalence assessment of technical materials is done according to Sanco/10597/2003. This guideline is not suitable for Straight Chain Lepidopteran Pheromones (SCLPs). Today (Nov. 2010) nearly two years after the SCLPs were included in Annex I there is still only a draft version of a proposed guideline. The industry is therefore left in the dark when estimating future market activities and requirements. Pheromones are released in a very slow and controlled way by VP dispenser formulations, unfortunately more Member States apply R50/53 (R51/53) environmental classifications to these formulations applying the calculation method according to Directive 1999/45/EC. The only possible risk to the aquatic environment from dispenser products would be a systematic opening of the packaging, cutting hundreds of dispensers into pieces and then extract the content from the pieces and put it into the water, which is a totally unlikely happening. Applying the calculation method is therefore not only scientifically unjustified but is also contrary to the whole concept of classification and the real intentions behind the legislation. Here the guidance for testing toxicity to aquatic organisms does not fit dispenser products. Concerning National Action Plans in the framework of the sustainable use directive, the R50 classification has been mentioned as a possible parameter for decision making. An unjustified classification under one regulation would here penalise a PPP under another directive. Conclusion: it is needed that regulators and authorities adjust in advance (specific guidelines) legislation to cases for which the legislation does not fit, and if problems come up due to non-fit situations scientifically justified and not bureaucratically based decisions should prevail.

RA05 - Integrated science: Key to risk assessment

RA05-1

Assessing the environmental risks associated with contaminated sites: 'ERICA: Environmental Risk Index for a Chemical Assessment'

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A risk assessment strategy considering the impact of chemicals on the whole ecosystem has been developed in order to create a sound and useful method for quantifying and comparing global risks posed by the main different hazardous chemicals found in the environment. This index, called the Environmental Risk Index for a Chemical Assessment (ERICA), merges in a single number the environmental assessment, the human health risk assessment and the uncertainty caused by missing or unreliable data. ERICA uses a scoring system with parameters for the main characteristics of the pollutants. The main advantage is that it preserves a simple approach by condensing in this single value an analysis of the risk for the area under observation.
The availability and reliability of the data is an important part of the work done to build the index. Experimental and predictive data were compared to evaluate the reliability. Data was derived both from literature sources (experimental models mainly) or predictive models. ERICA can be considered a diagnostic and prognostic tool for environmental contaminants in critical and potentially dangerous sites, such as incinerators, landfills and industrial areas or in broader geographical areas. The application of the proposed integrated index provides a preliminary quantitative analysis of possible environmental alerts due to the presence of one or more pollutants in the investigated site.

RA05-2

Integrated risk indices to assess water quality

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The European Water Framework Directive (WFD) 2000/60/CE prescribes a series of tasks for properly assessing and managing river basins with the ultimate aim of achieving a good ecological and chemical status of surface waters by the end of 2015. The Ecological Status (ES) of each water body has to be evaluated and classified by using biological Quality Elements (QE) as key parameters and physico-chemical, chemical as well as hydromorphological QE as supportive parameters. Moreover, the Chemical Status (CS) has to be evaluated by comparison of measured concentrations of priority substances in water, sediment and biota compartments with Environmental Quality Standards (EQS) set at EU-wide level.
The overall assessment asks for an approach able to integrate different types and sources of information in order to identify the stressors playing a major role in affecting the ecosystem and to guide future management actions.
In this context, one of the main objectives of the MODELKEY project was the development of an Integrated Risk Assessment (IRA) methodology implemented into a Decision Support System (DSS) guiding decision makers in assessing and managing river basins according to the WFD requirements (i.e. MODELKEY DSS). In particular, Integrated Risk Indices (IRI) based on Multi Criteria Decision Analysis (MCDA) methods and Weight of Evidence approaches (WoE) were developed and calculated in order to evaluate and classify the ES and CS at site-specific scale (i.e. sampling stations). The developed IRI implemented into a DSS module (i.e. IRI module) were applied to several river basins in Europe.
In this paper, after introducing the IRI module we will focus on main results obtained by its application to Elbe and Danube River Basins, in order to highlight difficulties which are usually encountered in assessing water quality and to suggest how to overcome them by means of integrated risk-based approaches and decision support tools.

RA05-3

Standard and non-standard tests for environmental risk assessment of human pharmaceuticals

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The presence of pharmaceuticals in the aquatic environment has gained increasing attention over the last years, and concerns have been raised over possible negative impact on aquatic organisms since pharmaceuticals are designed to interact specifically with biological processes already at low concentrations. Indeed, the synthetic estrogen ethinylestradiol causes skewed sex ratios and impaired reproduction in fish and frogs at environmentally relevant concentrations. Since the regulatory requirements for pharmaceutical efficacy and safety are relatively far-reaching, their pharmacological effect, mode-of-action, and potential side effects in humans are generally well described. However, currently available standard test methods for deriving regulatory ecotoxicity data are in many cases not sensitive enough to measure the specific (pharmacological) effects that are expected.
We have analysed the relative sensitivity of standard and non-standard ecotoxicity tests to identify pharmaceuticals of concern to non-target species in the aquatic environment. Our results show that test results exhibit considerable variation between standard and non-standard tests performed for one and the same substance. In many cases, non-standard tests employ more specific end-points, yielding increased sensitivity. An illustrative example is ethinylestradiol where a comparison of available data shows that non-standard endpoints are 100 to 200 000 more sensitive than standard endpoints. In other cases, standard tests were more sensitive. However, for most pharmaceutical substances very few ecotoxicity studies exist and a comparison could not be made.
We conclude that the lack of relevant, robust, and sufficiently sensitive test methods is a major deficiency in regulatory environmental risk assessment of pharmaceuticals. Appropriate test methods are fundamental for risk identification and thus a crucial part of the risk assessment process. As we see it, there are at least three potential ways forward to improve the situation: (1) To develop new standard tests, (2) To adjust existing standard tests i.e. supplementing them with additional endpoints, or (3) To increase the use of non-standard tests.

RA05-4

Application of a weight of evidence Triad approach in assessing impacts of diffuse contamination at regional scale

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Triad-based ecological risk assessment aims to estimate environmental impacts due to contamination, encompassing chemical, ecotoxicological and ecological data. In fact, measuring biological effects allows assessing impairments on biota at different levels of biological organization (i.e. molecule/cell, tissue/organism, population/community) and estimating environmental risk and biological vulnerability.
Goal of this study is to evaluate environmental risk and biological vulnerability at regional scale in soils from Campania, Italy, that, as known, are impacted by deposition of organics. Levels of metals and metalloids, PAHs, PCDD/F and PCBs have been determined in 48 superficial soil samples; a 3-tiered ecotoxicological and ecological investigation has been realized. Triad data have been integrated by an Expert Decision Support System (EDSS) into 3 indexes: Environmental Risk Index, EnvRI, Biological Vulnerability Index, BVI, Genotoxicity Index, GTI.
In some samples, chemical results have shown concentrations of contaminats above the Italian maximum permissible concentrations for residential use. Ecotoxicological endpoints at high level of biological organization (i.e. survival and reproduction) haven't shown dramatic alterations. High variations of both functional and structural community parameters among sites have been evident. Changes in sublethal biomarkers have underlined the presence of bioavailable contaminants able to induce a sublethal stress syndrome.
Data have been compared to control (CTR) (i.e. lab control and pristine ecological values), and reference conditions (REF) (i.e. unpolluted sites) with the EDSS. The obtained risk values show variations of EnvRI ranging from 0.11 to 0.58 and from 0.06 to 0.51 respectively. BVI levels indicate a sublethal stress in model organisms, with values ranging from 0.26 to 0.51. GTI values allow individuating cases where significant effects on DNA are evident, with values ranging from 0.00 to 0.65.
Risk levels in the Campania region don't evidentiate critical situations (EnvRI below 0.75 in all sites). The contamination seems related to a general environmental degradation rather than to significant inputs of PCDD/F and PBCs from atmospheric deposition. However, BVI and GTI indicate sublethal stress conditions in the majority of sampling sites, suggesting that biological systems are suffering and that a decrease of pollutants input is necessary to reduce the risk of biodiversity loss in the study area.

RA05-5

Is ecotoxicogenomics useful in fulfilling the goals of environmental risk assessment?

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The development of experimental approaches which offer additional mechanistically relevant information is widely considered to be beneficial in environmental risk assessment. Toxicogenomics technologies are regarded as excellent tools for the investigation of the biological mode of action (MOA) of a chemical. However, the data gathered through transcriptomics analyses is not readily applicable in a regulatory framework. The hypothesis that similar chemical treatments are reflected by comparable gene transcription profiles opens up perspectives for the development of strategies to group chemicals according to their MOA; transcript fingerprinting). Gene transcription profiling can additionally assist in the unravelling of a chemical's toxic MOA.
In this particular study, the potential of transcriptomics is investigated in a cross-species setting using a group of structural analogues (chlorinated anilines; aniline, 4-chloroaniline, 3,5-dichloroaniline, 2,3,4-trichloroaniline). Although the toxicity of these group of polar narcotics is assumed to be similar, our previous study illustrated large interchemical and interspecies differences in toxicity. Knowledge on the mechanistic basis of toxicity is essential to understand why the different test species differ in their responses to the four structural analogues. These settings offer the perfect basis to investigate the potential added value of an across-species transcriptomics approach. Therefore, we aimed at evaluating whether the different toxic MOAs can be distinguished based on differential gene transcription data, and how transcriptomics data can give additional information that is essential to explain the interchemical and interspecies differences observed in the acute toxicity study. In a second part, the specific difficulties we encountered and the potential benefits of the application of transcriptomics in an environmental risk assessment context will be discussed in a broader perspecitive.
The results of this study illustrated that for simple structural analogues (chlorinated anilines) MOA data can not be extrapolated among different species (daphnia versus fish). It is obvious that additional biological information provided via transcriptomics studies is beneficial in a risk assessment context. It is however still too early to suggest one standardized procedure to be implemented in a risk assessment context e.g. under REACH.

RA05-6

A review of methods for analysing standard data: is there a place for non-standard methods?

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Even if new types of bioassays and thus of data arrive on the ecotoxicology market, standard data and associated statistical analyses are still worth interesting, because they are still widely used within laboratory routine and regulatory context. In particular, several classical toxicity thresholds are established from standard procedures, carried out according to the OECD either to collect experimental data or to implement appropriate analyses. The No Observed Effect Concentration (NOEC) and the 50% Effective Concentration (EC50) are listed among the preferred in terms of environmental policy and risk assessment. Some years ago, an alternative, still non-standard, emerged: the No Effect Concentration (NEC).

Nevertheless, statistical analyses, classically recommended to deal with standard data [5], are far to be clear and easy to use, and call for careful thought. We propose to review standard statistical analyses that are classically performed on standard survival data to estimate NOEC and LCx, adding a non-standard NEC estimate method, and discussing pros and cons of these different methods. For each threshold type, several methods are compared. In case of NOEC, three hypothesis test methods are compared: the Jonckheere-Terpstra's test, the Cochran-Armitage's test if a monotone dose-response is expected, and else the Fisher's exact test with the Bonferroni-Holm correction. In case of LCx, three dose-response models are fitted in parallel: the log-logistic, the log-probit and the Weibull models. In case of NEC, we chose one threshold model with a three-phase linear stress function.

We illustrate our words with nine standard survival data sets from literature, all concerning the freshwater invertebrate Daphnia magna exposed to copper, zinc, cadmium, potassium dichromate, chlordane, ivermectine, oxolinic acid, streptomycin or sulfadiazine. For each threshold type, pros and cons are discussed, and the most appropriate method is emphasized. Such a review finally aims to warn regular users against a misuse of p-values, against the consequences of the prior choice of an arbitrary value (the x in LCx), or against the consequences of the choice of a particular model. Hence, we suggest to use [0 - LOEC] confidence intervals, possibly to use LCx values with a wittingly chosen x, or even better to use NEC-type toxicity thresholds, which combine all advantages and statistical properties to become reliable and relevant in the framework of environmental risk assessment.

RA07 - Monitoring data and post-registration studies for PPPs: generation, compilation and use in the environmental risk assessment and management

RA07-1

Birds & mammals: balanced decision-making is a social responsibility. Field effect studies & incident reporting provide the link to the real world. Example of OP insecticide chlorpyrifos.

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Over-precaution has a cost to society. In recent years, some active substances in EU have been heavily restricted due to lower-tier risk assessments for birds & mammals. Plant Protection Products (PPP's) are tools to enable efficient production of food, to feed a growing population. In this context, it is our responsibility to make balanced scientifically-robust decisions based on 'real risks' to decide if specific PPP's should be available to EU farmers. Chlorpyrifos is an OP insecticide, applied as a spray in many EU countries on a wide range of crops. 'Tier 1' assessments indicate potential high risk. A large program of field studies has been run: brassicas (Poland), pome fruit (Italy & Czech Rep.), grapevines (France) and citrus (Spain). These include acute and long-term studies on both birds & mammals. The results will be presented. There are two publications ([1] [2]). Overall, no effects of chlorpyrifos were seen. There are no reported poisoning incidents relating to registered spray uses (annual use around 2.7 million ha). The evidence indicates a low risk, enabling regulators to judge actual risk for this important insecticide. Overall it can be concluded that a holistic view of data from the lab and field is needed before judging unacceptability or acceptability of the risk. A holistic view of regulatory decisions is also needed as alternative pest control options (if there are any) may present a greater risk.

RA07-2

A post-registration study in France investigating effects of an insecticide-seed treatment on the honeybees

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The placing of Plant Protection Products (PPP) on the market requires, as mandatory and in accordance with Directive 91/414/EC and the updated related Regulation to come, an assessment of the risks posed by their uses in crop protection to the environment. This risk assessment usually rely worst case risk scenarios, in estimating exposure conditions and exposure levels that are safe to the group of organisms or ecosystem considered. The most refined risk assessments, even relying on up-to-date exposure measurements, ecotoxicological studies and field studies may not address all the uncertainties associated to the occurrence of exposure and effects in the field. In the case of honeybees and other pollinating insects, these uncertainties are related to the conditions for exposure that are the rule in the field (multi-exposure to PPP but also to other background chemicals and veterinary products), indirect effects of crop management techniques (management of field boundaries) and extrapolation of a risk assessment based on the honeybee to other pollinating insects. A post-registration survey has been performed in France, which investigates the effects of the exposure to maize fields grown from insecticide-coated seeds in apiaries. Practical aspects of this survey implementation will be presented, together with the advantages and limits of the approach, in comparison to dedicated field studies and monitoring approaches.

RA07-3

Pesticide pollution monitoring in streams for the identification of appropriate risk mitigation measures

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Risk mitigation measures are becoming increasingly important especially as regulatory frameworks like the European Water Framework Directive or the EU-framework for sustainable use of pesticides require higher standards. Their effectiveness under field conditions are currently examined at specific stream catchments in intensive agriculture in Germany. At a first step stream catchments were monitored to detect pesticide pollution and to develop proposals for appropriate risk managment strategies.

The monitoring was carried out in 2009 at 10 sampling sites in an intensive field crop region in central Germany and at 9 sampling sites in a vineyard region in south-west Germany. Pesticide residues in the water and sediment phase were determined following heavy rainfall events. Moreover an ecological monitoring (macroinvertebrate community) and a landscape monitoring (water body and riparian buffer strip characterisitcs) was performed.

In the field crop region maximum permissible concentrations of insecticides (mainly alpha-cypermethrin, lambda-cyhalothrin, pirimicarb) and fungicides (epoxiconazole, spiroxamine, tebuconazole, fenpropimorph) were exceeded mainly in the sediment but also in the water phase. In the vineyard region, mainly fungicides (especially folpet, tebuconazole, azoxystrobin, fludioxonil) accounted for the pesticide pollution. The evaluation of macroinvertebrate data using the SPEAR-concept, showed that in both study areas water quality is predominantly "poor"or "bad"(field crop region: 7 sampling sites; vineyard region: 6 sampling sites).

In the field crop region the sampling sites with a higher pesticide pollution differed from less polluted sampling sites regarding riparian buffer strip width and vegetation, the number of erosion rills and neighbouring fields of presumed increased higher exposure potential. In the vineyard region, the concrete road network and associated erosion rills leading the runoff in the water body are regarded as main pesticide entry routes. Runoff samples exemplarily taken in erosion rills showed the same pesticide residues as samples from the stream but in higher concentrations.

The different patterns of pesticide pollution in the two study regions show that a differentiated approach for the identification of appropriate mititgation measures is required. At identified higher polluted stream sections appropriate measures are planned to be implemented and evaluated in a further monitoring phase.

RA07-4

Pesticide and nutrient discharge from greenhouses in Norway: preliminary investigations 2007 and 2008

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Preliminary investigations of pesticide and nutrient discharge from Norwegian greenhouses indicate that these effluents can influence water quality and biological diversity in downstream recipients.

During the autumn of 2007 a total of 29 water samples were grabbed in small streams and ditches downstream 9 greenhouses, mainly producing flowers. Pesticide residues were found in 90 % of the samples. A total of 18 different pesticides were detected; 9 fungicides, 5 herbicides and 4 insecticides. Six fungicides (cyprodinile, propiconazole, iprodione, azoxystrobin, prochloraz and vinclozolin) and 3 insecticides (pirimicarb, diazinon and chlorfenvinphos) were found in concentrations exceeding the Norwegian standard for environmental effects. The fungicides pyrimethanile, iprodione and imazalile were detected in concentrations higher than 1 µg/l. The grab samples that contained high concentrations of pesticides also contained high concentrations of nutrients typical for a greenhouse effluent (0.8 - 7.4 mg P/l and 6 - 42 mg N/l). Further investigations in 2008 included grab samples in the drainage system of 10 greenhouses, but also samples in streams and ditches downstream greenhouse plants. A total of 49 water samples were analysed for pesticides and nutrients. Pesticide residues were found in more than 90 % of the samples. A total of 25 different pesticides were detected; 11 fungicides, 7 herbicides, 6 insecticides and 1 growth regulator. Two fungicides (cyprodinile and imazalile) and four insecticides (pirimicarb, endosulfan, chlorfenvinphos and lambdacyhalotrine) were found in concentrations exceeding the Norwegian standard for environmental effects. The maximal nutrient concentration in drainage water was 30 mg P and 650 mg N/l.

The results confirm that greenhouse discharge may contain harmful concentrations of pesticides and nutrients. The results demonstrate a huge variation in effluent concentration as a function of production, operation, water handling system and water consume. The total discharge of pesticides and nutrients are expected to be efficiently reduced when including environmental aspects in planning, construction and operation of the greenhouses. Optimization of the water handling system (irrigation and drainage) is especially important.

RA07-5

Pesticides in surface and groundwater - Italian monitoring data 2007-2008

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ISPRA since 2003 carries out the national report on the presence of pesticides in surface and groundwater in order to provide on a regular basis information on the quality of waters in relation to the risk of these substances. The report is the result of a complex activity involving regions and regional environmental protection agencies (ARPAs) that perform surveys and transmit data to ISPRA, which coordinates the activities providing methodological documents, guidelines and evaluating data.

Pesticides are chemicals used to control weeds, insects and other pests in agricultural areas, and a variety of other land-use settings. In the European Union, from a regulatory point of view, we can distinguish substances used in plant protection products and biocides, which are used in various fields (disinfectants, wood preservatives, pesticides for nonagricultural use, antifouling, etc.). The use of these substances raises issues about possible adverse effects on humans health and the environment, because most of them are molecules generally hazardous to all living organisms.

During these years, the activities of planning made it possible to focus the survey on substances actually used and to identify priorities in relation to the potential risk. This activities provided the basis for harmonization of regional monitoring programs and at the same time, the realization of a national system for managing information on the topic.

The results of national monitoring of surface and groundwater held in the years 2007-2008, will be showed. We present the contamination level found, for comparison both with the limits fixed for drinking water, and for comparison with environmental quality standards defined in recent years by the EU and national legislation.

RA07-6

Could sustainable use directive provide solutions for a better pesticide risk management?

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Introduction

In September 2009 the Council of the European Union adopted the so called pesticides package (PP). The PP includes the Directive 2009/128/EC (SUD). The objective of the SUD is to achieve a sustainable use of pesticides by implementing a plan for risk and use reduction.
OPERA is a research centre of the Università Cattolica del Sacro Cuore (UCSC). It is an independent, not-for-profit scientific think tank, committed to the successful integration of agro-environmental measures within European legislation. Within this context OPERA reviews and advise in the implementation and measurement of risk reduction methodologies, which are crucial for the successful implementation of the SUD.
Activities
Several parallel activities have been implemented by OPERA to contribute to the success of the implementation of the SUD:
- Selecting the right risk indicators
- Developing mitigation measures
- Reforming the CAP
Results
Relating to the selection of the right risk indicators, OPERA has put forward a proposal of a possible pool of indicators to select from when elaborating the NAP. Risk Indicators are expected to help national regulatory bodies to assess trends in pesticide risk reduction and to judge the effectiveness of their programmes. The risk indicators issue is the subject of two OPERA publications. Regarding the example of field margins, the efficiency of such a solution to protect the environment and human health depends on multiple factors. It has emerged that the implementation needs to take into account and coordinate with other legal requirements to establish such buffer zones. OPERA has treated this subject in its publication "Multifunctional Landscapes".
Related to the CAP, agriculture has to be perceived as a strategic sector due to its multifunctional role for the society. We have created a participatory process to debate with stakeholders the most important bottlenecks for the future policy. This resulted in a series of documents, available on the OPERA website, to elaborate on the issues of concern as well as in a opinion paper submitted to the European Commission in the process of public consultation on the future of the CAP.

RA08 - PBT substances

RA08-1
Screening for PBT chemicals in the set of chemicals in commerce
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One important innovation of REACH, the new European chemicals legislation on the Registration, Evaluation, Authorization and restriction of chemicals, is that REACH includes a quantitative assessment of chemicals concerning their persistence, bioaccumulation potential, and toxicity (PBT assessment). Key questions for the PBT assessment under REACH are: how many PBT chemicals are there in total and what types of chemical structures will be present in this class? What are suitable approaches to identifying these chemicals? Here, we present a screening for PBT chemicals in a set of 100'000 chemical structures. We establish a PBT scoring system and, to apply this system to the set of chemicals, estimate the chemical property data needed using methods from the Estimation Programs Interface Suite [1]. To obtain PBT characteristics of these 100'000 chemicals, we defined a PBT scoring system. For each chemical, subscores for P, B and T were calculated and integrated into one overall score. Each subscore is the ratio of a chemical property value and the corresponding REACH threshold (a degradation half-life of 60 days, a BCF of 2000, and a NOEC of 0.01 mg/l). The subscores were added and their sum divided by three. A PBT chemical (all three thresholds exceeded) has three subscores of 1 and also a total score of 1. Using the PBT scores, we defined four hazard classes into which we binned all 100'000 chemicals. The class "PBT"contains chemicals with a score of 1, "nonPBT2"chemicals with two subscores equal to 1, "nonPBT1"chemicals with one subscore equal to 1, and "nonPBT0"chemicals with all three subscores below 1. The four classes contain 2919 (2.8%), 8194 (8.0%), 28019 (27%), and 63'673 (62%) chemicals for PBT, nonPBT2, nonPBT1, and nonPBT0, respectively. Many chemicals in the PBT class contain chlorine, bromine or fluorine, and also highly branched alkyl substituents and nitro groups are observed. To investigate the influence of uncertain chemical property data on the PBT scores, we varied first the half-lives, then the BCF values, and finally the toxicity data of all chemicals by a factor of two and re-calculated the PBT scores. This showed that the number of chemicals in the PBT class is most sensitive to changes in the BCF, followed by the biodegradation half-life, whereas the influence of the toxicity data is much weaker.
[1] US EPA. 2009. Estimation Programs Interface Suite. http://www.epa.gov/opptintr/exposure/pubs/episuite.htm. Accessed 29/11/2010.

RA08-2
Estimation of kinetic rate constants from water-sediment standard tests
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Data from water-sediment tests are often exploited by estimation DT50 and DT90 values for disappearance from water, sediment (where applicable) and total system and categorization the compounds according to the total amount of non-extractable residues (NER). However, there are at least five different processes that have to be separately considered: (i) transfer of the substance from water to sediment (back and forth), (ii) primary degradation in the water phase, (iii) primary degradation in (bulk) sediment, (iv) emergence of non-extractable residues (NER), and (v) mineralization to $^{14}CO_2$. We determined kinetic rate constants from data of various pesticides in water-sediment test systems that were made available by the German Federal Environmental Agency (UBA, Berlin). Data sets were fitted to a first order kinetic process model that separately considers the five processes mentioned above. A non-linear optimization algorithm (Levenberg-Marquardt) with least-square fit was used. Most of the data sets could be successfully fitted to the model with realistic rate constants for metabolization in sediment, increase in NER (decrease in extractability) and CO_2 evolution. These rate constants can be transformed to half-lives that allow for much more realistic assessment of persistence by avoiding misinterpretation of DT50 values as biodegradation half-lives in water.
In general, it turned out that biodegradation of parent compounds basically occurs in the sediment. Rate constants for degradation and mineralization in water were always estimated to be zero, unless the compound was prone to abiotic hydrolytic degradation. Thus, first-order rate constants for loss from water describe the diffusive transfer into sediment and should not be used to derive degradation half-lives. Instead, bulk degradation rate constants for sediment can be determined from the model fit. These degradation rate constants are often quite different from DT50 values for total system that were used as an assessment criterion for persistence in official documents. Another important factor is the phenomenon of non-extractable residues that occur for some of the compounds. Model simulations indicate that the process behind the observed decrease in extractability is kinetically determined and not or only very slowly reversible. This is manifested by the steady increase of NER fraction over time.

RA08-3
Organic pollutants with long-range transport potential in oceans are persistent
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According to the Stockholm Convention on Persistent Organic Pollutants (POPs) [1] substances are prone to long-range transport with atmospheric currents if they are characterized by a half-life in air of more than two days. In addition, pollutant transport may also occur within the water compartment. An analogous half-life criterion is, however, not given. Therefore, we derived half-life criteria characterizing LRTP in water based on flow velocities of European rivers and ocean currents running into the Arctic Ocean. The well-established concept of characteristic travel distance (CTD) calculated by a multimedia model takes partitioning between environmental compartments and degradation behaviour into account [2] and was transferred to substances primarily transported within the water compartment. On a global scale, which is especially significant as far as an international chemical assessment is concerned, derived half-life criteria for LRTP in oceans are significantly smaller than current persistence criteria in water as defined by the Stockholm Convention and the European chemical legislation (REACH). Consequently, organic pollutants prone to LRTP in oceans are already registered as substances of very high concern by the persistence criterion. Simulations with the multimedia model ELPOS [3] of perfluorooctanesulfonic acid (PFOS) which is included in Annex B of the Stockholm Convention as a persistent organic pollutant show a higher CTD in water than in air. Nevertheless, simulation of the waterborne POP PFOS supports the LRTP and POV boundaries derived by Klasmeier et al. [4] with a well-established set of reference chemicals basically transported by atmospheric currents.
[1] UNEP, 2001. Stockholm Convention on Persistent Organic Pollutants. United Nations Environment Programme. Geneva, Switzerland. (http://www.pops.int).
[2] Beyer et al., 2000. Environ. Sci. Technol. 34:699-703.
[3] ELPOS (Environmental Long-range Transport and Persistence of Organic Substances) is available free of charge via http://www.usf.uos.de/usf/arbeitsgruppen/ASW/ELPOS.de.html.
[4] Klasmeier et al., 2006. Environ. Sci. Technol. 40: 53-60.

RA08-4
Hexachlorobutadiene marine risk assessment for the North Sea and evaluation of secondary poisoning risks
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Hexachlorobutadiene (HCBD) is a chemical of historic interest, and was utilised in several processes by the chemical industry. Due to concerns about its persistence, bioaccumulation potential and toxicity, the use of HCBD in industrial applications in Europe has essentially ceased. It can also be generated as a by-product from some chemical syntheses. Emissions of the substance in Europe are currently extremely low, on the order of 140 kg/year to water (2008) (E-PRTR). Given that discussion of this substance is ongoing in various forums, it was decided to conduct a risk assessment of this substance. The risks for the marine environment were assessed according to the methodology laid down in the Guidance Documents of the EU Existing Substances Regulation 793/93. The risk is indicated by the ratio of the Predicted Exposure Concentrations (PEC) and the Predicted No-Effect Concentrations (PNEC) for the marine aquatic environment. For the North Sea risk assessment a PNECwater value of 130 ng/l and a PNECsed value of 24.4 µg/kg dw were derived. From the available monitoring data a PEC_{water} of 6 and 12 ng/l and PEC_{sed} of 1.1 and 4 µg/kg dw were derived for a typical and a worst case situation, respectively. As all PEC/PNEC ratios are well below one, overall the data are supportive of the conclusion that the levels of HCBD in the marine environment do not pose an unacceptable risk. As HCBD has PBT properties it is appropriate to also consider risks for secondary poisoning. The SETAC POP/PBT workshop (Integr Environ Assess Manag, October 2009) recommended that one method to assess this risk is the use of Critical Body Burden (CBB). CBB values could be derived from the available literature data, which allowed the determination of the risk to marine species from the measured exposure. With regards to the CBB calculation, it can be shown that the measured body burdens are seven orders of magnitude below the most sensitive calculated CBB. This represents a wide safety margin between actual and critical body burdens.

RA08-5
Accumulation of persistent organic pollutants in the Mediterranean population of Catalonia
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Persistent Organic Pollutants (POPs) are typically produced for agricultural and industrial applications, possessing notable toxicity potential and persistence, therefore representing a threat to human health and the environment itself. The most relevant POPs are the organochlorine (OC) pesticides β-HCH, 4,4'-DDT, its major metabolite 4,4'-DDE and HCB, PCBs and PBDEs. They are commonly encountered in humans, and blood serum analysis is the simplest method for assessing their body burden.
The present study reports the occurrence of these compounds in Catalonia, a Mediterranean country of 32,000 km^2 and 7.5 million inhabitans, with notable agricultural and industrial sectors. It is based on a public health survey conducted by the Government of Catalonia in 2002, that included a health exam and a blood testing for 919 people.
All studied individuals from the population of Catalonia show detectable levels in one or several organochlorine compounds (OCs, n=919), with a detection over 75% of the samples. Regarding PBDEs (n=310), the most abundant congener is BDE-209, with almost 60% of detection level. In general terms, all these OCs concentrations tend to be slightly higher than in many other previously studied populations, such as the U.S., some asian studies and the European populations from Germany and the U.K. The concentrations are however lower than in populations from economies in transition such as Romania and Slovakia, or from those that have a particularly high exposure to OC-related chemicals: a population from the Canary Islands and the Flix township in Catalonia itself. Regarding PBDEs, these concentrations also tend to be higher than european and asian studies, but lower than the U.S. population.
The concentration of OCs in the population of Catalonia increases with age. Regarding PBDEs, this age effect is not clearly observed. Sexual differences are also observed in the present study. The most notorious observation is a much higher concentration of several OCs in women, particularly HCB and β-HCH (both of them with statistically significant differences). In the

case of 4,4'-DDT, 4,4'-DDE and PCB-118, their concentrations are also higher in women. In contrast, men have moderately higher concentrations of PCB-138, PCB-153 and PCB-180. Finally, PBDEs do not show differences between sexes, although men tend to have slighly higher concentrations.

RA08-6

Influence of physical-chemical properties on the transference of organochlorine compounds from serum to breast milk

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Introduction and objectives. Human milk is lipid-rich and a good store of lipophilic pollutants such as organochlorine compounds (OCs). These pollutants are circulated throughout the body by the venous system and retained in breast milk due to their lipophilic properties. Studies addressing the influence of the specific physical-chemical properties of these pollutants on breast milk accumulation are needed. The present study is based on the analysis of a large number of pairs of colostrum and serum samples in which the concentrations of OCs have been determined. The resulting database is used to assess the dependence of breast milk retention on these properties.

Methodology. 361 paired colostrum and serum samples were analysed for HCB, four HCH isomers, DDT and its metabolites and 7 PCB congeners. For those compounds detected in at least 50% of samples in both cohorts, colostrum:serum concentration ratios were calculated using the ng/g lw values, and then compared with physical-chemical properties of the compounds, such as log Kow, log Koa and molecular weight.

Results and discussion. Concentrations of OCs in serum and colostrum were significantly correlated ($p<0.001$). The colostrum:serum ratios exhibit certain variability between subjects but a well defined compound dependent trends is observed, being PCB 180 and β-HCH the compounds with highest and lowest median ratios, respectively. This compound-dependence has been found to be related to the log Kow and molecular weight values. More lipophilic compounds and with heavier molecular weight were preferentially transferred to breast milk. The pesticides exhibited a clear trend with lipophilicity and PCBs seemed to be more influenced by their molecular weights. Colostrum lipid concentrations were 5-fold higher than serum, this difference in lipid content between both matrices seemed to be relevant for the distributions of these compounds and their transference from one fluid to the other.

Conclusions. The paired concentrations of OCs from colostrum and serum samples were significantly correlated. Calculation of colostrum:serum ratios for each individual compound showed that the more lipophilic and heavier OCs were those preferentily transferred from serum to breast milk during the first days of lactation. The different lipid contents of these two fluids were consistent with these observations.

RA09 - Risk assessment in the marine environment and regulation

RA09-1

Assessing the risk of chemicals in the marine environment: regulation and science issues

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The risk assessment of chemicals in marine ecosystems is becoming more and more a mandatory part of regulations for chemical management. The new European chemical legislation REACH will gradually produce a large amount of information on the hazardous properties, use and exposure of chemicals. Other environmental policy frameworks, in particular those where environmental quality standards are used to safeguard environmental quality, will benefit from this accelerated generation of information. On the other hand, marine species are mandatory for risk assessment under the OSPAR regulation without excluding freshwater data. Furthermore, each regulation is covering parts of marine waters from coastal to offshore waters while those waters can have deep environmental parameter differences (turbidity, salinity, ecological traits, etc.). The aim of this presentation is to describe briefly regulatory data requirements and related methodologies and to highlight scientific basis of hazard and exposure assessments in coastal waters and offshore waters. The second aim of this presentation is to stress gaps of knowledge for sound risk assessment in the marine environment. The third aim is to induce discussion about risk management at the boundaries of the regulations.

RA09-2

Composing batteries of ecotoxicological tests to assess marine sediment quality

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In the frame of the activity of UNICHIM (Italian Organization for Standardization in Chemistry), a committee was constituted to evaluate the possibility of devising one or more batteries of ecotoxicological tests, in order to support monitoring programmes of the quality of sediment from marine coastal waters and transitional waters. The committee produced a 5 parts document, which is now proposed to the scientific community for a peer review.

The document provides not only practical examples of batteres of ecotoxicological tests suitable for the assessment of the quality of sediment from marine coastal waters and transitional waters, but in addition it suggests a way to compare different batteries, on the basis of their respective scientific and practical relevance.

The document addresses also the problem of combining the results of tests concurrently applied to the same sample, and indicates how to summarise those results on both a toxicity and a risk scale.

RA09-3

A proposed minimal saltwater ecotoxicity data set that can be tiered based on risk assessment needs

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There are no specific saltwater testing requirements in the major European environmental legislation regulating chemicals (REACH), pesticides (Directive 91/414/EEC or Regulation (EC) No 1107/2009) or pharmaceuticals (Directive 2001/83/EC). Due to the inherent physiological differences between freshwater and saltwater organisms it is logical to hypothesize that one of the groups of organisms will be more sensitive to a specific chemical than the other. Therefore, it is important to provide sound saltwater testing paradigms since marine organisms can be more sensitive than freshwater organisms. It is difficult to predict which chemical substance will be more toxic and simply applying safety factors can provide a false sense of security or can be over protective when not warranted. One basic paradigm is founded on developing a data set on a limited number of taxa that adequately protects the low end of the species sensitivity distribution as well as helps define the chemical response curve. The objective of this presentation is to demonstrate how the two marine species, the sheepshead minnow (Cyprinodon variegatus) and the mysid (Americamysis bahia) when combined with a basic freshwater data set can provide one example on how to develop a tiered saltwater testing paradigm that takes advantage of the principles of species sensitivity distributions, dose-response relationships for a given species-chemical combination and the principle that some species will be inherently sensitive to most chemicals. Based on exposure assessments and associated toxicological concerns additional marine species will be recommended to systematically expand the data base. Due to time limitations marine algae and plants, and marine benthic dwelling organisms will not be discussed.

RA09-4

Assessing the acute effects of an Hazardous and Noxious Substance (aniline) on the common prawn Palaemon serratus: swimming behaviour and biomarkers

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In the last decades, the concern about the spills of oils and Hazardous and Noxious Substances (HNS) in the sea has been increasing due to both short and long-term ecological and economic impacts. Aniline a very toxic compound and one of the HNS transported at high volumes by ship. Despite the studies that have been done on the toxicity of this HNS, a considerable lack of knowledge still exists on its effects on marine invertebrates. Therefore, the objective of this study was to assess the effects of aniline on the common prawn (Palaemon serratus), a species with a wide distribution in the Atlantic (from Denmark to Mauritanea), where it can be found in estuaries, coastal areas and offshore waters until a depth of about 40m. It is an important prey of several fish, some of them with high economic interest. In a first bioassay to calculate 50% lethal concentrations (LC50), animals were exposed for 96h to several concentrations of aniline, in semi-static conditions with appropriate medium renewal, in a photoperiod (16h light: 8 h dark) and temperature (20±1ºC) controlled room. In a second bioassay, animals were exposed in similar conditions to high but sub-lethal concentrations to assess effects on behaviour and several biomarkers, namely, the activity of the enzymes acetylcholinesterase, lactate dehydrogenase, isocitrate dehydrogenase, several anti-oxidant enzymes and glutathione S-transferase, and lipid peroxidation levels. The 96h-LC50 of aniline to P. serratus was 18.3 mg/l (95% CL: 11.2-29.2 mg/l). The HNS was able to decrease the swimming velocity of the common prawn and caused significant alterations in several biomarkers. These results indicate that aniline spills may have a direct acute impact in exposed populations of this species since intoxicated animals may have a decreased capability of avoiding predators and capture preys, as well as reduced capabilities of performing physiological functions determinant for their survival and performance. This study was done in the scope of the project RAMOCS funded by the Portuguese Foundation for the Science and Technology (FCT) and FEDER funds (ERA-AMPERA/0001/2007, EU ERA-NET AMPERA ERAC-CT2005-016165) and was also supported by FCT (European Social Fund and national funds from the "Ministério para a Ciência, Tecnologia e Ensino Superior"- MCTES) though a research grant to Luis Luis (SFRH/BI//51043/2010).

RA09-5

Altered timing of metamorphosis in early life stages of Antarctic krill (Euphausia superba) exposed to p,p'-DDE

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p,p'-DDE is a persistent organic pollutant (POP) found to accumulate in wild populations of a key Southern Ocean species, Antarctic krill. Despite the ecological significance of Antarctic krill, there is little ecotoxicological data available for environmental risk assessment of the species. This study evaluated the effects of p,p'-DDE aqueous exposure (1 - 20 µg/L, nominal) on the earliest larval stages of Antarctic krill (N1, N2, MN and C1). In tests A to C, larvae were exposed to p,p'-DDE for 1, 3 and 9 days post hatch (dph) in order to target A) developmental progress of N1, B) metamorphosis to N2 and C) metamorphosis to MN. In test D, larvae were exposed to p,p'-DDE for 5 dph and monitored in clean seawater until 21 dph (to reach the C1 stage). Developmental timing was accelerated in p,p'-DDE exposed larvae from N1 to MN; i.e. the proportions of A) N1 with premature tail spines, B) fully metamorphosed N2 and C) fully metamorphosed MN were significantly ($P < 0.05$) higher in p,p'-DDE treatments compared to solvent controls. Some p,p'-DDE exposed N2 larvae were further found to have unusually protruding tail ends. The results of test D support acceleration of larval development in p,p'-DDE exposed larvae until the MN stage, whereas metamorphosis to C1 was impeded. No mortality was observed in tests A to C. In test D, mortality was however, increased in the p,p'-DDE treatments from the onset of metamorphosis to MN and continued to increase throughout the MN stage until 18 dph. These findings indicate that the MN stage is more vulnerable to toxicant induced stress than the preceding larval stages. The observation of aggravated effects in Antarctic krill larvae long after exposure has ended further demonstrates the significance of delayed effects of contaminant exposure. The observed delayed responses could be the first in a cascade of indirect effects caused by the initial stimulatory stress. All developmental endpoints were significantly induced from the lowest p,p'-DDE exposure concentration tested (1 µg/L) resulting in an internal lowest observed effect concentration (ILOEC) of 10.1 µmol/kg wet weight (1 dph). The obtained effective body residue for Antarctic krill larvae is comparable to those known to cause developmental effects in temperate species and one order of magnitude lower than the residue leading to sublethal narcosis in larval krill, strongly highlighting the importance of developmental endpoints for environmental risk assessment.

RA09-6

Tools for assessing the contaminant situation in the Baltic Sea - reproduction disorders in amphipods

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Assessing the effects of contaminants on ecosystem health requires not only measuring the concentrations of contaminants in the environment or biota, but also to study their effects on ecologically relevant biomarkers in species naturally occurring in the study area. It is crucial to rely on robust biomarkers in risk assessment and marine monitoring, i.e. ones that respond solely to the contaminant imposed stress being investigated but not to environmental fluctuations in abiotic or biotic conditions. This study is part of the BONUS project "BEAST"which aims to identify a broad range of such biomarkers on various species in the Baltic Sea.
In the marine environment organisms are exposed to a mixture of contaminants with different modes of toxicity and potential interactions. It is therefore fundamental to investigate the total toxicity of sediments and understand their effects on marine organisms in order to give recommendations. Reproduction disorders in amphipods measures as embryo malformations is a sensitive and early warning bioindicator to contaminant exposure. Effects of potential co-occurring stressors as oxygen deficiency, food availability and temperature have been thoroughly examined and found not to affect frequencies of embryo malformations.
In this study for the first time we directly link embryo malformations with a large span of contaminants (trace metals, PAHs, PCBs, DDT, PBDE) analysed in both whole crustaceans and sediment. Amphipods were sampled in contamination gradients in the Bothnian Bay, the Gulf of Riga and the Gulf of Gdansk, in 14 sites in total. Chemical analyses are under progress but the six contaminated sites are expected to display a broad contamination profile. The hypothesis is that some contaminants might have a larger influence on the embryo malformations than others. Suitable multivariate analysis will be performed to quantify their specific influence on the response variable.
The results show that there is a clear increase in levels of embryo malformations in contaminated sites for both the Bothnian Bay amphipods and the gammarids from the Gulf of Riga and the Gulf of Gdansk. This study shows that amphipods of different species can be used as tools to measure contaminant stress and that background levels of embryo malformations do not differ between species. Thus this method can be used in various parts of the Baltic Sea for risk assessment and to determine the ecosystem health in specific areas.

RA10 - Risk assessment of chemical mixtures: how can we crack the nut?

RA10-1
Risk assessment of POP mixture: the case study of the Arctic
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Persistent organic pollutants (POPs) are global contaminants, capable to reach remote areas, and particularly cold regions, trough long range transport patterns. Therefore, detectable concentrations may be measured in sites very far from direct emissions, such as polar areas (Arctic, Antarctica) and high mountains. Several groups of chemicals have physical chemical characteristics that allow reaching remote areas. Some of them are already regulated under the Stockholm Convention (the so-called Dirty Dozen); many others are still in use in relatively large amounts. Therefore remote areas are exposed to complex mixtures of POPs.
In this Case Study an exercise is described to assess and quantify the composition of a "realistic"mixture, likely to occur in a remote area like the Arctic. The hypothesis on which the exercise is based is that in remote areas, far from emission sites, where only long range transport may occur, only environmental and geographical characteristics may affect environmental concentrations. It may be assumed that, due too many characteristics (relatively uniform cold temperature, absence of dry-land, etc.), the Polar Arctic Circle is a relatively homogeneous area. Therefore, POPs concentrations may be assumed as relatively homogeneous and data from different literature sources may be considered as comparable.
A simple trophic chain has been considered. Literature data on POP concentration have been collected for water, fish (polar cod, Boreogadus saida), ringed seal (Pusa hispida), and polar bear (Ursus maritimus) as one of the most representative Arctic top predators. Three levels of mixture risk were taken into account: (1) fish exposed to mixture in sea-water; (2) seals eating fish; (3) bears eating seals.
The results show that the risk for fish exposed to the low concentrations present in water is negligible. However, the transfer in the trophic chain may produce significant risk for predators. Considering that most POPs are known to be endocrine disrupting chemicals, damages to reproduction and development are likely to occur.

RA10-2
Quantifying in vitro bioassays data: Mathematical methods to estimate bio-equivalents introduce a high degree of uncertainty
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Quantification is a key issue when interpreting in vitro bioassay data. Commonly, so-called bio-equivalents (e.g. estradiol or dioxin equivalents) are used to quantify the endocrine potential of complex human or environmental samples in relation to a well-characterized, prototypical reference compound. However, there is no generally accepted data analysis strategy for estimating bio-equivalents from this type of studies.
Therefore, within this presentation we will (I) review mathematical procedures for the derivation of bio-equivalents from the body of literature, (II) critically assess the accuracy of the most common models to predict bio-equivalents, and (III) propose measures to reduce uncertainty in the calculation of bio-equivalents.
By scrutinizing the literature covering the period of 1990 to 2010 we compiled a data base of more than 200 publications that report bio-equivalents. From the published data we extracted three main data analysis strategies to calculate bio-equivalents. These models are based on linear or nonlinear interpolation, and the comparison of EC_x values.
To assess and compare the models' accuracy, we employed simulated data sets in different scenarios. This theoretical calculations indicate that the linear and the EC_x model considerably misestimate bio-equivalents in most of the scenarios (-20 to 2,000% deviation). Compared to that, the less employed nonlinear model predicts correct bio-equivalents in all cases. Following the actual estimation, bio-equivalents are commonly transformed to a defined reference volume or mass of the sample (e.g. one kilogram sediment). Since this is achieved by linear extrapolation additional inaccuracy is generated.
Taken together, our evaluation indicates that data analysis by itself introduces a considerable degree of uncertainty in the derived bio-equivalents. To increase accuracy in bio-equivalent estimation, we will present strategies to improve data analysis as well as experimental design of in vitro bioassays.

RA10-3
Interactions and their impact on the applicability of concentration addition for environmentally realistic mixtures
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Regulatory guidelines make heavy use of Concentration Addition (CA) as a tool for predicting and assessing the toxicity of chemical mixtures. CA assumes that the compounds in a mixture do not interact with each other, neither in their toxicokinetic nor in their toxicodynamic phase. However, several of those interactions are described in the scientific literature for almost all major groups of environmental chemicals. These result in essentially synergistic or antagonistic mixture toxicities, i.e. higher, respectively lower mixture toxicities than expected by CA. With a view on the regulatory risk assessment of chemical mixtures, it is hence important to quantify the range of expectable synergisms, respectively antagonisms.
I discuss the quantitative consequences of interactions for the predictive power of CA using two published studies on the hazards and risks of environmentally realistic mixtures. One case study concerns the human health effects of a mixture of anti-androgens, the other the ecotoxicity of a pesticide mixture. Based on a series of simulation studies in which interactions were gradually assumed to occur in the mixtures, I outline the limiting cases (worst case situations) as well as the fundamental relationship between expectable deviations from CA and number of mixture components, mixture ratio and number of interacting substances in the mixture.

RA10-4
Working with the regulator to determine key risk-driving compounds
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Risk assessment is currently underway for a contaminated site in Northern Ireland with a wide variety of compounds of potential concern (COPCs). The risk assessment follows the UK CLR11 framework for management of contaminated sites, leading the site from investigation to remediation and ensuring a "suitable for intended use"outcome. The experience we aim to share is the process of determining key risk-driving compounds for the site while involving the regulator throughout the entire decision making process.
The current phase of works is focussed on assessing the risks to human health and the water environment from multiple COPCs. With this large number of substances detected over several years of investigation at a relatively large site, one of the initial challenges has been clearly evaluating and then communicating the relevance of these compounds so as to focus on the key issues and potential risks.
Rather than deriving generic assessment criteria (GAC) for a large number of COPCs under multiple land uses, the approach adopted was to first identify and focus the assessment on the key risk-driving compounds. Compounds were evaluated based on receptor (humans, aquatic and wildlife species) and with regard to toxicity, physical and chemical properties, spatial distribution, frequency of detection and magnitude of exceedence of adopted target concentrations. Compounds detected in association with each pollutant linkage were placed into chemical groups and, by working through the evaluation procedures, one or two key risk driving compounds were identified within each group.
Ultimately, of the compounds identified originally, approximately 30 were identified as likely to be key risk-drivers and were carried further into the risk evaluation process. Efforts to derive GAC will then focus on this manageable list of key compounds.
Discussions with the regulatory agency have provided the basis for streamlining the assessment. The regulator was engaged during the early stages of developing the compound evaluation approach and provided with the opportunity to review documentation progressively. Transparency in communication and decision making is key and ensures continued alignment as the risk assessment progresses in a timely manner.

RA10-5
Risk assessment of single substances and mixtures of micropollutants in the Belgian coastal waters
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Assessing mixture toxicity is a complex issue and therefore not implemented in the current EU legislations. It is practically not feasible to assess experimental mixture toxicity with all combinations of chemicals present on the market and in the environment. In the existing INRAM (Integrated Risk Assessment and Monitoring of micropollutants in the Belgian coastal zone, 2006-2010) project, one of the aims is to study the possibilities of assessing mixture toxicity through an integrated risk assessment approach. More than 100 micropollutants (e.g. pesticides, endocrine disruptors, pharmaceuticals) were analyzed in the Belgian coastal harbors. A database was subsequently developed summarizing all toxicity and ecotoxicity parameters of these chemicals (e.g. PNEC, DNEL, BCF, TDI). Both a single substance risk assessment (PEC/PNEC) approach and a mixture toxicity approach based concentration-addition were performed for the analyzed substances.
The main conclusions of this study are: the lack of basic data to support both the exposure and effects assessment, the need to quantify uncertainty, better justification of environmental quality standards, field evidence for the occurrence of mixture toxicity and development of tools to assess the trade-off between conservatism and uncertainty. A pragmatic approach is needed to determine the level of conservatism in exposure and hazard assessment for the implementation of mixture rules in the current EU legislations without accumulating conservatism.

RA10-6
On the use of mixture toxicity assessment in REACH and the Water Framework Directive
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This review seeks to connect scientific theory of mixture toxicity to its implementation within

different regulatory frameworks both within Europe and the US. The aim is to demonstrate how mixture toxicity assessment can be more thoroughly integrated into the European chemical regulations, REACH and the Water Framework Directive (WFD), using the experiences gained through other regulatory frameworks. The paper consists of 1) an examination of the scientific underpinnings of the range of methods used to assess the joint action of chemicals; 2) how such methods have been used in different regulatory frameworks; and 3) how such methods could be applied within REACH and WFD. It is concluded that REACH and WFD include mixture toxicity assessment to a very limited extent at the moment. However, it is shown that it is both scientifically feasible and regulatory practicable to integrate a more holistic mixture toxicity approach in both legislations. This will require that a limited number of chemicals are used in each mixture toxicity scenario. Those with individual PEC/PNEC > 0.1 is recommended to be included. Furthermore the construction of a database that includes data on chemicals in the European environment could help determine the relevant chemicals for each mixture toxicity assessments.

RA11 - Risk assessment of chemicals within REACH integrating alternative methods and non testing strategies

RA11-1

Threshold of toxicological concern assessment: investigation of possible improvments by means of in silico methods

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The Threshold of Toxicological Concern (TTC) approach is a screening tool based on the principle of establishing human exposure threshold values for chemicals, below which no significant risk to human health is expected. According to this approach, a safe level of exposure can be identified for many chemicals based on their chemical structure and the known toxicity of chemicals which share similar structural characteristics. This generic approach was first developed for the endpoint of cancer by the US FDA in the 1980s and adopted in 1995 as the 'Threshold of Regulation' for food contact materials. Since then, the TTC concept has evolved over the years to take into account extensive analysis of available data on mainly the oral toxicity of substances for toxicological endpoints other than cancer. Using a structure-based decision tree, the Cramer classification scheme, together with information on human exposure, the TTC approach has been applied, mainly in the food area. It has been used to evaluate flavouring substances, food contact materials, genotoxic impurities in pharmaceuticals and for the risk assessment of other chemicals. In 2010 the European Food Safety Authority (EFSA) funded the present research project aimed at investigating how the applicability of the TTC approach can be improved by incorporating physicochemical data and toxicity data generated by non-testing methods. The Cramer classification scheme, which is used to classify chemicals according to their structural chracacteristics, was analyzed to assess whether an alternative classification based on molecular descriptors might be useful in refining or replacing the original Cramer classes.In the current study the two major TTC datasets were retrieved, verified and analyzed, the Carcinogenic Potency Database (CPDB), including additional carcinogens based on Kroes et al , provides TD50 values, and the Munro dataset covering non-cancer toxicological endpoints. The chemical space covered by the two datasets was characterised using several molecular descriptors and explored by means of a variety of chemoinformatics techniques. The chemical space of the datasets was also compared against the wider universe of chemicals, as represented by the Distributed Structure-Searchable Toxicity (DSSTox) Database developed by the U.S. Environmental Protection Agency (EPA) as a reference. Finally, a refinement of the Cramer structural classes was proposed which identify structural subclasses.

RA11-2

Exemplification of the integration of tools within REACH: the CADASTER project

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Implementation of REACH requires demonstration of the safe manufacture and use of chemicals. REACH aims to achieve a proper balance between societal, economic and environmental objectives, and attempts to efficiently use the scarce and scattered information available on the majority of substances. Thereupon REACH aims to reduce animal testing by optimized use of in silico and in vitro information on related compounds.
The REACH regulation advocates the use of non-animal testing methods, but guidance is needed on how these methods should be used. The procedures include alternative methods such as chemical and biological read-across, in vitro results, in vivo information on analogues, (Q)SARs, and exposure-based waiving. The concept of Intelligent Testing Strategies for regulatory endpoints has been outlined to facilitate the assessments. Intensive efforts are needed to translate the concept into a workable, consensually acceptable, and scientifically sound strategy.
CADASTER aims at providing the practical guidance to integrated risk assessment by carrying out a full hazard and risk assessment for chemicals belonging to four compound classes. A Decision Support System (DSS) will be developed that will be updated on a regular basis in order to accommodate and integrate the alternative methods mentioned above. Operational procedures will be developed, tested, and disseminated that guide a transparent evaluation of four classes of emerging chemicals, explicitly taking account of variability and uncertainty in data and in models.
The aim of this presentation is to exemplify the integration of information, models and strategies for carrying out safety-, hazard- and risk assessments for large numbers of substances. It will be whosn how real risk estimates can be delivered according to the basic philosophy of REACH of minimizing animal testing, costs, and time, thus showing how to increase the use of non-testing information for regulatory decision whilst meeting the main challenge of quantifying and reducing uncertainty.

RA11-3

Towards guidance on how to characterize prediction uncertainty in QSAR regression models within the CADASTER project

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RA11-4

A framework for hazard assessment under data-scarce conditions: the transformation product problem

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The European Union's REACH legislation requires that transformation products be included in chemicals assessment for chemicals produced or imported in amounts exceeding 100 tonnes per year. However, including transformation products in such assessments could be considered an intractable problem, given the paucity of available data and the difficulty of predicting the most likely transformation route from the many possible degradation products of a complex parent chemical (the so-called 'combinatorial explosion' problem). We have developed a scheme that seeks to overcome these data and knowledge gaps to identify transformation products that substantially contribute to the persistence of a parent chemical and its substance family. This is accomplished through the integration of methods to predict biodegradation products, to estimate physico-chemical properties and environmental half-lives, and to calculate the joint persistence, a metric that includes both the emitted chemical and its transformation products. Our results highlight that the 'combinatorial explosion' problem can be managed, but there remains a serious need for better quality data, particularly for the environmental half-lives of chemicals.

RA11-5

ITS implementation and acceptance: investigating the stakeholders' perspective?

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The comprehensive data requirements of REACH, the animal welfare concerns put forward by REACH, and the large number of industrial chemicals that are going to be tested require a "paradigm shift"of the current risk assessment and risk management regime for industrial chemicals from a extensive hazard testing to a risk-driven approach. In this context, Intelligent or Integrated Testing Strategies (ITSs) (i.e. sequential combinations of existing human and physicochemical data, testing and non-testing information, and exposure information) have been considered appropriate tools for more efficient and flexible toxicity testing.
However, little information has become available so far about the users' perspective on ITS development and implementation. This is the more important as the ITSs that have been developed within the past years will be used only if they respond to the users' needs.
The aim of this study is, therefore, to investigate the stakeholder's view on the development, use and implementation of ITSs and to investigate the needs for further research. For this purpose qualitative, semi-directed stakeholder interviews were performed with members from different stakeholder groups (industry, science, regulatory agencies, consultancies, animal protection interest groups). The paper presents and discusses the results of the interviews.
Our results show that there does not exist a commonly agreed definition of what an ITS. Furthermore, we observed that the stakeholders have different viewpoints regarding the potential use, the strengths and the limitations of ITSs in a regulatory context. Our study confirms that ITSs are generally considered useful tools for reducing the need for animal testing. The stakeholders emphasized various aspects for a more systematic development and use of ITSs.
In addition, our study underlines the crucial role of risk and uncertainty communication in order to improve ITS implementation and acceptance.

RA11-6

The OECD QSAR Toolbox - version 2.0

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The first version of the OECD QSAR Toolbox was publicly released in March 2008. The Toolbox is a software application intended to be used by governments, industry and other stakeholders to fill gaps in (eco)toxicity data needed for assessing the hazards of chemicals. It contains databases with results from experimental studies, grouping engine, a library of QSAR models, tools to estimate missing experimental values, and report editor.
Since October 2010 the OECD QSAR Toolbox version 2.0 is available for free and could be downloaded from the OECD website. For the first time in Version 2.0 there is a distributed version along with a stand-alone version. Also, with Version 2.0, new downloads are accessible via auto-updates. This release is part of a four-year collaborative project between OECD, ECHA, LMC and other partners.
The aim of this presentation is to elucidate the improvements of the main functionalities of the new version of the Toolbox: Input, Profiling, Endpoint, Category Definition, Data Gap Filling and Report, as well as the new features introduced in version 2.0 of the Toolbox. Key change in second version compared to the first version is the improved user interface to make the Toolbox more intuitive and user-friendly. The key mechanistic profilers were refined, and new databases were added. The new version of the QSAR Toolbox allows also users to import/export data from and to IUCLID 5, and suitable reporting of the results. The system infrastructure was also improved in order to address the numerous other requirements of the growing number of users. Currently, based on the user comments, we are working on the next version 2.1 which will be releases in mid February 2011

RA12 - Risk communication for environmental protection: Scientific and regulatory needs

RA12-1

Development of realistic and effective risk mitigation measures within authorisation of hu-

man and veterinary pharmaceuticals

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Human and veterinary pharmaceuticals are released continuously into the environment in amounts which are comparable to those of pesticides. Therefore, potential environmental risks have to be assessed according to legal requirements within registration and authorisation procedures for pharmaceuticals. If an unacceptable risk for the environment is indicated, risk mitigation measures (RMM) should be proposed by the applicant and implemented within risk management procedures.

The authorisation of a veterinary pharmaceutical can be refused due to specific environmental concerns, whereas for human pharmaceuticals a refusal due to environmental concerns is not envisioned. So far, RMM have been applied only for veterinary pharmaceuticals. Depending on the registration procedure (decentralised, centralised, mutual recognition or national procedures), the year of authorisation and the reference member state, RMMs for the same active ingredient can differ significantly. For human pharmaceuticals, feasible and effective risk mitigation measures are not yet available.

Therefore, within a R&D-project funded by the German Federal Environment Agency (UBA), risk mitigation measures to reduce environmental risks posed by veterinary and human pharmaceuticals were identified, evaluated and further developed. The RMMs will be assessed according to their practicability, efficiency, sustainability, verifiability and their compliance with national and European regulations. As one of the outcome of this project a catalogue of risk mitigation measures will be compiled which serves likewise applicants and authorities within authorisation procedures of human and veterinary pharmaceuticals. The application of these RMM may lead to more harmonisation, transparency and equal treatment within authorisation of medicinal products.

RA12-2

Communication about environmental risks of human pharmaceuticals and the therapeutic desires of patients: is there an overlap?

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Potential environmental risks of human pharmaceuticals are a subject of growing interest in the scientific and regulatory community as well as in the general public. Evaluation of the environmental risk assessments and the discussion on consequent management options focus primarily on the environmental impact but rather little on the desire of the patients for optimal therapeutic treatment. This produces uncertainties amongst the stakeholders in the public health system about the actual extent of the problem and the urging needs for regulatory actions.

In this context, a classification system of medicinal products was introduced in Sweden as a tool for communicating environmental risks to the involved parties, in order to support the possibility of selecting the medical treatment with the potentially lowest environmental impact. This system is accessible to the public and provides information in form of summary statements of environmental risks or hazards as well as detailed study results for the specialist. It was proposed to be extended across the European Union. However, results of a previous survey on the success of this system to influence prescription and use patterns are not very promising. One reason for this lack of success may be the dilemma that the environmental concerns are not in accord with the desire of patients for optimal treatment even in a society such as the Swedish with a reputation of high environmental concern.

As a better communication method, an assessment which describes benefits, potential environmental and socio-economic risks, and possible management options may provide a more balanced view on public health needs and the legitimate concerns for an intact environment.

As an example, we summarize a socio-economic impact assessment of the steroid hormone ethinylestradiol, a component of the birth control pill, which has been has been described as an endocrine disruptor in surface waters, but which benefits are rarely been discussed in similar scrutiny outside the field of medical specialists.

In essence, we propose a socio-economic impact assessment as an appropriate approach to communicate benefits, potential environmental and socio-economic risks, and management options. Although rather complex, this approach may better serve the differentiated information requirements about the various aspects of the use of certain groups of medicines as just a classification system regarding environmental risks.

RA12-3

The application of CLP/GHS to pesticides: impact of new hazard communication element

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CLP Regulation is the new EU Regulation on Classification, Labelling and Packaging of chemical substances and mixtures. It introduces a new system for classification and labelling of chemicals, based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), an attempt to provide an international guidance on standardized label elements and safety data sheets.

CLP/GHS criteria establish new hazard communication elements which are, to a great extent, different from those currently used in Europe. As a consequence, each chemical substances placed on the EU market will be checked and then reclassified and relabelled as needed.

New elements will be applied to all chemicals used in the workplace as well as by consumers, and in particular to pesticides. Pesticides labelling is an important device to provide practical information about their hazards and handling, as well as to ensure a safe use and management of risks for man and the aquatic/terrestrial ecosystem. Plant protection products (PPP), in fact, represent mixtures with intrinsic hazardous properties which are intentionally released into the environment.

The implementation of CLP/GHS in EU will involve a long transition period to allow pesticide manufacturers to comply with new criteria, and customers (farmers) to become familiar with new hazard communication elements. Thorough information campaign targeted to stakeholders in chemical supply chain, including consumers, should be performed and the level of response continuously monitored.

Aim of the project is to investigate difficulties and probable gaps encountered by downstream users of the pesticides supply chain in complying with the new CLP/GHS classification rules and with the new hazard communication elements. Five different targets (pesticide retailers, trade-unions, personnel employed in Public Occupational Health Services, pesticide professional users, and pesticide manufacturers) established in Lombardy Region (Italy) were selected. Questions were related to the pictogram meaning, the right check of PPP labelling, the need of specific training course, the need of press aimed campaigns.

Preliminary results concern basic knowledge or any information about the new regulation. Some gaps and problems emerged, and results were used as a basis to produce a simple illustrated leaflet displaying the incoming changes, to be distributed to professional users and to consumers.

RA12-4

To inform or not to inform, that is the question

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Risk communication is an area that has received great attention in the last few years as it is recognized that communication is a necessity for addressing pressing environmental concerns. The ambition of risk communication can also be a bit more modest or local, where a need for risks to be properly communicated between producers and a larger audience is important for reducing risks to the environment or health on a local level. This contribution presents the results from findings from a survey study performed among producers of articles in order to investigate how they inform or communicate potential risks with their customers (N=101). Five types of articles were identified: textiles, shoes, tires, home electronics, and PVC flooring. The results show that producers of articles that may contain chemicals that can pose a threat to either human health or the environment have good information about what their products contain. This information is primarily used for the internal environmental work, and not for communicating with consumers. However, customer interaction is high and the most producers in this study are willing to make changes in their production, when this is possible, as a result of this interaction. However, the overall impact of consumer or public perception has weaker influence on actions taken to reduce the negative environmental impact of production. The most common way of communicating or informing consumers about what the products contained was trough labeling and tables of content. The least utilized way of reaching consumers was providing contact information. It Is concluded that that the most common way of reaching consumers with information on possible negative environmental and health impacts with goods and articles were by providing information on the product itself and the least common was to provide contact information, which in practice can deter consumers from communicating directly with the producers. It is argued that legislation seems to be the most powerful tool in encouraging producers to reduce possible negative effects with their production, the requirement and form for communication must be formally regulated in order to reach its intended impact.

RA12-5

How can the cautious policy of acceptance 'case-by-case' become a mechanism of progress towards the rigorous, successful and extensive use of QSARs within REACH?

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Current policy on the use QSAR models within REACH is to accept each use 'on a case by case basis'.[1] This is also described as a 'learning by doing approach'.[1]

This familiar 'case by case' policy can be viewed by industry, regulators and QSAR developers as a necessary and responsibly cautious approach, but it can also be viewed as a frustrating barrier to regulatory clarity and the increased use of QSARs. The process of industry learning from cases also becomes challenging in a context where cases are diverse and subject to commercial confidentiality.

In the presentation we therefore contribute to the discussions of what is needed to support this important process of 'learning by doing' and so hasten the 'case-by-case' progress towards the rigorous, successful and extensive use of QSARs to reduce animal testing.

Specifically, we will draw out key themes from the ECHA guidance for industry registrants, and from ECHA's initial observations from registration dossiers (presented in seminars and available online). We will also draw on insights from an interview and discussions with ECHA and from a survey and an international workshop with industry, regulators and QSAR developers. The first author draws on research experience in applied linguistics and science & technology studies (STS), specifically on science communication, learning professional genres, science and documentation issues in environmental assessment, and public debate around the 'case by case' UK GM crop trials. The presentation is therefore the result of dialogue, and applies social science insights to the policy, science and technology of toxicology and QSARs.

We suggest that the existing ECHA guidance documents provide a strong basis for potentially flexible and responsive practice, and that ECHA's initial seminar 'observations from registration dossiers' are a first step in the process of 'documenting the learnings'.[1] The further challenge is to understand the 'unacceptable' submissions in order to guide a diverse network of professionals in a contingent and reflexive activity. We offer a conceptual framework to support and develop this process.

[1] ECHA 2008. Guidance on information requirements and chemical safety assessment: Chapter R.6: QSARS and grouping of chemicals. pp12 & 27

RA12-6

How to improve the safe use, explanation and acceptance of QSAR models for REACH?

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There is a large debate about the possible use of QSAR models for REACH. Indeed, REACH requires that no animal experiment is done, unless other approaches are not possible or have failed. QSAR models can be useful for many reasons: they bring innovation; they counteract problems related to the issues posed by animal tests for the necessary time, animal lives, number of laboratories; they are cheaper, can be used for thousands of chemicals allowing fast prioritisation, and offer possibility to plan safer chemicals in advance.

Within REACH QSAR models are foreseen for 1) risk assessment, 2) classification and labeling, and 3) prioritization. There are several QSAR models with different features, which are appropri-

ate for one or more scope.
The use of QSAR for risk assessment within the substance registration is a demanding task. In this case the model uncertainty has to be low. It is important to compare the uncertainty associated to the experimental model, with that of the QSAR model.
The use of QSAR models for classification and labeling (C&L) has different requirements, regarding the use of continuous or categorical values, and the associated uncertainty. QSAR models may offer a systematic way to provide C&L of the substance.
Prioritization has also different requirements. In this case the uncertainty of the model is not a major barrier. The tools for prioritization can be also different from those used within the C&L approach, since it may be useful to summarize the results for different endpoints.
These different requirements, use and kind of QSAR models, demands different ways to communicate the results, in particular for the uncertainty and the details of the QSAR models. However, some general issues have to be addressed. As requested by REACH, a description of the limitations of the model has to be done, addressing the applicability domain of the model. To clearly explain this has a major influence on the QSAR acceptability.
Further discussion will address the features, use and possible acceptance of QSAR models depending on the case where they are used within a conservative or realistic perspective, which has also implications on the strategies of integrating results from different models. The role of QSAR experts, and how to make objective the explanation on the results, will be also addressed.

RA13 - The future of ecotoxicological risk assessment - biological traits, ecological vulnerability, improved SSDs, indirect ecological effects

RA13A-1
Considering protected aquatic non-target species in the environmental risk assessment of plant protection products
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Maintaining biodiversity when using plant protection products is aimed for by the new regulation for the placing of PPPs on the market (EC, 1107/2009). A relevant aspect of biodiversity are endangered and also protected species that have a scattered but widespread distribution in agricultural habitats. Therefore, they need to be considered within the risk assessment framework of PPPs. Here we explore whether the current risk assessment framework safeguards protected species as defined in the Habitats Directive (92/43/ EEC, 1992).
Reviewing information on the effects of toxicants, we conclude that protected species with regard to their toxicological sensitivity seem to be comparable to non-protected species. However, sensitivity of protected species may be increased in the environmental context, as typically found for individuals and populations under stress. Also population recovery is often slower compared to many non-protected species as protected species often have comparably long generation times.
We conclude that the toxicological context sensitivity on the individual- and the population level of endangered and protected species is higher compared to non-protected species. Therefore, we suggest exploring whether for endangered and protected species a higher safety factor in addition to the existing ones should be introduced when extrapolating from laboratory standard test systems. We also suggest not to consider recovery of populations in environmental risk assessment.

RA13A-2
Ecotoxicology and macroecology - time for integration
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After almost 50 years of development of ecotoxicology, it has become clear that this discipline cannot answer its central questions, such as, "What are the effects of toxicants on biodiversity and ecosystem functions and services?" We argue that if such questions are to be answered, a paradigm shift is needed; the current bottom-up approach of ecotoxicology should be merged with a top-down macroecological approach. Furthermore, we show that such integration is currently methodologically possible. To tackle the current pressing challenges, ecotoxicology has to search for the truth using both the bottom-up and top-down approaches, similar to digging a tunnel from both ends at once.

RA13A-3
Cross-effects of metallic contamination and parasitism on zebra mussel physiology
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In the framework of environmental risk assessment, parasites could represent a confounding factors interacting with other stress factors, like pollution. Among freshwater macroinvertebrates used in bioindication procedures, zebra mussels (Dreissena polymorpha) are considered a reliable bioindicator species. However, like many other organisms, it can be parasitized. Our previous studies underlined a positive correlation between metallic contamination, particularly by Chrome and Nickel, and the infection prevalence of Ricketsiales-like organisms (RLOs). Moreover, our in situ observations revealed that parasitism can be a confounding factor in ecotoxicological by modifying host physiological responses. We thus performed experiments in laboratory controlled condition specifically focused on Dreissena-Ophryoglena and Dreissena-RLOs systems to investigate the cross effects of parasite infection and nickel contamination.
Zebra mussel specimens were sampled in a site on the Meuse River with low trace-metal concentrations. After acclimatization, the mussels were exposed to 0, 20 µg Ni.L-1 (French legal limit of concentration in drinking water) and 500 µg Ni.L-1 (French legal limit of concentration of industrial discharge) during 48 hrs. Parasite infection capacities were assessed all along the experiment (0, 24 and 48 hrs). After 48 hrs of exposure, cell parameters in the digestive gland such as structural changes of the lysosomal and peroxysomal systems, and neutral lipid and lipofuscine granule accumulation were measured by histochemistry and automated image analysis. The immune capacities of mussels (i.e. total hemocyte counts, differential hemocyte counts, phagocytosis capacity, production of reactive oxygen species and the lysosomal activity) were also evaluated by flow cytometry. An increased prevalence rate and a decreased infection intensity were observed: at the end of the experiment, there were two-times more infected mussels presenting a lower number of parasites per individual. This indicates a rapid infection from the initially infected mussels. Moreover, a tendency to a decrease of the immune system capacities was measured (e.g. a reduction of phagocytosis, more reactive oxygen species produced). In a larger context, this study highlights the potential risk of Bivalvia species subject to multiple environmental stress factors such as parasitism and chemical contamination.

RA13A-4
Xplicit, a novel approach in probabilistic spatiotemporally explicit exposure and risk assessment for plant protection products
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The quantification of risk, the likelihood and extent of adverse effects, is a prerequisite for regulatory decision making for Plant Protection Products and the goal of the Xplicit project. In Xplicit, realism is increased in the exposure and risk assessment (ERA), first by using real-world data on, e.g., landscape and agronomic factors affecting exposure; and second, by taking the variability of key factors into account. Spatial and temporal variability is explicitly addressed. Scale-dependencies (e.g., of variability) are taken into account. This allows for risk quantification at different scales: e.g., at landscape scale, an 'overall picture' of the potential exposure of non-target organisms can be derived (e.g., for all water bodies or off-crop habitats in a given landscape); at local scale, exposure might be relevant to assess recovery and recolonisation potential; intermediate scales might best refer to population level and hence, might be relevant for risk management decisions (e.g., water body stretch, or individual off-crop habitats). The Xplicit approach is designed to comply with a central paradigm of probabilistic approaches, namely that each individual case that is derived from the variability functions employed should represent a potential real-world case. This is achieved by operating spatiotemporally explicit. Landscape factors affecting the local exposure of habitats of non-target species (=receptors) are derived from geodatabases. Variability in time is resolved by operating at discrete time steps, with the probability of events (e.g., application) or conditions (e.g., wind conditions) defined in Probability Density Functions (PDFs). The propagation of variability of parameters into variability of exposure and risk is done using a Monte Carlo approach. Among the outcomes are expectancy values on the realistic worst-case exposure (Predicted Environmental Concentration, PEC), the probability P that the PEC exceeds the EAC (Ecologically Acceptable Concentration) for a given fraction of habitats, and risk curves. The outcome can be calculated at any ecologically meaningful organisation level of receptors. Xplicit uses a modular and object-oriented software design that facilitates cooperation between the different disciplines. An example application of Xplicit is shown for a hypothetical risk assessment for Non-Target-Arthropods (NTAs), demonstrating how the risk quantification can be improved compared to the standard deterministic approach.

RA13A-5
Effects of time-variable exposure of isoproturon on green algae under flow-through conditions - modelling and experimental results -
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Due to the impact that plant protection products may have on aquatic ecosystems, extensive laboratory toxicity studies and risk assessments are required before a product can be registered in the European Union. Predicted environmental concentrations in surface water bodies (PEC_{sw}) are calculated based on realistic worst-case exposure scenarios defined by the FOCUS group. They reflect that aquatic exposure is often characterized by multiple peaks of variable height and duration, driven by spray drift input as well as run-off and drain flow events. Relating the results of standard ecotoxicity tests performed under constant exposure to FOCUS time-variable exposure patterns is only possible by gross simplifications and the use of worst-case assumptions. In order to refine this approach, knowledge about the responses of aquatic organisms (e.g. green algae) to time-variable exposure needs to be gained for more realistic but nevertheless conservative risk assessments. One way forward is the use of population models within the higher-tier risk assessment, in close combination with ecotoxicological experiments. In order to address the above problems in the case of algae, the aim of this work was to establish a flow-through test system in combination with mathematical modelling for the assessment of effects of time-variable exposure of pesticides on populations of the green algae *D. subspicatus* and *P. subcapitata*.
The flow-through system was developed and applied to assess the effects of isoproturon on the selected algae. A FOCUS exposure pattern was generated for isoproturon and a simplified version was used in the test system. In parallel, an algae population model was developed that is able to describe the population density of relevant algal species in dependence of variable environmental conditions (nutrients, light, and temperature) and the presence of toxicants.
The experimental results show a recovery of the algae after each exposure peak. The developed algae model is able to describe population dynamics in a flow-through system under exposure of isoproturon in close agreement with the experimental data. The model predictions were successfully verified by the experiments and the flow-through system in combination with the algae population model is a promising higher-tier tool to assess the effects of time-variable exposure on algae. The results make it possible to evaluate the potential of an algae population to recover after pulsed exposure patterns.

RA13A-6
Setting environmental quality criteria for groundwater: a comparison of current approaches
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Environmental quality criteria for groundwater have to date been largely based on guidelines for surface water protection, drinking water standards, or the analytical detection limits of some pollutants, with little consideration of the inherent value and protection of groundwater ecosystems per se and their unique biota. Importantly, risk-based approaches, such as species sensitivity distributions, that are widely advocated and used for setting surface water criteria have not been used for setting criteria for groundwater ecosystems, largely because of the lack of toxicity data specifically for groundwater biota.
Using toxicity data derived for groundwater invertebrates and microbial assemblages from aquifers in eastern Australia, we derive environmental quality criteria for common groundwater contaminants using a risk-based approach. We compare the degree of protection offered by the various approaches for criteria setting discussed above, compare our criteria for groundwater against existing criteria used in Australia and Europe, and consider future directions for groundwater ecosystem protection in light of the paucity of relevant toxicity data now and into the future.

RA13B-1

SSDs - Good idea, bad practice

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The concept of a Species Sensitivity Distribution (SSD) appeared more than 20 years ago. Since that time it has been widely used as the basis for establishing so called 'safe concentrations' for chemicals in the environment. While seemingly well-grounded in statistical theory the SSD lacks a credible nexus between statistics and ecology. Furthermore, the integration of statistics and ecotoxicology has been patchy resulting in a lack of statistical rigour in some aspects of ecotoxicological practice - the impact of which is neither fully understood nor appreciated.

RA13B-2

A Bayesian approach to probabilistic ecological risk assessment using SSDs: risk comparison of nine toxic substances in Tokyo surface waters

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Quantitative risk comparison of toxic substances is necessary to decide which substances should be prioritized to achieve effective risk management. We compared the ecological risk of nine toxic substances (ammonia, bisphenol-A, chloroform, copper, hexavalent chromium, lead, manganese, nickel, and zinc) in Tokyo surface waters by adopting an integrated risk analysis procedure using Bayesian methodology. Species-sensitivity distributions of these substances were derived by using four separate models. These models were different in the assumption about sensitivity differences at taxonomic group level. We then computed deviance information criterion values of the models and compared them to select the model with the best predictive ability. In the estimation of SSD, an advantage of our approach is that we are able to correct potential biases in the proportions of taxonomic groups in the ecotoxicity data. Environmental concentration distributions were derived by a hierarchical Bayesian model that explicitly considered the differences between within-site and between-site variations in environmental concentrations. Medians and confidence intervals of the expected potentially affected fraction (EPAF) of species were then computed by the Monte Carlo method using Markov chain Monte Carlo samples of the posterior distributions of species-sensitivity and environmental concentration distribution parameters as input values. The estimated EPAF values suggested that risk from nickel was highest and risk from zinc and ammonia were also high relative to other substances. The risk from copper was highest if bioavailability was not considered, although toxicity correction by a biotic ligand model greatly reduced the estimated risk. The risk from manganese was highest if a conservative risk index estimate (90% upper EPAF confidence limit) was selected. The presented risk analysis procedure using EPAF and Bayesian statistics is expected to advance methodologies and practices in quantitative ecological risk comparison.

RA13B-3

Developing a Species Sensitivity Distribution curve with Arctic species exposed to petroleum compounds and comparison with temperate species

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To identify and manage potential impacts associated with increased activities in arctic regions reliable risk assessment approaches for the arctic environment are required. Toxicity data on arctic species are, however, lacking and it is unsure if available toxicity data on temperate species also represent the sensitivity of arctic species. We performed toxicity test with 2-methyl naphthalene with 11 arctic and 6 temperate species from different functional groups. Based on the test results we constructed an arctic and a temperate Species Sensitivity Distribution (SSDs). The distributions were compared to determine whether arctic and temperate species differ in their sensitivity to 2-methyl naphthalene. Median estimates for the hazardous concentrations affecting 5 and 50 percent of the species (HC5 and HC50) based on both no effect concentrations (NEC) and 50% lethal effect concentrations (LC50) were up to a factor 2 higher for temperate species then for arctic species. However, due to overlapping uncertainty intervals around the median estimates of the arctic and temperate HC5s and HC50s, we cannot conclude that this factor of two actually indicates a difference in sensitivity between arctic and temperate species.

RA13B-4

Extending the SSD concept to explore some foundational model limitations: a Bayesian hierarchical approach

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Species sensitivity distributions (SSDs) are statistical constructs which model interspecies variation of sensitivity to a particular toxic stressor. The current REACH technical guidance document permits the application of SSDs in risk assessment, subject to a number of criteria. Notwithstanding noteworthy criticism received, the SSD is considered by regulators to be a pragmatic model for extrapolating to environmental toxicant concentrations of concern. The manner in which SSDs are currently applied is implicitly dependent on a number of (overlapping) statistical and ecological assumptions. These include (but are not limited to): (1) the measured species toxicity values being precisely known; (2) independence of SSDs for each separate chemical risk assessment; (3) *a priori* exchangeability of species toxicity values; (4) no correlation between species.

In this research we propose a model which generalizes the SSD concept to include chemical effects and shared species effects. It offers flexibility to address or refine each assumption by hierarchically adding layers into the model. Models are fitted to RIVM and US EPA acute-effect toxicity databases under a Bayesian statistical framework to allow for transparent quantification of and flexible propagation of uncertainty. Important insight is gained from the inclusion of 'species effects' modelling which, expectedly, indicates increasing differences as taxonomic distances in SSDs increase. The magnitude of measurement error estimated, based on within taxa homogeneity, which also properly accounts for censored measurements, is likely to be of significance to risk assessors and warrant further consideration in either modelling framework. The current quasi-meta-analysis approach towards aggregating multiple chemical-species data points is untenable from an uncertainty viewpoint.

Initial results indicate deficiencies in the current SSD concept, thus reducing the credibility and meaningfulness of any subsequently derived hazardous concentrations. Other recent model proposals which act as precursory tools to SSD modelling may not sensibly propagate uncertainty and/or succumb to modelling contradictions. A hierarchical model may overcome this, however will require a more radical approach to defining protection goals and environmental concentrations of concern.

RA13B-5

Species sensitivity distributions: how they can be improved?

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Species sensitivity distributions (SSDs) are cumulative distributions of species' physiological sensitivity to toxicants. An attractive attribute of SSDs is that they allow the severity of a particular concentration of a chemical to be estimated in terms of the potentially affected fraction of species. The severity of a pollution event is a critical component of risk: the severity of an undesirable event and the probability that it will occur. However estimates of the potentially affected fraction of species from SSDs are contingent on several assumptions that are unlikely to be met in conventional uses of SSDs. Recently various novel experimental, field and/or statistical methods have been suggested that will result in SSDs that better meet their assumption. At the same time there is growing recognition that physiological sensitivity of species to toxicants (as measured in laboratory tests) is only one aspect which will influence the response of populations to toxicants. Modelling a species' population will provide better estimates of the likely effect of chemicals on its population but is unlikely to be feasible for a large and representative sample of species from environments/regions of interest. The combining of resilience traits (e.g. generation time and dispersal capacity) and an avoidance trait (e.g. spending a significant part of the life-cycle removed from the contamination) and physiological sensitivity has proven very useful in developing biological indexes that can indentify community level impacts from specific classes of chemicals. Here we examine how these traits might be combined with physiological sensitivity to make SSDs that describe both types of sensitivity and review suggested developments to SSDs. We conclude that there are modifications that will improve the use of SSDs but that their widespread adoption is not a purely technical issue.

RA13B-6

On correct interpretation of Species Sensitivity Distribution output through eco-epidemiological analyses

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Species Sensitivity Distributions are statistical distributions which relate the concentration of a toxic compound (X) to the Potentially Affected Fraction (Y) at that concentration, with their oldest use being the derivation of protective Environmental Quality Criteria from laboratory ecotoxicity test data. Such protection-aimed EQCs, like the HC5 (the Hazardous Concentration for 5% of the species, also known as the 95%-protection criterion), are used in environmental policy to protect ecosystems from ecotoxic effects. There are also EQCs which trigger further research or remediation (e.g., the HC50). Despite this use in ecosystem protection or risk reduction, there is no ecology in these SSDs at all. Hence, questions have been posed on the whole idea of SSDs in relation to ecosystem protection and management.

This paper is concerned with the interpretation of SSD-output.

We have studied various large-scale (bio)monitoring data sets, in which each of the sampling points is characterized by a suite of environmental parameters as well as toxic compound mixtures. We found, that there is an association between the predicted toxic pressure of mixtures across sites and the magnitude of local impacts on the local taxa. Various types of association are shown. While this type of approaches evidently does not 'validate' the SSD-model, our analyses certainly give answers to questions like: (1) is there any relation between SSD-predicted impact and observed impact? (2) if so, is the relationship as expected (higher toxic pressure, higher impacts)? and (3) if so, what is then the use of SSDs in practice? We argue, that SSD-output is of use as ranking tool in various contexts, ranging from protection of ecosystems against chronic exposures, as well as environmental disasters involving toxic chemicals.

RA13C-1

Purposefully-quantified traits and kinetic parameters as predictors for intrinsic sensitivity

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Current ecological risk assessment (ERA) practices are based on the assumption that taxonomically-related species will show similar sensitivity to toxicants, using safety factors or species sensitivity distributions to extrapolate from tested to untested species. In community ecology, taxonomic approaches place limitations on our description and understanding of species assemblages in nature. Thus, it has been proposed that the systematic inclusion of species traits in ERA could provide a useful alternative insight for prospective approaches. At the same time, there is a growing recognition that the use of conceptual, mechanistic and quantitative models in ERA may improve predictive and extrapolative power. In order to better understand how structural and functional system facets may facilitate inter-species extrapolation we explore how purposefully-quantified traits can be linked to mechanistic effect models in order to predict intrinsic sensitivity. For this we use previously published data on the sensitivity, toxicokinetics and toxicodynamics of a range of freshwater arthropods exposed to chlorpyrifos, employing a purposefully-generated trait data set. The results of a quantitative linking of seven sensitivity endpoints and traits demonstrates that while it is possible to establish meaningful quantitative links between traits and/or trait combinations and process-based (toxicokinetic) model parameters, predicting classical sensitivity endpoints is more elusive. Future research needs include a quantitative linking of toxicodynamic parameter estimations and physiological traits, requiring further consideration of how mechanistic trait-process/parameter links can be used for prediction of intrinsic sensitivity across species and substances in ERA.

RA13C-2

Using functional traits to characterize the effects of pesticides on aquatic macroinvertebrate communities

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Biological and ecological traits reflect the ability of species to deal with environmental constraints and opportunities. Therefore, they can potentially be used to explain species perfor-

mances and co-existence with other species under particular environmental conditions, including the presence of toxicants. The potential of traits to support a mechanistic link between biological responses and disturbances make them relevant tools to study the relationships between biodiversity and ecosystem functioning. In this 20-month study, the impacts of four realistic pesticide exposure scenarios on trait composition of aquatic macroinvertebrate communities and on leaf litter processing were assessed in outdoor pond mesocosms. The responses of quantitative trophic functional diversity indices combining biological traits and species abundances (QRao and FDis) were also studied. In addition, a new index (Iadd) designed to estimate leaf breakdown rate from the structure of communities was developed and tested. This index is based on the combination of quantitative measurements of a performance trait (shredding efficiency) and abundances for keystone species for litter breakdown. Treatments caused serious impacts on functional attributes of communities and leaf decay. Respiration type was the more discriminating traits influencing the response of macroinvertebrates, which is in accordance with the fact that gills facilitate the assimilation of dissolved toxicants. However, some traits were found relevant for species sensitivity only because they were correlated with other traits, which highlights the importance of considering interrelations between traits to reduce potential errors in interpretation. Both quantitative functional diversity indices showed that trophic diversity was significantly reduced following pesticide treatments, due to an important decrease in the abundance of dead plant feeders. The reduction of leaf decay in treated ponds was a consequence of the effects on dead plant feeders abundance. Iadd proved to be a powerful predictor of leaf breakdown and therefore a promising method for forecasting litter breakdown dynamics from the abundance of keystone species. This work reinforced the idea that trait-based approach may be an efficient tool for understanding the mechanistic effects of chemicals on organisms and their consequences on ecological processes.

RA13C-3

In situ ecological risk assessment using taxonomic metrics and life history traits of macroinvertebrates

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The European Water Framework Directive (WFD) requires that Member States evaluate the quality of their water bodies using bioindicators like macroinvertebrates. In this work, we aimed at identifying relevant taxonomic and life history based metrics from which we built a first version of a new national tool intended to help experts in identifying stresses from survey of macroinvertebrate communities.
Faunal data comes from the application between 2005 and 2008 of a normalized protocol on 1795 sites distributed through all France (4190 sampling units). Chemical characterization of sites was performed considering 10 anthropogenic stress categories. A set of taxonomic and life history based metrics was calculated for each sampling unit. Considering the available information (chemistry and biology), we selected at least 6 "reference"sampling units (i.e. the least impacted as possible) for each river type (68 river types).
We transformed metric values in standardized deviations from reference. Then we used conditional trees, i.e. dichotomic classification method using Monte-Carlo permutation tests (α=0.01; 999 iterations) to decide if groups based on stress status are significantly different considering biological metrics. For metrics that showed a significant response to stress we quantified their Discrimination Efficiency (DE). We selected "generalist"metrics (DE > 30% for at least 7 stress categories) and "specific"metrics (DE > than 30% for at most 3 stress categories). The selected metrics were used to build conditional forests with different combinations of metrics based on their specificity and their DE. These models predict the risk for a sample to come from a stressed station according to its metrics values.
All our "specific"models are pretty good in classifying the samples from reference sites but classify samples from impaired sites with lower efficiency (True Negative Rate between 0.0952 and 0.6892). Incorporating generalist metrics increases the TNR of the models but decreases their specificity.
In this work, we showed that it was possible to construct models that help at establishing a diagnostic on the nature of stress from taxonomic and life history based metrics, we also demonstrated that metrics exhibit a trade-off between specificity and sensitivity and that life history metrics are more important than taxonomic ones in models intended to identify stress rather than detect stress.

RA13C-4

Development of a benthic macroinvertebrate flow sensitivity index for Canadian rivers: can biological traits support the interpretation of species responses?

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Anthropogenic pressure on flow regimes has been recognized as a leading threat to the health of Canadian river ecosystems and their protection requires guidelines based on sound science. To develop ecologically meaningful approaches for the management of riverine ecosystems the interaction between biota and flow variables is a critical step. However, this relationship is poorly understood and, as a consequence, over-simplistic hydrology-based guidelines for river management have been adopted without establishing clear indicators of their success or failure. Here, we support the improvement of guidelines for flow management by presenting a macroinvertebrate-based flow-sensitivity index for Canadian rivers. 2700 biological samples with associated environmental variables were extracted from Environment Canada's Canadian Aquatic Biomonitoring Network (CABIN) database. In addition, biological traits were extracted by two major sources of information on freshwater taxa. A Canadian Ecological Flow Index (CEFI) was developed based on current velocity preferences of common benthic invertebrate families. Tested in a multi-stressor environment, the index strongly responded to changes in hydraulic conditions. The index was further validated using two data sets from the west and east of Canada, indicating its potential nationwide applicability. Biological traits were used to validate the current velocity optima and to explain the response of both the scores and the overall index, helping understating biological properties of the response. In conclusion, we have developed a practical approach to evaluate relationships between flow and an important component of the river ecosystem measured in biomonitoring programs. This has facilitated the development of an index which has good potential as an indicator for the ecological effects of flow alteration. Moreover, we have identified how biological traits can help explain the responses of biological taxa to flow and we have identified a sub set of trait modalities potentially interesting for a development of a traits-based diagnostic for flow alteration.

RA13C-5

Trait-based approach and vulnerability analysis of soil communities affected by pesticide application

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The commonly used tools to assess ecotoxicological risk are lacking ecological realism, because they cannot predict the real consequences for natural communities. In recent years, ecotoxicology has moved towards an improved ecological realism developing new tools capable of accounting for the complexity of ecosystems. In particular, the concept of ecosystem vulnerability is now considerd an essential component of site-specific risk assessment. Vulnerability is defined as a function of sensitivity, susceptibility to exposure and recovery potential after a stress. Trait-based assessment is a tool for predicting vulnerability based on the hypothesis that the vulnerability of different species to a stressor can be predicted from their characteristics.
Though ecosystem vulnerability and trait-based assessment are two promising concepts and are used in modern ecotoxicology, only few studies are available in the literature, especially on the soil compartment.
A field survey was conducted in a vineyard in Northern Italy, under application of pesticides. Microarthropod communities were sampled from the vineyard and from two "control"stations 4 and 10 m outside the plant rows, and identified to the level of taxa (order, sub-order or family level). Morphological, life cycle, physiological and ecological traits were collected for these taxa, to create a big trait matrix. The matrix was intersected with the field survey results to identify which are the traits that cause a major or minor vulnerability to the stress due to pesticide application. The taxa more or less vulnerable were identified according to these traits.
In an alternative approach, traits were divided according to the three components of vulnerability. A score was assigned to each trait according to its relation with vulnerability, which was quantified applying these scores and the trait matrix, using an index. Results were compared to those obtained when using the trait based approach. Similarities and differences between the two approaches will be discussed.

RA13C-6

Soil invertebrates, the poor cousin of TERA ?

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The evaluation and monitoring of soil biodiversity is imperative to assess, understand, model and predict soil functions and its changes. Currently, soil quality measurements take little account of biological components. This is partly since taxonomy-based descriptions of ecosystems limit our ability to depict ecological responses to stress. One way to solve this problem would be to challenge the conventional view of biocenosis towards a perception of invertebrate through their biological/ecological attributes. The challenge is to define how functional traits interact with their environment and each others. This platform presentation we will be split in two parts.
First, we will discuss the reasons why soil invertebrates TERA is not yet developped to date. First, soil is probably one of the most species-rich habitats of terrestrial ecosystems but has received relatively little attention. The second problem is the difficulty of identifying soil invertebrates. One emergent way to solve these problems is the development of metagenomics. Another problem lies on trait ontology inconsistency. Finally, Statzner & Bêche (2010) identified that the greatest obstacle in TERA is the creation of a trait database for many taxa. This difficulty would be overcomed in few years since different intiatives emerge to collect and organize informations in multi-trait, multi-taxon databases.
In a second time we will present some encouraging results on this topic. In a first work, we aimed at elucidating soil macro-invertebrate response to trace metal (TM) contamination. We demonstrated that the shape of functional traits-TM concentration relationships varied, reflecting additive or threshold effects. A second study was dedicated to the early pedogenesis of a technosol built with former coking plant and a combination of thermally treated industrial soil mixed with exogenous organic materials. Three years after Technosol building, our results permit to elucidate different colonisation strategies, and demonstrate the increase of sapro- and geophagous invertebrate that are crucial to organic matter dynamics in the system.
In this communication, we brough some evidences that soil invertebrate TERA can be an alternative to traditional assessement of invertebrate responses to environment and environment changes. However, the improvement of soil invertebrate TERA will require a coordinated international effort to achieve its full potential, by surmounting the currently cited brakes.

RA13D-1

Pollution-Induced Community Tolerance (PICT) in ecological risk assessment - future prospects

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There is a gap between the European regulatory systems for hazard and risk assessment of chemicals, and the systems for assessment of ecological and chemical status of ecosystems. This is partly because chemicals in the ecosystems occur as everchanging environmental mixtures for which the joint toxicity is rarely estimated, and partly because short-term, physiologically oriented test systems have only little coupling to important ecological events and processes, including natural selection. Short-term test systems may provide knowledge about the instantaneous sensitivity of a clone or a group of individuals, and might be used to describe mechanism of action and the metabolic syndrome associated with the toxicity of an individual compound. Such test systems also ignore crucial ecological interactions as well as the ability of natural populations to adapt to stressors. In the environment however, organisms will be subject to a variety of selection pressures (natural as well as antropogenic including toxic chemicals). These selection pressures shape populations and the biotic communities of contaminated ecosystems.
Toxicants cannot damage a biotic community in an ecologically relevant way without exerting selection pressure on species or strains of its populations. PICT is an essentially ecological tool based on detection of the outcome/consequences of selection at the community level. During the long-term selection phase of a PICT study, toxicants eliminate sensitive individuals and species and replace them by tolerant ones [1]. In the detection phase any hereby induced community tolerance to suspected toxicants is quantified in short-term tests, in a way that combines the Koch´s rule elements. We suggest that Pollution-Induced Community Tolerance due to its sensitivity, its potential causal links, and its connection to selection processes should be used as a complement to present approaches for describing ecological status.
The aim of the present paper is to outline the procedure for PICT studies in risk assessment and to discuss the possibilities of coupling PICT to other approaches like species sensitivity distributions and metagenomics.

RA13D-2

The effects of varying environmental conditions on the toxicity of mine effluent constituents to coldwater fish

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Due to method standardization, current regulations may not afford protection against adverse effects of mine developments under non-standard pH, temperature, and hardness conditions of arctic waters. These effects may be due to liberated minerals, processing by-products, and service community wastes. Canadian mines are controlled under the Fisheries Act, regulating "Any substance that is[3DOTS]deleterious to fish or fish habitat or to the use by man of fish that frequent that water". These regulations limit effluent parameters based on toxicological evidence produced via assay methods published by Environment Canada. For coldwater fish, rainbow trout is used as a surrogate and employs conditions optimal to that species. In contrast, locations of concern exhibit temperatures 10°C colder than standard test conditions, hardness 2-10 times lower than dechlorinated water, and variable pH due to contamination and natural geology.

The study aims to characterize the variability of effluent toxicity across a range of species and conditions while assessing whether the current standard test is protective enough to cover this variation by comparison of standard and 'amended standard' (varied conditions) assays. Eighteen contaminants (NaCl, NaNO2, NaNO3, Na2SO4, KCl, CaCl2, Cd, Cr, As, Cu, Zn, Mg, Ni, Al, MoO4, Se, NH4OH, NaClO, and toluene) were tested using five species of fish native to Northern Canada (arctic charr, lake trout, lake whitefish, round whitefish, and arctic grayling) across a range of pH (5.5, 6, 6.5, 7, 7.5, and 8), temperature (5,10, and 15ð-ðC), and hardness (30, 60, and 120ppm) conditions and their observed effects modeled as 24,48,72, and 96-hour LC50 values.

By comparing these results to each other and to those of the standard rainbow trout test, we have assessed the effect of pH, temperature, and water hardness on contaminant toxicity and time-to-toxic effect within and between species while characterizing the ecological protection afforded by current regulations. The data suggests that, in some instances, the standard has failed to capture the potential toxicity exhibited in non-standard species and non-standard, real-world conditions. Should this variability of non-standard conditions exceed the resiliency of the standard test to act as a surrogate, the mandate of the Fisheries Act to provide adequate protection to fish, fish habitat, and human use of fish will not be met. This potential risk is guiding the project's second phase of research.

RA13D-3

SPEAR index adaptation to lentic/outdoor pond mesocosm studies: an assessment of long term vulnerability to pesticides

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Lentic outdoor pond mesocosms are valuable tools for the ecological risk assessment of toxicants, although few synthetic metrics exist to describe overall effects of treatments on the invertebrate communities they host. SPEcies At Risk (SPEAR) metrics have originally been developed for the assessment of the response of river benthic invertebrate communities to toxicant exposure. However, their use for non lotic ecosystems has not been evaluated so far. Such application requires considering the differences between lentic and lotic ecosystems in the species traits used. Both community exposure pattern as well as species mobility were reconsidered in a lentic variant (LSPEAR) we developed. Results of the application of the original SPEAR method, as well as its LSPEAR variant on a dataset obtained during a 16-month long outdoor pond mesocosms study are presented. Benthic invertebrate communities were sampled every 3 weeks in a series of 16 outdoor ponds (9m3, max. depth 0.90m). 12 ponds were split into two groups (CS and LS) treated respectively with mixtures of pesticides corresponding to two alternative Wheat-rape seed crop treatment strategies and 4 ponds were kept as controls. Each of the two classification systems (SPEAR/SPEnotAR and LSPEAR/LSPEnotAR) were compared with the effects on the abundance of macroinvertebrate taxa deduced from Principal Response Curve analysis performed separately for each strategy. PRC showed significant differences between control and treated ponds communities immediately after the beginning of treatments in November 2008 for CS ones and in spring 2009 for LS ponds. All treated ponds communities were restored by late august 2009. According to the original version of SPEAR, 12 taxa out of 54 were supposed to be at risk whereas only 4 taxa met the criteria to be at risk in the LSPEAR. For both treatment strategy, no significant difference was shown for mean bk values between SPEAR and SPEnotAR taxa (Kruskal-Wallis test, $p>0.05$) whereas a significant difference was shown between LSPEAR and LSPEnotAR taxa, therefore indicating that LSPEAR successfully grouped the most affected taxa according to the PRC. This suggests that the adaptation of SPEAR to lentic systems is feasible but that it necessitates reconsidering the traits used in its construction. It also emphasizes how species composition of pond invertebrate communities may be determinant for their sensitivity and restoration ability after exposure to a toxicant.

RA13D-4

Sensitivity analysis and calibration issues for an aquatic ecosystem model

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In order to assess potential effects of toxicants at the ecosystem level, modeling reveals to be a powerful tool by considering species interactions, and by predicting toxic effects on non-target species populations (indirect effects). The aims of our work are: (i) to develop a new mathematical model which comprehensively describes a whole aquatic ecosystem accounting for species interactions with a clear set of equations including both abiotic and biotic factors; (ii) to perform a sensitivity analysis, i.e., to highlight parameters having the greatest influence on calculated target endpoints in order to simplify further calibration; (iii) to optimize model parameter values using semi-field experimental data from mesocosms; (iv) to incorporate perturbation functions on chosen processes within the model in order to predict potential toxic effects on populations and to identify functional groups at risk.

Following an extensive literature we build a compartmental ecological model for a whole aquatic ecosystem. Compartments include primary producers (macrophytes and algae from phytoplankton and periphyton), primary consumers (juvenile fish and invertebrate grazers, shredders and collectors) and secondary consumers (invertebrate predators and fish). All compartments are related within a food web as well as to abiotic factors like light, temperature and nutriments. We first calibrated and then tried to validate the model on three experimental data sets from mesocosms (specifically, the data from the control mesocosms).

During the talk, the sensitivity analysis method as well as the calibration step approach will be presented in detail for the 'Periphyton-Grazers' sub-model, which constitutes a small prey-predator entity, with a smaller number of parameters (~20) than the complete model (~260). The model calibration provided new values for the parameters that were found to have an important influence on the model output. These values will be presented, during the talk, as well as simulations of the ecosystem dynamics under the non-contaminated conditions. We will finally discuss how the choice of the perturbation functions impacts the ecosystem dynamics, and thus illustrate how useful such a modeling approach could be for ecological risk assessment.

RA13D-5

Reviewing 15 years of European risk assessment: what if we had used models to assess ecological effects?

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Ecological effect assessments estimate threshold concentrations of chemicals that do not result in adverse effects on ecosystems. Two types of models that use single species effect concentrations (ECs) have been proposed to assist in such assessments: species sensitivity distributions (SSDs) and food web models. Here, we review the risk assessments of 123 existing chemicals evaluated between 1996 and 2009 to examine if SSDs and food web models could have been useful in obtaining ecological threshold concentrations for these chemicals. From the 123 chemical we considered, an effect assessment could only be performed for 77 chemicals, largely because of inadequate data availability. Median hazardous concentrations for 5% of the species (HC5) estimated by the food web model were better predictors of community NOECs measured in micro- and mesocosms than HC5s produced by SSDs, although community NOECs were only available for 9 out of 77 chemicals. Precision, quantified as inverse of the 90% confidence interval (CI) of the HC5s, could only be compared between both modelling approaches for chemicals with > 10 ECs available as significant SSDs could not be constructed for chemicals with less data. Precision of the food web model decreased with increasing sample size (from 1 to 10 ECs) but remained relatively constant at sample sizes > 10, at which it was comparable to that of chronic SSDs.

RA13D-6

Microsporidia parasites in Gammarid: a confounding factor in the evaluation of cadmium toxicity

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Microsporidia are obligate intracellular parasites thought to be closely related to fungi. They are infecting a wide range of hosts inhabiting several environments (freshwater, marine or terrestrial). Microsporidia may use horizontal and/or vertical transmissions. Vertically-transmitted parasites are common in freshwater amphipods, where they can reach high prevalence and have several effects on host sexuality and behaviour. Because of their ubiquity and their obligate parasitic status, they are supposed to disrupt physiology of their host, but curiously, few studies have been devoted to search for the effects of parasite on energy reserves and how they interact with the antitoxic defence capacities of their host. Here, we investigated how parasites of Gammarus roeseli affect total lipid and glycogen contents, in parallel with reduced glutathione concentration, γ-glutamylcystein ligase activity and metallothionein concentrations as defence capacities. Toxic effects were highlighted by means of malondialdehyde measured as a biomarker of toxic effect. This study was carried out in physiological conditions and after cadmium exposure in laboratory conditions.

Two microsporidia species were detected in G. roeseli: Dictyocoela roeselum (prevalence = 68%) and Dictyocoela muelleri (32%). No male was infected while 72% of females harboured one of the two parasite species. In physiological conditions, there was no significant difference between uninfected and infected females for the different parameters, except for metallothionein concentration which was significantly higher in D. roeselum-infected females. After the exposure for 96 hours at two concentrations of cadmium (2 µg Cd.L-1 and 8 µg Cd.L-1), differences in energy reserves and antitoxic responses were observed between microsporidia-infected females and uninfected ones. The general observed pattern is that infection by microsporidia led to a larger antitoxic defence mobilization, as if the presence of parasites in the host cytoplasm (a biological "toxic") acted in synergy with the chemical toxic. Therefore, the detrimental effect of these parasites on host condition was evidence only under additional stress (here the cadmium pressure). More practically, this study highlights the necessary to take into account parasitological infections in ecotoxicological studies.

RA14 - Waste fluxes around the world and the associated risks

RA14-1

Environmental and health risks of chemical additives and recycling materials

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1. Introduction

Products undergo a recycling process and make their ways into a recovered material with unpredictable and not foreseen health and safety problems. The primary aim of RISKCYCLE is to identify future R&D needs required to establish a risk-based assessment methodology for chemicals and products that will help reduce animal testing while ensuring the development of new chemicals and product management pattern leading to minimized risks for health and the environment. The project is focussing on consequences due to the behaviour of chemicals and their release during recycling of the six fractions: paper, electronics, leather, lubricants, plastics, textiles.

2. Specific objectives of the project RISKCYCLE

The global trade of chemicals and products containing chemical additives such as paint, cosmetics, household cleaners, paper and cardboard, plastic toys, textiles, electronic appliances, petrol, lubricants etc. has resulted in a substantial release of harmful substances to the environment with risk to man and nature on a worldwide scale. Unpredictable and not foreseen health and safety risks due to recycling processes of products, which make their ways into a recovered material, are major issues of today`s waste management.

In spite of some common efforts to harmonize the safety assessment of chemicals and products a new problem with Recovered Material. The new threat is coming from closing the loop in a global scale. Plastic, paper and cardboard, lubricants and other products undergo a recycling process and make their ways into a recovered material with unpredictable and not foreseen health and safety problems.

The critical points throughout the products life cycle for the release of chemical substances and the hazardousness of the material set free will be evaluated. Beyond this it is also important to know if the effects caused by the chemicals have a global or only local meaning and if the release

of specific substances in the circular economy is an actual risk or a perceived risk.
Within the project the following key pieces of information will be required and collected: where are critical points throughout the products life cycle (for the release of chemical substances), how hazardous and toxic is the material set free, effects caused by the chemicals (global or regional meaning), risk (actual or perceived) of the release of specific substances in the circular economy.

RA14-2

Review of models used for human health and environmental risk assessment

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The coordination action RISKCYCLE aims at establishing and coordinating a global network of international experts and stakeholders to define together future needs of R+D contributions for innovations in the risk-based management of chemicals and products in a circular economy of global scale. RISKCYCLE is focused on the fate and behaviour of additives used in six industrial sectors (textile, electronics, plastics, leather, paper and lubricants). Some of these additives are nonylphenoxy acetic acid, polybrominated diphenylethers, hexabromocyclododecane and triclosan, among others.
The goal of risk assessment of a chemical emission is to evaluate if the risk to any receptor (environmental compartment, population, individual) will be acceptable or not. For the last twenty years different models and methods have been developed to predict the impact of a chemical released into the environment in the context of risk assessment. Within the framework of RISKCYCLE project, a selection of risk assessment models has been done. The analysed models have been Ecopoints, ECOSENSE, EDIP method, Eco-indicator 99, USES-LCA/EUSES, Caltox, GLOBOX and USEtox, among others. Their weaknesses and the strengths have been analysed.

RA14-3

Life cycle assessment and additives: state of knowledge

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Concerns about possible toxic effects from additives/impurities accumulated in globally recycled waste/resources like paper and plastics was one of the main reasons for starting up the EU FP7 Coordination Action project RiskCycle (www.wadef.com/projects/riskcycle). A key aim of the project is to identify research needs within this area focusing on both risk assessment (RA) and life cycle assessment (LCA). Besides the sectors on paper and plastics also lubricants, textiles, electronics and leather are included in RiskCycle. On plastics a literature review regarding the state of knowledge on additives/impurities in LCA has been performed within RiskCycle. Several inventory databases (LCI data) have been investigated and the result shows that most LCI databases use PlasticsEurope data for plastics production. Most of these data are aggregated and do not include additives. Regarding the production of additives only data on metals and DEHP was identified. As regards LCAs on plastics 110 papers has been reviewed. Only 25 of these mention additives but they are not included in the emissions list. Only a few studies include additives in the impact assessment and additives are never mentioned as important for the outcome. Regarding LCAs on printed matter (including paper) only a few studies have been done - mostly focusing on the energy part. However, one of the most recent and comprehensive studies actually include toxic impacts from chemical emissions - mostly printing chemicals like printing ink of which some components may accumulate in recycled paper. Even though recycling is included in this recent study there is no special focus on the additives/impurities in the recycled paper. Anyway, the study shows that potential toxic impacts from the production and use of chemicals like pigments, solvents, metals, AOX and biocides may play a very significant role in the impact profile of printed matter. Regarding the life cycle impact assessment (LCIA) part an investigation of the availability of characterisation factors (aquatic ecotox) for the about 17 additives/impurities to be included in RiskCycle have been done. These additives belong to 15 chemical groups/chemicals like PFOS, PFOA, NPAA, HBCDD, triclosan and more. The best practice LCIA "consensus"model USEtox was chosen. For only nine of the 17 substances characterisation factors exists and four of these are preliminary (interim). Regarding the rest, factors have to be calculated - if possible.

RA14-4

Occurrence assessment of PFCs in food, drinking water and biological human samples in Spain, and global comparison

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Perfluorinated compounds (PFCs) comprise a large group of compounds widely used in industrial applications that are characterized by a fully fluorinated hydrophobic linear carbon chain attached to one or more hydrophilic head. PFCs repel both water and oil, and are therefore ideal chemicals for surface treatments. PFCs have been manufactured for more than 60 years, and released into the environment following production and use. Two of the most important PFCs are perfluorooctane sulphonate (PFOS) salts, components of fire-fighting foam concentrates, and perflorooctane acid (PFOA), primarily used as emulsifier in industrial applications.
PFCs are ubiquitous environmental contaminants, which persist and may bioaccumulate through the food chain. These compounds have been detected worldwide in sediments and biota. In recent years, an increasing number of papers report high levels of PFCs in blood, tissues, and breast milk from both occupationally and non-occupationally exposed human populations.
The most important exposure pathways of perfluorinated compounds for humans are thought to be intake of drinking water, food and inhalation of dust. Due to the widespread distribution, environmental degradation, and metabolism of the PFCs released into the environment, a very complex exposure situation exists. As a result, the relative contribution to human exposure from different routes or from a single source is not yet known. Because of their bioaccumulation and potential health concerns including toxicity, and their possible contribution to cancer promotion, non-governmental organizations, national and international authorities have addressed the PFCs problem by several pressure and legislative actions.
This communication will present the concentration levels of 18 PFCs different type of matrices including drinking water and food, as well as, the levels encountered in breast milk, urine, cord blood and hair of donors from Spain. The analytical techniques used for their analysis along different studies performed by our group will be presented. Finally, results of exposure and human bioaccumulation in Spain will be compared at global scale by comparison with results reported worldwide. Relations between occurrence levels and ambient factors, drinking water pollution and type of diet will be as well discussed.

RA14-5

Toxprint: an easy tool to translate process mass flow into toxicological impact

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In the last years, an huge number of techniques has been developed to estimate the possible adverse effects on human health and ecosystems induced by the release of chemicals by human activities, in particular incinerators, landfills and industrial plants. The most important and spread techniques are human risk assessment (HRA), ecological risk assessment (ERA) and Life Cycle Assessment (LCA). HRA and ERA evaluate the induced risk accounting of the direct exposure of targets while LCA, instead, assess each impact associated with all the stages of a process. These tools are often very difficult to perform and require time to form experts and to perform the analysis. In order to save money and time, we are proposing Toxprint, an easy way to evaluate the toxicological impact of plant processes using data on their mass flow and their chemical characterization. Toxprint is a simplified tool that integrate the mass flow of a process, its toxicological and ecotoxicological analysis and the environmental fate of the chemicals characterizing the process. From the analysis of process mass flow, some compounds are selected as indicators of the entire process based on their toxicological profiles, environmental properties and quantity. For each chosen indicators, the Equivalent of Toxicity (ET) will be defined, depending on the nature of the compound (toxic or cancerogenic chemical). The Index of Toxicological Impact (ITI) and the Index of Ecotoxicological Impact (IEI) are then calculate for each compound as ratio between the quantity of compound and its ET or PNEC, respectively. The Environmental Fate and Transport (EFI) analysis of the compound is based on its water solubility, mobility, volatility, bioconcentration factor and persistence. The Toxprint of each indicator is calculated by the formula Toxprint_compound= (ITI+IEI)[GREEKX]EFI and, finally, the Toxprint of the process is estimated adding the single toxprint of compounds.
The integrated approach is applied to the leachate of a landfill in the Northern Italy which was subject of a 10-years monitoring program performed by our laboratory. Thirteen chemicals were selected as indicators due to their abundance and their frequency of finding, including heavy metals, phenols and other organic compounds. For the investigated site, the most concerning leachate components are copper and iron.
The proposed tool was partly founded by the EU project RISKCYCLE and Italian project FIRB.

RA14-6

Application of an integrated approach to evaluate health risks for toxic chemicals by linking multimedia environmental and PBPK models

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The paradigm of health risk assessment may consist of two main pillars, i.e., the exposure and dose-response assessments. Human exposure to chemicals via multiple pathways can be estimated by environmental multimedia models, which calculate the distribution of chemicals in the component media, i.e., air, water, soil, plants, and animal media. Combined with the information about human behaviors such as dietary habits, time spent outside, and etc, the multimedia models can provide an estimation of the daily chemical intake by inhalation or ingestion by humans. Physiologically based pharmacokinetic (PBPK) models are used to estimate the body burden of toxic chemicals throughout the entire human lifespan, integrating the evolution of the physiology and anatomy from childhood to advanced aged. The use of such PBPK models overcomes the limitations that dose-response modelling holds, e.g., it simply determines the relationship between the dose and the probability of an effect.
The European project 2-FUN (Full-chain and UNcertainty Approaches for Assessing Health Risks in FUture ENvironmental Scenarios) aims at improving the approaches currently used in exposure and dose-response assessments. According to the aim of that project, an environmental multimedia model and a generic PBPK model are coupled as an integrated tool (2-FUN tool) and built up on a platform system, Ecolego®. This study presents here the first application of the integrated tool to perform the full-chain risk assessment of a chemical for human health, considering multiple exposure pathways of chemical via inhalation of out-door air, and ingestion of water and foods. For this application of the tool, a case study was designed based on the information available in a region situated on the Seine river watershed, downstream of the Paris megacity and Benzo(a)pyrene (B(a)P) was selected as a target chemical substance. This study focuses especially on the propagation of uncertainty and inter-individual variability along the modelling chain. A probabilistic simulation was then performed to identify the input parameters and exposure pathways sensitive to model outputs (e.g., internal effective concentrations in organs).

Poster abstracts

EC01 - Advances in passive sampling and dosing techniques

MO 002

Passive sampling for the monitoring of organic pollutants (PAHs, BTEX) in groundwater. Application to a former industrial site.

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Classical techniques for groundwater sampling can affect the measurement of chemical composition of water. Sampling devices such as low-flow peristaltic pumps can sample water slowly from wells to obtain representative samples but can also involve sorption of hydrophobic compounds to the tubing and losses of volatile compounds through volatilization. In this context, passive sampling technology presents several advantages associated with a low perturbation of the sample, including simplicity, low cost, no power requirement and possible estimation of time weighted average (TWA) concentration of pollutants.

The aim of this work is to present results obtained with two passive sampling devices, an integrative passive sampler (SPMD - Semi Permeable Membrane Devices) and an equilibrium passive sampler (PDBs -Passive Diffusion Bags) for respectively the estimation of PAHs and BTEX concentrations in groundwater at a former industrial site. Results were compared with those from classical analysis (LC/UV/fluorescence and ITEX/GC/MS for PAHs and BTEX respectively) on water samples obtained with a discrete interval sampler. The discrete sampler allows sampling with minimal disturbance of the water in comparison with classical sampling. SPMD were deployed for several time durations to estimate the kinetic accumulation of compounds. PDBs were deployed to estimate the BTEX concentrations in groundwater. First results demonstrated that BTEX concentrations in PDBs samplers were in good agreement with those estimated in water samples with the discrete interval sampler. This demonstrates that PDBs can estimate the VOC concentrations in the well at a defined depth. For most of PAHs, the kinetic accumulation was linear over the deployment period. For some PAHs, time average concentrations (TWA) estimated with SPMD could be in good agreement with those found in water samples.

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MO 003

Applicability of passive sampling to groundwater monitoring

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Passive sampling technology has become of great importance in the field of environmental monitoring for several years, due to its well-known advantages (low perturbation of the sample, time weighted average concentration estimation [3DOTS]). Although passive samplers have been successfully used in a variety of field studies in surface waters, only a few studies have tested their applicability in groundwater. Indeed, groundwater presents specificity such as a low velocity of water which might affect the uptake of compounds in passive samplers. Moreover, the use of passive samplers in groundwater supposes the water in the well to be representative of the whole groundwater.

The aim of this work is to test several passive sampling devices (DGT, POCIS, PDBs) for the monitoring of metals, pesticides, and volatile organic compounds (VOC) in groundwater in order to identify the pros and cons of this technique for groundwater applications. Several campaigns of measurements were conducted in order to compare the results obtained by passive sampling with those obtained by classical sampling. All passive samplers were deployed in replicates in a well and at different depths to study the stratification of pollutants. First results demonstrated that:

- A good repeatability is observed on the passive sampling results,
- VOC concentrations obtained by PDBs are in accordance with those obtained by classical sampling in the well. These results highlighted that PDBs allow the measurement of the VOC concentrations at different depths easily,
- In some cases, the low water velocity seems to limit the uptake of compounds for integrative passive samplers. This factor has thus to be taken into account for the calculation of the concentration in water.

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MO 004

Occurrence and fate of pesticides in Arcachon's bay using the passive sampler approach

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Conventional water sampling techniques are time consuming and cost effective, especially when heavy logistical organisation is required to assure a high frequency sampling that is necessary to take into account the variability occurring in pollutant contamination.

Indeed, the chemical characterisation of coastal waters and of transition water systems such as estuaries or bays is complex because important dilutions and important temporal variability usually occur. To overcome the problem posed by the temporal variability of the water concentration, various passive sampling techniques were developed allowing the estimation of the mean water concentration of the pollutants over the exposure time.

The present work thus aims to evaluate the presence and the sources of pesticides in the Arcachon Bay (South West coast of France). Various environmental problems have occurred these last few years in this quite complex and vulnerable ecosystem with regression of sea grass, alteration of oyster physiological condition... In view to understand the potential role of chemical contamination on these phenomena, water bay contamination was monitored. Indeed among all the potential parameters that can affect water quality, chemical contamination is crucial to document and among all the potential suspected contaminants, pesticides can play a significant role. To evaluate pesticide sources and to precisely characterize their presence, antifouling and various herbicides were monitored in the main tributaries and in also directly in Arcachon Bay by discrete and passive samplings. Both types of sampling were compared. Furthermore tributary contribution to the Bay contamination was evaluated.

MO 005

Improvement and application of the polar organic compound integrative samplers (POCIS) using Performance reference compound approach for pesticides and pharmaceutical substances

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Since few years, the application of POCIS to monitor hydrophilic compounds in dissolved medium is commonly used. Unfortunately, the sampling rates of these tools are affected by several environmental parameters like hydrodynamic conditions, temperature and bio-fouling. Consequently, the sampling rates calculated in laboratory experiments are not adapted for the majority of environmental assessments. To overcome this phenomenon, the performance reference compound approach was developed during the 90's for the Semi Permeable Membrane Devices (Prest et al. 1997) and the same approach was later adapted to the POCIS tools for the pesticides using the Desisopropyl Atrazine d5 as reference compound (Mazzela et al. 2007/2010).

The PRCs are put into the receiving phase of the sampler before the exposure. During the exposure, the PRCs are eliminated from the receiving phase. Their elimination rates allow to determine the specific sampling rates of target analytes in the in situ condition.

In this study the PRC approach was developed for the pharmaceutical pollutants sampled by POCIS. A first experiment was performed to determine the pharmaceutical compounds which can be used as PRC, among which the Caffein C13, Salbutamol d3 present a satisfactory behaviour. In a second time, a laboratory experiment was realised to calibrate the previous PRCs and the Desisopropyl Atrazine d5. Simultaneously the sampling rates of pesticides and pharmaceutical compounds were evaluated . Finally, the devices were exposed in the Gironde estuary during one year to evaluate the pesticide and pharmaceutical contamination of this ecosystem.

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MO 006

Pesticide monitoring of Arcachon bay using various passive sampling techniques

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The present work focuses on the study of the pesticide contamination of the Arcachon bay in the south west coast of France. This hydrological system is extensively used for a large number of activities (fishing, agriculture, tourism). In addition, many tributaries which receive drainage and runoff waters from the surrounding irrigated fields of various cultivated areas contribute to the water bay potential contamination. In this context, and since few years, several research programs have investigated the water contamination to evaluate pesticides pressure on this ecosystem. For this purpose, the passive sampling approach was frequently used to assess the global contamination of the water bay since it overcomes drawbacks of spot sampling approach which gives only the contamination at the time of sampling and not at all integrated assessment of it..

In this study, the monitoring of the water bay pesticide contamination was completed using simultaneously the grab sampling and passive sampling approach during one year.

Three types of passive samplers were used : The Silicon rubber samplers which can trap the most hydrophobic pesticides, the polar organic integrative samplers (POCIS), and an adaptation of the classical POCIS which are made with a highly porous membrane to reduce the mass transfer resistance and improve the detection limit of this tool. These three passive samplers exposed in the same environmental medium allow to evaluate the performance of each, their advantages and drawbacks for various compounds present in the water of the bay.

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MO 007

Screening for Stockholm Convention POPs in Bosnia and Herzegovina using passive methods

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In contrast to the significant quantity of information available regarding the occurrence of persistent organic pollutants (POPs) in the European Union (EU), there is a paucity of data concerning concentrations of such compounds in Bosnia and Herzegovina (BiH). Additionally BiH has just recently signed and ratified the Stockholm convention, although its implementation hasn't started yet largely due to the country's complex socio-political situation. In an attempt to improve this we have carried out a suite of screening studies in BiH during the period 2007-2011. Two of the primary aims of these projects are capacity building for regular environmental monitoring and the identification of point sources and compounds of concern. In this regard the use of passive sampling devices (PSDs) is an advantageous approach allowing, unattended time integrated measurements, low detection limits and relatively inexpensive and straightforward laboratory procedures. In the present work we present data from several studies where semipermeable membrane devices (SPMDs) were deployed in two major rivers; the Neretva and the Bosna. Target compounds included polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs). Additionally we tested the applicability of cheaper low density polyethylene PSDs including the use of fluorinated PAH (F-PAH) as performance reference compounds and briefly compared concentrations to those found in locally caught fish and large volume water samples with extraction by polyurethane foam (PUF). Levels of POPs were generally in the low pg L-1 range, with a clear gradient in exposure shown downstream in the Neretva River with higher and more variable concentrations found in the more industrially influenced Bosna River. Similar to previous results LDPE accumulations compared well to those of SPMDs where deployment equipment, surface areas etc. were similar. Differences between PSDs and the other matrices examined are discussed in terms of their chemical and phase selectivity and of their suitability for addressing the aims of the project.

MO 008

Combining passive sampling (PSII herbicides) and remote sensing (water quality) data on the Great Barrier Reef, Australia

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The Reef Rescue Marine Monitoring Program (MMP) was established to assess any change in water quality on the World Heritage Area Great Barrier Reef (GBR). Annual monitoring

of the concentrations of photosystem II inhibiting (PSII) herbicides such as diuron, atrazine, hexazinone and tebuthiuron has been conducted at inshore reef sites along the GBR for up to five years using passive sampling techniques. As a result of this a unique set of data is now available that provide seasonal and regional trends of monthly or bimonthly average concentration estimates of these chemicals in the water column at these sites. In addition to pesticide monitoring at relatively few sites, remote sensing has been used to provide high spatial resolution data on the concentrations of water quality parameters (total suspended sediment, chlorophyll-a and coloured dissolved organic matter). One of the dominant contributors to declining water quality within the GBR are terrestrial discharge derived inputs of elevated nutrients, sediments and agricultural chemicals from adjacent catchments. There is some evidence for synergistic effects between specific water quality parameters and PSII herbicide concentrations on crustose coralline algae which may influence the structure and functioning of coral reefs. The aim of this work is to provide a preliminary evaluation of the statistical relationships between remote sensing derived water quality parameters and the concentrations of specific PSII herbicides on the GBR. The information gained from this work may be used to inform research into the effects of multiple stressors such as a decline in water qualtiy and exposure to PSII herbicides, on reef ecosystems. Furthermore it may eventually provide a tool that can be used for predictive purposes and to evaluate whether changes in pesticide usage and/or land management results in future differences in the observed relationships at these specific locations.

MO 009

Comparison between in situ Stir Bar Sorptive Extraction and the Polar Organic Chemical Integrative Sampler for the passive sampling of agricultural pesticides

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The monitoring of organic micropollutant contamination in water bodies and the determination of reliable concentration estimates have become challenging issues in Europe, since the Water Framework Directive (WFD, European Commission, 2000) has aimed to improve and protect european rivers by 2015. For the determination of the concentrations of micropollutants such as pesticides, several sampling techniques can be employed. The pertinance of grab sampling can be questionable in case of temporal variations of these concentrations in rivers, when it is performed at low frequencies. Automated or active sampling allows more reliable estimates of the contamination because averaged samples can be taken over a large time period. Nevertheless, this technique is often time-consuming and expensive for the purchase, as well as for the utilization and the maintenance. Integrative or passing sampling has recently been developped in order to obtained, at lower cost, realistic estimates of the contamination levels of the aquatic environment. The polar organic chemical integrative sampler (POCIS) is one of the main devices used for the passive sampling of the moderately polar organic compounds. Its efficiency in the determination of time-weighted average concentrations of hydrophilic pesticides has been reported in the literature. Stir bar sorptive extraction (SBSE) is a solvent free sample preparation technique dedicated to organic compounds in aqueous samples. It is composed of a magnet enclosed in a glass tube coated with a thick film of polydimethysiloxane (PDMS). The *in situ* application of this technique has also been reported in the literature.
Stir bars and POCIS were deployed for two weeks in two rivers of a french agricultural watershed. The aim of this study was to assess the performances of the SBSE applied *in situ*, and to compare them with the POCIS results, regarding the passive sampling of agricultural pesticides in surface waters. Good repeatability and sensitivity of the *in situ* SBSE have been obtained for the hydrophobic pesticides, and increasing concentrations of micropollutants have been determined from the up-stream to the down-stream of the rivers sampled. Differences in the nature and the amount of the target compounds accumulated on the two sampling devices have allowed the authors to suggest the SBSE as a complementary passive sampling technique for the monitoring of more hydrophobic pesticides in the aquatic environment.

MO 010

Affinity of Chemcatcher's receiving phase for polar herbicides sampling

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The occurrence of agrochemicals in water resources requires a regular monitoring for water quality. The conventional method used for monitoring relies on discontinuous spot sampling at fixed time intervals. However this method is not an efficient way to assess periodic fluctuations of pollutants concentration. Throughout the last decades, an alternative sampling technique called passive sampling has been used successfully for various pollutants monitoring in aquatic environments. Passive samplers represent a promising alternative to grab sampling as they continuously accumulate dissolved organic pollutants from water and allow the measurement of time weighted average (TWA) concentrations.
Different types of samplers are commercially available among which we focused on Chemcatchers®. It consists of a polytetrafluoroethylene sampler body in which different solid phase materials and diffusion membranes can be incorporated in order to optimize the accumulation of a wider set of target compounds. This accumulation depends on diffusion mechanisms, particularly into the receiving phase.
The presented work addresses the topic of polar herbicides occurring on different aquatic compartments (surface and groundwater for example). The objective is to select the most appropriate receiving phase with the higher affinity for the studied target pollutants. Thus, we compared the efficiency of different solid materials as receiving phase (Empore© disks) incorporated into Chemcatchers®. Devices were exposed to aqueous solutions containing the selected herbicides in order to evaluate kinetic accumulation of analytes. This work focuses on the comparison of kinetic constants and total mass analytes accumulated on each Empore© disk (as for example C18, SDB-XC, SDB-RPS).
Based on this study, a Chemcatchers® calibration step, at laboratory scale, could be carried out with the selected receiving phase in order to estimate the sampling rates (Rs) of pollutants. This calibration is necessary prior to use Chemcatchers® for field studies (water monitoring).

MO 011

Determining pesticide uptake rates for the polar organic chemical integrated sampler using field data

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A crucial issue in using passive sampling techniques like the polar organic chemical integrated sampler (POCIS) for environmental monitoring of pesticides and pharmaceuticals is the determination of reliable uptake rates. Often uptake rates are determined under lab conditions not reflecting natural conditions. Knowing that turbulence, temperature, salinity and biofouling can have an impact on the uptake rates of passive sampling devices, calculations of water concentrations using only lab-derived uptake data might be heavily biased. Therefore, auto sampler campaigns have been run in parallel to POCIS deployments in four Luxembourgish sewage treatment plant effluents as well as their receiving rivers during 24 hours. Substance specific uptake rates have been calculated by using data of the POCIS extracts and results of the auto sampler campaign analyzed via LC-MS/MS. It can be shown that these "field-derived"-Rs-data are between the values obtained during the calibration in the lab. The latter values showed large variations and were strongly dependent on the stirring i.e. on the turbulence of the system during the experiment. Therefore, a sound determination of the time weighted average concentrations for the investigated compounds should be based on uptake rates, which have been verified on-site. In the same study, POCIS also have been exposed during 14 days at the same sites as a witness of the pesticides' presence during and between the grab sampling. It turns out that the limits of detection for POCIS are in the range of 1 ng/l for most of the 20 monitored substances. Therefore, the use of passive samplers can improve the limits of quantification by one order of magnitude compared to the measurement of grab samples. Using on-site verified sampling rates, POCIS allow for an integrative monitoring down to the trace level. Within the 14 day sampling period, even more substances could be detected than have been found in the 24 hour samplings. This combined approach with 24 hour and 14 day deployment of POCIS also allows for a rough calculation of the variation and possible contamination peaks during the investigated period.

MO 012

Laboratory calibration of POCIS: kinetic accumulation of 59 polar organic contaminants and evaluation of candidate performance reference compounds

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POCIS (Polar Organic Chemical Integrative Sampler) is a new emerging tool for sampling polar organic micropollutants in water (Alvarez et al., 2004). It is still in development concerning its domain of validity (e.g. molecules sampled, type of water studied, optimal exposure duration) and its performances, including the definition of molecules sampling rates, repeatability, accuracy of the evaluation of time-weighted average (TWA) concentrations. Performance reference compounds (PRCs) enable to decrease the effect of variable environmental conditions, so they can be used to obtain more reliable TWA concentrations in situ. To our knowledge, for POCIS, only one PRC has been identified to date: deisopropylatrazine-d5, that has been used and proved efficient for polar herbicides monitoring in freshwater (Mazzella et al., 2007, 2010).
In order to obtain in situ TWA contaminant concentrations, POCIS needs to be calibrated in laboratory for each molecule of interest. Our work was based on laboratory calibration of POCIS for 5 alkylphenols, 9 hormones, 12 pesticides, 29 pharmaceuticals, 3 phenols and 1 UV filter in a flow-through system. This system was composed of 2 aquaria (50 L) containing i) spiked tap water (circa 3 µg/L) and non-spiked POCIS for the determination of accumulation kinetics or ii) non-spiked tap water and spiked POCIS for the determination of desorption kinetics. In both experiments, water temperature, pH, conductivity and dissolved organic carbon were controlled. Agitation in the aquaria was ensured via a submerged pump. The resulting flow velocity was 10±5 cm/s and was directed perpendicularly towards POCIS surface. Triplicate POCIS were analyzed at 0, 1, 3, 6 and 12 hours and at 1, 3, 7, 11, 14, 21 and 28 days.
We will present various results depending of molecules such as the duration of linear accumulation phase and sampling rates, sampling repeatability and possible PRCs. As for example, linear accumulation phase for estrogenic hormones seems to be 21 days, since at 28 days the equilibrium regime begins to be reached. For betablockers, linear accumulation phase is shorter: less than 14 days. Sampling rates for this 2 families of compound vary from 0.058 L/d for sotalol to 0.537 L/d for 17-a-estradiol. Concerning the desorption experiments, atenolol-d7 and sotalol-d6 for example show a good potential as PRCs. Indeed, a desorption of 70% for atenolol-d7 and 50% for sotalol-d6 was observed in 24 days.

MO 013

A method for the in situ calibration of a passive phosphate sampler in estuarine and marine waters

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Passive samplers for phosphate were calibrated in the laboratory over a range of flow velocities (0-27 cm s 1) and ionic strengths (0-0.62 mol kg 1). The observed sampling rates were between 0.006 and 0.20 L d 1. An empirical model allowed the estimation of these sampling rates with a precision of 8.5%. Passive flow monitors (PFMs), based on gypsum dissolution rates, were calibrated for the same range of flow velocities and ionic strength. Mass loss rates of the PFMs increased with increasing ionic strength. We demonstrate that this increase is quantitatively accounted for by the increased gypsum solubility at higher ionic strengths. We provide a calculation scheme for these solubility's for an environmentally relevant range of temperatures and salinities. The results imply that co-deployed PFMs can be used for estimating the flow effect on the in situ sampling rates of the phosphate samplers, and we expect that the same may hold for other passive samplers.

MO 014

Determination of deployment specific chemical uptake rates for SPMD and PDMS using a passive flow monitor (PFM)

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Monitoring of micro-pollutants in the aquatic environment remains a challenge. In the last decade passive sampling techniques have been developed that facilitate the time integrated monitoring of target chemicals through the use of selective receiving phases. These techniques rely on the implementation of methods that will negate the effects of environmental factors (such as flow, temperature, etc.) or that will facilitate the quantitation of site and chemical specific rates of uptake into the passive sampler employed. This uptake follows first order, linear kinetics when either an ad- or ab- sorbent is employed as a receiving phase. The use of performance reference compounds (PRCs) has been established for the in-field calibration of passive samplers when the target analyte is absorbed. We have adopted an in situ calibration technique based on the dissolution of gypsum to measure the average water velocity a sampler has been exposed to. We have demonstrated that the loss of gypsum from the passive flow monitor (PFM) can be applied

to predict changes in Rs dependant on flow when using the absorbent SPMD (semipermeable membrane device) or PDMS (polydimethyl siloxan) passive samplers. The study further demonstrates the applicability of the PFM in situ calibration method when quantifying the effect of flow/turbulence on chemical specific uptake kinetics. The application of this tool will enhance the accuracy when calculating and reporting environmental pollutant concentrations measured through the use of passive sampling devices.

MO 015

The performance of Passive Flow Monitors and phosphate accumulating passive samplers when exposed to pulses in external water flow rate and/ or external phosphate concentrations

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Passive samplers are typically calibrated under constant flow and concentration conditions. This study assessed whether concentration and/or flow pulses could be integrated using a phosphate passive sampler (P-sampler). The assessment was conducted in three 21 day experiments featuring a pulse in flow rate, a pulse of Filterable Reactive Phosphate (FRP) concentration and a simultaneous concentration and flow rate pulse. FRP concentrations were also determined by parallel grab sampling and the P-sampler calibrated with Passive Flow Monitors (PFMs) and direct measurement of flow rates. The utility of PFMs as a flow-monitoring device with variable flow conditions was successfully demonstrated. Good agreement was observed between the grab and passive measurements of FRP concentration when exposed to a pulse in flow (6% overestimation) or concentration (2% underestimation). However, a 32% overestimation occurred with a simultaneous concentration and flow rate pulse. Results obtained were consistent with a lag time for accumulation within the P-sampler

MO 016

Atmospheric input of persistent organic pollutants into soils close to the urban Rhein-Main-Area (Germany)

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Persistent organic pollutants like Polycyclic Aromatic Hydrocarbons (PAH) are ubiquitous in the environment. PAH are mostly generated by incomplete combustion processes, i.e. urban emissions from traffic, industry and residential areas (particularly house heating in winter). Even in rural areas the enrichment of organic pollutants in soil is often dominated by atmospheric deposition Yet, only little is known on the impact of urban short-range PAH deposition and accumulation in soil.

The Rhein-Main region in Germany is one of the most densely populated and highly industrialized areas in Europe with the adjacent Taunus mountains (25 km W of Frankfurt) as one of the most probable receptors of urban emissions. The aim of the project was to investigate the impact and the seasonal variation of the short-range transport and deposition of PAH.

The atmospheric input at three test sites (urban influenced, mountain crest and rural) was investigated using passive samplers for particles (sigma2 samplers), air (PUF disks) and atmospheric bulk PAH deposition. A typical sampling period lasted 3 months. Furthermore, soil samples were analysed for PAH.

The aerosol composition as determined by environmental scanning electron microscope analyses (ESEM) revealed the urban impact on the urban exposed area and on the mountain crest. The rural test site showed a clearly different aerosol composition and thus lower urban impact. The fraction of sulfate particles, as a typical component of urban aerosol, decreased at the test sites from ca. 50% (urban) to below 5% (rural) within a distance of ca. 10 km.

Air and bulk deposition samples showed higher PAH deposition rates in winter at all test sites. While the air depositon rates were slightly higher at the mountain crest, the bulk PAH deposition was significantly higher at the urban site and on the mountain crest. These observations probably results from additional sources in winter (heating period) and decreased degradation. Soil samples from the mountain crest showed the highest PAH contents. Samples from low altitudes at the urban and the rural test side showed significantly lower but comparable PAH contents. This indicates that the mountain range is scavenging the persistent PAH. To evaluate the mass transfer of pollutants in the study areas, future work will focus on input-output balances and assessment of pollutant storage in soils.

MO 017

Air monitoring of POPs near a waste incineration plant. Suitability of air passive sampling devices.

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High-volume active samplers are generally used as conventional sampling techniques for monitoring persistent organic pollutants (POPs) in air. The relatively high cost of this equipment, together with some technical requirements such us a pump and source electricity, are important disadvantages of these sampling methods. A potential alternative of high interest is the use of passive samplers. These devices allow semi-quantitative comparisons of the atmospheric POP levels. Taking into account their low cost and simple manipulation, polyurethane foam (PUF) disks are especially attractive. In recent years, the viability and suitability of using passive samplers, as a substitute of conventional systems, have been studied in Catalonia. Both techniques have been alternatively used to monitor the environmental impact of waste management plants (e.g., incinerators, landfills, etc.) by analyzing the levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). Between March and June 2010, 8 PUF devices were deployed at different sampling sites around the municipal solid waste incinerator (MSWI) of Tarragona (Catalonia, Spain). The airborne concentrations of PCDD/Fs, as well as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs), were analyzed. Following the passive samplers theory, the sampling rate for the pollutants analyzed here was previously acquired from the scientific literature. The mean flow rate for the different PCDD/F congeners was approximately 2 m3/day, while that established for PBDEs, PCBs and PCNs was around 3 m3/day. The reported concentrations of PCDD/Fs in air ranged between 6.95 and 22.3 fg WHO-TEQ/m3. PBDE average level was 41.9 pg/m3, and PCN mean concentration was 3.10 pg/m3. Regarding PCBs, the concentration of dioxin-like congeners accounted for between 0.18 and 6.17 fg WHO-TEQ/m3, and the mean levels of the 7 environmental marker PCBs was 57.7 pg/m3. According to the results of all POPs in air samples, we can still consider that the MSWI does not have a significant impact on the area under its direct influence, with respect to the emissions of PCDD/Fs, PBDEs, PCBs and PCNs. The concentrations found here are similar or even lower than those values obtained in other studies conducted in areas near waste incinerators and other industrial/urban areas.

MO 018

Concentrations of polychlorinated biphenyls (PCBs), polibrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) using passive air sampling in Tuscany region, Italy

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Passive air samplers consisting of polyurethane foam disk were deployed over a 3-month period from April to July 2008 at 19 locations throughout the Tuscany Region in central Italy. Sampling sites were selected based on different criteria including urban, rural and agricultural sites. The purpose of the study was to assess the gas-phase concentration of PCBs, PBDEs and PAHs on a local and regional scale. Derived air concentrations (pg/m3) ranged from below detection limit (BDL) to 304 (52 ± 98) for ∑6PCBs and from BDL to 104 (21 ± 34) for ∑8PBDEs. For PAHs the concentrations were much higher with values between 292 to 4670 pg/m3 (2051 ± 1316). The highest PCBs concentrations (pg/m3) were observed at urban sites, peaking at Piombino (304), follow by the rural sites at Fogliano Grosso (45). At the agricultural sites (Siena Nord, Montalcino, Val d'orcia, Abbadia San Salvatore and Arcidoso) the concentration were BDL for all the sites. The highest concentration of PBDEs was recorded at Scarlino (104 pg/m3), a rural site, though there were not big differences between the three categories. The highest level of PAHs was found at Siena Nord (4670 pg/m3), close to the Siena-Florence highway. The PCBs congeners composition frequently detected for PCBs included 28/31, 52, 99, 105, 118 and 149; the congeners with 5-cl were the predominant accounting for the 91%, followed by the 4-cl with 7%. For PBDEs the predominant congeners were 15, 49, 66 and 100. PAH with 3-6 benzene rings were analysed, the most abundant was 3 ring-congeners accounting for 76 ± 6 % of the total PAHs composition. Phenantrene alone accounted for the 45 ± 11 % of the total PAHs composition. The percentage for 4 rings and 5 ring-congeners was 24 ± 6 % and 0.1 ± 0.3 %, respectively. The concentration of PCBs and PBDEs showed a big variation between the different sites probably showing local emissions and highlighting localised hotspots. This study shows the feasibility of using PUF disks as time-integrated passive samplers, assessing local and regional scale for concentrations of POPs in central Italy.

MO 019

Influence of passive air sampler configuration on chemical uptake by the sampling medium

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Passive air samplers (PASs) of various configurations have been widely applied to monitor semi-volatile organic compounds (SVOCs) in the atmosphere. Trying to understand how sampler design affects chemical uptake, we investigated different configurations of a PAS, which relies on cylindrical, sorbent-filled mesh cylinders as sampling medium. Silica-gel and XAD-2 were used to sample water vapor and SVOCs respectively. Water uptake by silica-gel was measured gravimetrically, which enables numerous replicated measurements in a short period. Experiment results show that the PAS sampling rate (R, m3/d) is (i) determined by the surface area of the sampling medium exposed to air, and (ii) influenced by the distance of the sampling medium to the opening of the PAS shelter. Due to their high molecular diffusivity, water molecules are expected to be uniformly distributed within the sampling medium. The experimental data on water uptake indeed conform well with a PAS uptake model that assumes uniform distribution. However, more and more evidence questions the validity of this assumption for the uptake of SVOCs in PAS. In order to test this assumption, experiments are being conducted to characterize the distributions of SVOCs within the two sampling media most commonly employed in PAS (polyurethane foam and XAD) by separately analyzing axially and radially segmented sampling media. Results indicate inhomogeneous axial distribution of PCBs within XAD-filled mesh cylinder at wind still conditions. Wind blowing towards the opening of a PAS shelter greatly increases R and tends to homogenize the axial PCB distribution within XAD-filled mesh cylinder.

MO 020

Use of chemical probes and passive samplers for the determination of the atmospheric input and fate of persistent organic pollutants (POPs) in soil

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Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) can be found ubiquitously in the environment. They are typically emitted from local and/or diffuse sources and enter the atmosphere either in their gaseous form or sorbed to carbonaceous particles. Removal from the atmosphere is due to wet and dry deposition and this can lead to an accumulation of the POPs in soils and sediments. However, after deposition climate-controlled desorption and evaporation processes may lead to a contaminant remobilization. It was speculated that especially in high altitudes remobilized contaminants may accumulate and contaminant patterns may change, due to the contaminants chemical and physical properties. These processes are summarized as cold condensation or cold trapping. In our study we used PAHs and PCBs as model compounds to study climate and altitude triggered fractionation processes in detail. In two remote alpine valleys and in a valley in the Black Forest test sites were equipped to (i) quantify wet and dry deposition of these compounds using bulk deposition samplers and air samplers, and (ii) to determine evaporation rates using chemical probes. Measured deposition rates were two orders of magnitude higher for PAHs compared to PCBs. However, while higher deposition rates for PAHs were found in winter, presumably due to increased combustion in the heating period, PCB deposition rates were higher in summer, indicating increased evaporation from sources at higher temperatures. For the chemical probes, various sorbents were preloaded with selected PAHs and PCBs (several mg/kg), filled into mesoporous ceramic tubes and deployed at the test sites. After 9 months the tubes were collected and the sorbents analyzed for evaporative losses. Evaporation rates were found to correlate with vapour pressure and were negatively correlated with altitude. For quartz sand as the sorbent, losses of naphthalene and phenanthrene were close to 100%. For stronger sorbents, e.g. charcoal and peat, losses were much smaller and in some cases even an increase in loading was observed.

MO 021

PCBs levels in the atmosphere of industrial, urban and rural areas of the Rhine Valley: case

of Strasbourg (France) and Kehl (Germany)

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Passive air samplers (PAS) with XAD-2 resin have been used to measure polychlorinated biphenyls PCBs concentration in the atmosphere. This study was performed in the Rhine Valley, in the urban environment of the cities of Strasbourg (France) and Kehl (Germany) close to an important industrial harbour and in a remote area. Main industries in the harbour zone are waste incinerators, a steel plant, a thermal power plant and a petrol harbour. 22 congeners were analyzed by GC-2ECD after extraction on accelerated solvent extraction (ASE) and purification on silica and acidified silica gel. More than 80 samples were collected between March 2009 and August 2010 with a collecting time period of 4 weeks, in the industrial harbour but also in the urban and remote areas. Mean PCB concentrations measured in industrial, urban and forested zone were 3.9 ng.PAS-1d-1 (n=21), 3.8 ng.PAS-1d-1 (n=18) and 1.3 ng.PAS-1d-1 (n=14), respectively. The similar proximity of Kehl and Strasbourg center to the industries explains the similar PCB concentrations. Spatial and temporal trends were observed. Background level was measured in a remote area in the Vosges Mountains: concentrations were low in April-September: 0.9 ng.PAS-1d-1 and higher in October-March period 1.9 ng.PAS-1d-1. Similar concentration profiles were measured at different sites in the urban or rural areas. Two particular events were measured in April 2009 and February-March 2010 with the highest concentration level in the industrial area 8.6 ng.PAS-1d-1, 6.5 ng.PAS-1d-1, 19.9 ng.PAS-1d-1 respectively. PCB emitters are present in the industrial zone but not clearly identified. Sampling rate coefficients determined in the laboratory allow to give results in ng.m-3 and to compare with other cities. Gas particle distribution is on a range of 50:50-90:10. Thus pollutants were not considered only in the gas phase and total PCB (gas+particle) have been calculated. The ranges of variation in industrial, urban and remote area were 0.16-2.1 ng.m-3, 0.08-1.9 ng.m-3, 0.01-0.28 ng.m-3 respectively. These values were median concentration level, the highest concentrations found during particular events.

MO 022

Applications of PDMS partitioning methods in the study of biodegradation of pyrene in the presence of dissolved humic acid

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Although there are reports on the inhibition of anthropogenic organic chemicals biodegradation due to binding to dissolved humic substances (HS), there is an increasing body of evidence pointing to an enhancing effect in the case of hydrophobic chemicals, like pyrene. The addition of humic fractions to contaminated soils often causes an enhanced biodegradation and desorption of these compounds from soils. Other mechanisms proposed as operating in HS-mediated enhancements of biodegradation include the promotion of compound solubility and a direct access to HS-sorbed chemicals due to the physical association of bacteria and HS. Here, we propose the use of partitioning techniques using poly(dimethylsiloxane) (PDMS) to study the effect of binding of pyrene to a dissolved humic acid isolated from soil on biodegradation of this PAH by a representative soil bacterium. The application of these techniques in biodegradation studies may solve many questions about enhancements in diffusive mass transfer, in capacity/speciation and in dissolution. Therefore, our study may provide new insights into the effects of HS on microbial degradation of polycyclic aromatic hydrocarbons (PAHs).

MO 023

In situ silicone tube microextraction: Undisturbed sampling of root-exuded artemisinin from Artemisia annua in soil

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The analytical determination of bioactive plant produced compounds in soil is complicated: First, the extraction from the soil can be difficult due to sorption. Secondly, it can be difficult to measure actual and representative concentrations in the soil due to a heterogenic distribution of the compounds. Thirdly, the removal of samples from the soil leads generally to disturbance of the root zone, which makes it difficult to monitor concentrations over time, e.g. a growing season. Artemisinin has well documented biological effects not just as a medicinal drug. The compound has also ecotoxicological effects, which makes it highly relevant to measure in soil. With the purpose of measuring the root exudation of the bioactive compound artemisinin from the medicinal plant *Artemisia annua*, 3 m polydimethylsiloxane (PDMS) microtube was placed in the root zone of 9 weeks old *A.annua* plants for repeated sampling without disturbance of roots and soil. The soil was covered to prevent leaching of artemisinin from the above ground biomass making root exudation the only artemisinin source. The two ends of the microtube were placed above the soil surface to allow methanol elution of the artemisinin from the PDMS microtube at various points in time while keeping the tube in place. The artemisinin content in the methanol extracts was measured using LCMS analysis. Pilot-experiments showed that 3 x 1 ml methanol were sufficient for a complete artemisinin elution. Sampling every second week over a full growing season (28 weeks) showed artemisinin contents up to 8.25 ng totally in one tube. The content in the soil peaked at week 15, whereas the artemisinin content in the above ground parts of the plant increased after week 15. Correlation between high water content in the soil and high content of artemisinin in the microtube, suggests that diffusive mass transfer in the water phase controls the kinetics of artemisinin in the system. It is surprising to find artemisinin in *A. annua* soil where root exudates is the only source, as it previously has been reported that *A. annua* does not produce or store artemisinin in the roots. In *A. annua* pots without cover, the content of artemisinin was up to 140 ng in PDMS tubes left in the soil for 28 weeks. This suggests that rain off and release from dead plant material is a major contributor of artemisinin to the soil environment.

MO 024

Use of different plastics to mimic earthworm uptake of polycyclic aromatic compounds - evaluation using chemical analysis and the H4IIE-luc bioassay

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The bioavailability of organic contaminants is an important factor in risk assessment of remediated soils. A method for estimating bioavailability is to measure the uptake of contaminants in earthworms. Earthworms are appropriate model organisms for bioavailability since they process large amounts of soil, have a thin permeable cuticle and play a major role in the transport of pollutants from the soil to organisms in the food chain. The use of earthworms as a model for bioavailability is quite laborious and time-consuming and the worms do not survive exposure to certain substances or concentrations. The aim of the present study was to design an easy applicable method for estimating the bioavailability of polycyclic aromatic compounds (PAHs) in soil, using plastic stripes to mimic the uptake by earthworms. Five different plastics were tested in the study, polyoxymethylene 55 µm (POM-55), polypropylene 500 µm (PP-500), polymethylpentene 76 µm (PMP-76), polyethylene therephthalate 50 µm (PET-50) and cyclic olefin copolymer 254 µm thick (COC-254). One gram of each plastic strips were mixed with approximately 40 g of a remediated PAH-contaminated soil and exposed during 14 and 28 days, in two sets, on a rotary shaker. To mimic the earthworm exposure similar conditions i.e. humidity, soil ratio, exposure time were conducted as was done in earthworm uptake studies. After exposure the plastic strips were removed from the soil and extracted with n-hexane or methanol depending on the plastic resistance. The extracts were analyzed using booth GC-MS and the H4IIE-luc bioassay. The results were compared with the PAH profile in earthworms, Eisenida fetida exposed to the same soil. Of the plastics tested, POM-55 and PP-500 agreed best with the PAH profile in the worms, both in the toxicity test using the H4IIE-luc bioassay (TEQ values) and PAH levels (ng/g).

MO 025

Improved analytical performance using semi-permeable membrane extraction of lipid rich matrix

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Lipids are good matrices to accumulate many non-polar persistent organic pollutants (POPs) due to the lipophilic nature of these chemicals. Dairy products are easily accessible and available world-wide and analysis of this matrix supplies estimation of human food chain exposure and, at the same time, reflects the contamination level of the environmental compartment from which they derive.The Stockholm Conventions and the Global Monitoring Plan encourage the production of monitoring data to effectively evaluate the presence of the POPs in all regions, in order to identify changes in levels over time, as well as to provide information on their regional and global environmental transport.

Often large sample amounts must be extracted to achieve sufficiently low detection limits of the priority POPs. Consequently, large amounts of lipids can be present in the extracts depending on the lipid content of the sample. The first step of analyzing lipid rich matrices is to extract the pollutants, preferably without extracting the lipids themselves. The presence of lipids, in the form of phospholipids, triglycerides and free fatty acids or cholesterol can disturb the chromatographic capabilities in the further clean up or fractionation steps, as well as suppresses the ionization at the chemical analysis.

Dialysis with a semi permeable membrane (SPM) enables a non-destructive separation technique of the analytes from matrix, i.e. it efficiently extracts the smaller non-polar compounds, and retaining the larger compounds in animal or plant fat samples. The membranes are made from low-density polyethylene film with approximately 1-nm pores; and enables permeation of small (analyte) molecules whereas dialysis of molecules of matrix compounds larger than this is not possible.

The aim of this study is to develop and evaluate an improved clean-up methodology for large volume lipid rich samples. 5 grams of lipids from butter has been extracted with SPM and with several different solvent compositions to identify the optimal conditions for POP analysis with sufficient recovery. The extract was further cleaned up with alumina (basic) and sulphuric acid SPE columns. The target compounds of this study are PCDD/Fs, PCBs, PBDEs, and OCPs subject to the Stockholm Convention as by 2009. Analyses are executed with high resolution GC/MS and quantifications will be carried out based on labeled surrogate standards.

MO 026

Pine needles as natural passive samplers - several approaches for the extraction of polycyclic aromatic hydrocarbons

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For more than two decades, numerous forms of vegetation have been studied as natural passive samplers for the incidence, bioaccumulation or travelling patterns of a wide range of persistent contaminants in the environment. Among other species, pine trees proved their effectiveness as biomonitors, essentially through the retention properties exhibited by the waxy layer of their needles. The problem is that the properties favouring the capture of such pollutants pose, at the same time, a series of barriers and limitations when it comes to the analytical steps.

In fact, the extraction and analysis of plant matrices involved in biomonitoring studies require effective extraction and clean-up procedures to retain the target compounds and eliminate the lipidic and other interfering elements. These technologies are suffering continuous development and improvement aiming for the lowest limits of detection associated with faster and more reliable methodologies.

It was our objective to compare the efficiency of several extraction (Soxhlet, ultrasonic extraction, microwave-assisted extraction, accelerated solvent extraction) and microextraction (solid-phase, hollow-fibre liquid-phase) approaches followed when needed by solid-phase extraction clean-up for the determination of the 16 EPA polycyclic aromatic hydrocarbons (PAHs) in pine needles (Pinus pinea L.). PAHs are ubiquitous carcinogenic and mutagenic contaminants resulting from natural and anthropogenic sources mainly associated to combustion processes.

All methods were compared in terms of validation parameters (detection limits, precision, recovery). Quantification was performed by gas chromatography-mass spectrometry (GC-MS), using deuterated PAHs as internal standards. Overall, the extraction approaches yielded slightly better results (with ultrasounic extraction on top) than the microextraction advances. However, the latter are also valid and not to mention much cleaner options.

MO 027

An accelerated solvent extraction method for passive air sampler PUF disks

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Poly-urethane foam (PUF) disks are widely used as a sorbent for e.g. passive air samplers to evaluate the air pollution with organic pollutants, such as polyaromatic hydrocarbons (PAH) or polychlorinated biphenyls (PCB). The cleaning before deployment and the extraction after the field application are usually done by standard soxhlet techniques, which last several hours or even days for each sample. Automated extraction methods for organic compounds such as accelerated solvent extraction (ASE) need usually less time for a comparable extraction efficiency and allows the repeated sequential extraction of a single sample.

The aim of this study was to develop a rapid ASE method for PUF disks. PAH and PCB standards were used as model compounds to proof the extraction efficiency. PUF disks (14 cm diameter and 1.35 cm thickness, density 0.03 g/cm^3, destruction temperature 180°C) were pur-

chased from Klaus Ziemer GmbH, Germany and a Dionex ASE 300 model was used to develop a method. 6 PUF disks wrapped in kimtech wipes were pre-cleaned with dichloro-methane in 100 ml extraction cells with the ASE 300 and the method parameters described below.
5 of the disks were spiked with a PAH standard (16 EPA PAH) and a PCB standard (6 Ballschmiter PCB) with an absolute mass of 500 ng for each compound. After evaporation of the solvent all disks were wetted with 20 ml natural water to simulate maximum field humidity conditions. One disk was used as a laboratory blank and treated like the spiked disks to evaluate background contamination.
Each sample was extracted with acetone under the following conditions: The cell was heated to 100°C at 100 bar for 10 min (static extraction step). Subsequently the cell was flushed with acetone (60% of cell volume) and purged for 100 s into a sampling bottle. The extraction was repeated and both extracts were combined. A third extraction was performed to reveal the extraction efficiency of the first 2 cycles.
The results showed an extraction recovery of 86 - 139 % for PAHs and 85 -105 % for PCBs (blank corrected values) with 2 cycles of the method described above. The third extraction showed concentrations lower than 5% of the target compounds. The blanks were usually lower than 1%. The total extraction time is ca. 40 minutes for each sample.

MO 028

Determination of bioavailable fraction of persistent organic pollutants in freshwater sediments using two single-phase passive samplers

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Until recently sediment monitoring has relied on the determination of total or normalized contaminant concentrations. This approach, however, does not distinguish between freely dissolved and bound molecules and aims to assess the presence of chemicals rather than their activity and availability. Since many laboratory and field studies have demonstrated that biological effects in benthic organisms are not generally related to the total concentration of contaminants in sediments, alternative and more representative measures of the bioavailable fraction of contaminants in sediments are required. Application of passive sampling to assess bioavailability of persistent organic pollutants (POPs) in sediment can be undertaken in situ with buried passive samplers or in batch experiments in the laboratory following grab sampling or coring (and sectioning). Thanks to their simple construction, sample preparation, low cost and possible re-use, single-phase passive samplers are a suitable tool for environmental monitoring of POPs. We present results from application of single phase passive samplers based on silicone rubber (SR) and polyoxymethylene (POM) to estimate the fraction of contaminants available for desorption within a time scale of three months of as well as fraction effectively contributing to the partitioning with pore water and/or biota. Single-phase polymeric materials such as SR and POM elicit similar affinity for hydrophobic compounds and simpler sample processing, when compared with the most commonly used and commercially available semipermeable membrane devices (SPMDs). Data obtained with passive samplers can be used in risk calculations for sediment-bound contaminants with regard to any need for remedial measures for contaminated sediments and these studies would be an important input with regard to environmental quality standards for contaminants in water proposed in the EU Water Framework Directive.

MO 029

Development of a new time-integrative sampler using in-situ solvent extraction for sampling polar organic compounds in water

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Due to the potential fluctuation of the concentration of water contaminants, passive sampling techniques have advantages over conventional grab sampling. In spite of great success of passive sampling devices for hydrophobic contaminants such as PCBs and PAHs, integrative sampling of polar organic compounds to measure a time-weighted average concentration is still challenging because equilibrium between water and most sampling material is reached in a short time. In this study, we developed a new time-integrative sampler using in situ solvent extraction for polar organic chemicals. The sampler was composed of a 15 cm poly(dimethylsiloxane) (PDMS) tubing with the internal diameter of 0.5 mm and the wall thickness of 0.5 mm and a flow-through system of an extraction solvent (acetonitrile) through the tubing. Three polar organic contaminants, sulfamethoxazole, diuron, and 17α-ethynylestradiol, were chosen for the validation of the sampler. Without using in situ solvent extraction, the PDMS tubing could accumulate three model compounds in a time-integrative mode for less than 12 h and equilibrium between PDMS and water was obtained in a day in our laboratory conditions. However, the sampler using in situ solvent extraction could accumulate three model compounds in a time-integrative mode without limitation of exposure time. Measured sampling rates for a compound at three different extraction flow rate (0.2, 0.5, 1.5 mL min^{-1}) were close to each other, indicating that the overall mass transfer from aqueous solution to extraction solvent is likely to be dominated by the internal diffusion in PDMS. In addition, a pulsed exposure experiment confirmed that the new sampler worked in a time-integrative mode when the environmental concentration was highly fluctuated.

MO 030

A novel methodology to determine the narcosis potential of contaminated sediments by using polyethylene samplers and comprehensive two-dimensional gas chromatography

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Narcosis has been identified as a potential toxic effect of hydrocarbon mixtures in contaminated sediments. This effect is believed to occur when the total hydrocarbon concentration in the lipid membranes of an organism reaches a critical level of approximately 0.01 g/g of lipid. We present a novel methodology to calculate the total lipid load of an organism exposed to a contaminated sediment, by using polyethylene samplers and two dimensional gas chromatography, coupled with flame ionization detector (GCxGC-FID). Previous research has shown that retention times on GCxGC can be used to predict chemical properties such as aqueous solubility and Kow. By using an appropriate training set, we extended this capability to the prediction of the partition constants between the polyethylene sampler and water (KPEW) and phospholipids and water (KPLW). With knowledge about these two partition constants, we can calculate the corresponding membrane lipid concentration for any given polyethylene sampler concentration. In addition, the amount of each compound present in the polyethylene sampler can be quantified by using the relatively constant response factor of the FID detector. The total lipid load can then be calculated by summing the contributions from individual compounds. We are applying this narcosis calculation to polyethylene sampler extracts from five sites around Boston Harbor and comparing our calculated lipid loads with toxicity data from fertilization essays and embryo development tests.

MO 031

Can polar organic chemical integrative samplers be adapted for polycyclic aromatic hydrocarbons water sampling?

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Polar Organic Chemical Integrative samplers (POCIS) are nowadays widely used for the monitoring of emerging hydrophilic contaminants such as pesticides and pharmaceuticals[3DOTS]. Even though their receiving phase has an affinity to aromatic groups, their accumulation capacity for polycyclic aromatic hydrocarbons is limited. In fact, laboratory calibration to assess the performance of POCIS to sample PAHs from aquatic systems showed a variability of the sampling rates over time, and that a constant accumulation rate is only achieved when the concentrations of target analytes in the POCIS membrane reach a steady state. This lag time indicates that the uptake of PAHs into the POCIS is mainly controlled by the physico-chemical properties of the polyethersulfone membrane.
POCIS extraction and analysis are relatively fast and easy comparing to the SPMD which are the conventional passive sampling tools for the monitoring of hydrophobic compounds in the aquatic system. Thus, finding an "adapted"version of POCIS to sample PAHs would be very interesting. Since we have shown that it is the POCIS membrane that does not allow the integrative kinetic accumulation of PAHs into the POCIS sorbent, the present developments consisted of changing the POCIS membrane: Nylon and polyethylene were used as replacements of polyethersulfone in an attempt to reduce mass transfer resistance, and calibrations were conducted to compare POCIS accumulation for PAHs with these three membrane types.
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MO 032

Comparison between classical and improved Polar Organic Chemical Integrative Sampler (POCIS) for alkylphenol polyethoxylates

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The European Union (EU) adopted the Water Framework Directive in 2000 which aims at improving and protecting the quality of the aquatic environment and its resources. The main objective is to achieve good water status by 2015. In order to control the release in aquatic systems of "priority", "priority hazardous"and emerging substances in a better way, it is necessary to develop new sampling tools which would make it possible to do without grab sampling. Indeed, despite its usefulness, grab sampling is not representative of a heterogeneous environment and of spatial and temporal variability. Passive sampling tools allow to get access to Time Weighted Average (TWA) concentrations during the deployment period. The Semi-Permeable Membrane Devices (SPMDs) and the Diffuse Gradient in Thin Film (DGT) are widely used and well calibrated. On the contrary, Polar Organic Integrative Sampler (POCIS), still require laboratory development before exposure in aquatic systems.
Alkylphenol polyethoxylates (APnEO) are ubiquitous pollutants and their concentrations are often above environmental guidelines. They were also chosen for this study due to their hydrophilic/hydrophobic properties. Calibrations of POCIS were conducted in the laboratory in tanks contaminated by APnEO with a flow through system under a controlled temperature and hydrodynamic conditions. During the first calibration, classical POCIS were studied. The uptake rate constants were determined and compared to the literature. But the results shown that APnEO were firstly accumulated in the hydrophilic PolyEther Sulfone (PES) membrane and then in the receiving phase. After 15 days, these compounds were always present in the PES membrane with a significant concentration. Thus first it appeared important to test the influence of sorbent recovery solvent on compounds elution present in PES membranes because in most studies, methanol is used as recovery solvent despite its eluant property and secondly to test other membranes which could increase the APnEO accumulation into the phase and not in the membrane In another calibration adapted POCIS-like were used with Low Density PolyEthylene (LDPE) and nylon membranes which are more suitable for the APnEO because they are more hydrophobic. These calibrations allowed the comparison of the uptake rate constants obtained with classical and adapted POCIS.
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MO 033

Continuous Flow Integrative Sampler (CFIS). A new approach for sampling for a better control of water quality.

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Most aquatic monitoring program relies on collecting discrete grab, spot or bottle samples of water at a given time. Often, where pollutants are present at only trace levels, large volumes of water need to be collected. The subsequent laboratory analysis of the sample provides only a snapshot of the levels of pollutants at the time of ampling. However, there are drawbacks to this approach in environments where contaminant concentrations vary over time, and episodic pollution events can be missed. Passive sampling methods have shown much promise as tools for measuring aqueous concentrations of a wide range of priority pollutants.
An important limitation of passive sampling techniques resides in the fact that only the dissolved fraction (bio available fraction) is sampled. The suspended solid and colloid fraction information is not given as required in different legislations.
In this presentation, an example of the final industrial version of CFIS device will be showed to the audience and the main performance characteristics of the new developed device will be presented as well as results of the infield evaluation of the device. Limits of detection are in the range from 1 to 50 pg/L with a precision below 20%. An in field evaluation of the CFIS with priority apolar compounds including PCBs, chlorinated pesticides and PAHs was carried out for a 20 days sampling period in the outlet of a waste water treatment plant showed comparable results to those obtained with a classical sampling method.

MO 034

Evaluation and calibration of a prototype passive sampler for perfluorinated alkyl acids in Sydney Harbour, Australia

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Perfluorinated compounds (PFCs) have been widely used in many applications since the 1950s. In recent years their environmental and health relevance has been revealed via population studies as well as biota studies which report continual low level exposure to these compounds. Combined persistence bioaccumulation and toxicity to some animals has prompted government agencies worldwide to place regulations and restrictions on some PFC congeners. In addition the PFCs perfluorooctane sulfonate and perfluorooctane sulfonyl fluoride were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in May 2009.
To date monitoring of PFCs in aquatic systems has been predominantly facilitated by grab or composite sampling. Environmental samples are traditionally extracted using a weak anion exchange (WAX) resin to trap the alkyl acids. In this study we investigate the use of such a WAX resin in a passive sampler configuration to achieve time-weighted-average concentrations for PFCs and stipulate its use for additional acid alkyl compounds.
Data on the suitability of WAX resins as sorbent phase for PFCs, to our knowledge, were not previously published. To apply it in a passive sampler however, calibration data such as sampling rates (RS L.day-1) under environmental conditions must be available for target compounds. The aim of this study was to evaluate and calibrate a WAX polymeric phase passive sampler for PFC in a field study at Sydney Harbour, Australia. Here we present data that indicates that the WAX sorbent has the potential to make a useful adsorbent for passive sampling PFCs.

MO 035
On the way to bringing equilibrium SPME off-shore: development of an in-situ sampling device for persistent organic pollutants in sediment porewater
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Sediments are complex and variable environmental matrices which can serve both as source of and sink for persistent organic pollutants (POPs) in aquatic ecosystems. Ecologically relevant processes such as bioconcentration and baseline toxicity of POPs are mostly controlled by the chemical activity of the substance rather than by its total concentration in the sediment. Therefore, we developed a passive sampling device based on equilibrium solid phase micro-extraction (SPME) of sediment porewater.
For the purposes of validation i.e. by parallel sampling into polymer layers of different surface area to volume ratios, the device is capable of carrying sampling materials with variable formats: polymer-coated glass fibres with a diameter of up to 500 µm as well as the more flexible PDMS (polydimethylsiloxane) hollow fibres. Sampling materials are chosen as to restrict equilibration times to approximately two weeks. Encased in a copper mesh, the fibres are immersed in the surface sediment and left there for equilibration. The casing allows for long-term exposure in natural sediment systems without fibres getting broken or damaged by abrasion or the PDMS being affected by biofouling. Due to its rather small size, the device can be flexibly positioned even alongside other sampling spots like off-shore hydrographic stations. In future, the deployment in open-sea sediments is aimed for. Such measurements will help to elucidate the large-scale distribution and bioavailability of POPs in marine sediments.
Recent field studies in harbour sediments demonstrated the reliable performance of the in-situ sampling device and yielded valid results which corresponded well to those of in-vitro bioassays. In-situ equilibrium SPME proved thus a useful complementary tool in the ecotoxicological assessment of POP-contaminated sediments.

MO 036
Determination of silicone rubber-water partition coefficients for a wide range of compounds using the cosolvent method
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To transform data from passive sampling into aqueous phase concentrations sampler water partition coefficients (Kpw) are required. These Kpws were determined for 80 compounds among which were phthalates, musk compounds, aryl phosphates, chlorobenzenes, chlorinated pesticides, some PCBs and a number of other compounds. Determinations of K_{pw}s were done by spiking silicone rubber sheets of 3 g with target compounds that were parallel equilibrated with water only and a range of water-methanol mixtures up to 50 %. From the measured concentrations K_{pmix} values were calculated for the different methanol water mixtures. The $LogK_{pmix}$ values have an inverse linear relation with the molfraction methanol and by regression the intercept is determined being the $LogK_{pw}$ of water only. Not for all compounds equilibrium seemed to be obtained as indicated by differences in water only equilibrations where compounds were spiked to the water and where compounds were spiked to the sheets. These differences seemed not to be related to hydrophobicity but more to the character of the compounds i.e. whether a compound in addition to a hydrophobic character also contained polar groups. It is suggested that diffusion inside the sampler may reduce the rate of exchange for such compounds. Of the 80 $LogK_{pw}$s determined 80 % showed sufficient low uncertainty $sLogK_{p}w<0.12$) to be used for the calculation of aqueous phase concentrations in passive sampling. This 80% $LogK_{pw}$ obviously show a relation with the $LogK_{ow}$, but the error of regression (0.4) is insufficient to allow prediction of K_{pw} from K_{ow}.

MO 037
What is the optimal extraction phase for your chemicals? Modeling and estimation approaches for partition coefficients between polymeric sorbents and water.
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The equilibrium partition coefficients between polymeric sorbent phases and water (*K*) play a key role in successful development of passive sampling techniques. A very low *K* value implies a weak affinity of the analyte for the passive sampler, and thus low sensitivity and a high detection limit. A very high *K* value implies high sensitivity, though it also leads to long equilibration times in an open system (due to the larger water volume the samplers would have to sample) and significant depletion in a closed experimental system, which prevents accurate measurement of the freely dissolved concentration. Thus, it is clear that knowing *K* values of target analytes into different polymer sorbent phases is essential for optimal selection of a passive sampling technique. However, phase selection is typically done based on time-consuming trial-and-error preliminary tests or (often not mechanistically based) 'expert guess'.
In this contribution, we present a series of experimental *K* values for different polymer phases commonly used for passive sampling, including polyacrylate (PA), polyoxymethylene (POM), polyethylene (PE), and poly(dimethylsiloxane) (PDMS). Polyparameter linear free energy relationships (PP-LFERs) are used to model and estimate *K* values. Fully predictive calculation approaches that use only the molecular structures of polymers and analytes as input parameters are also presented.
The first results show that PP-LFERs fit well to the experimental polymer-water partition coefficients and can be reliably used for estimation of *K*. The calibrated PP-LFERs suggest that PA has much higher capacities (1-4 log units higher *K*) than PDMS to extract H-bond donor compounds (e.g., phenols, amides, and many pesticides), but this is not so much the case for other types of polar compounds. Further, PA is a somewhat more efficient phase than PDMS for hydrophobic aromatic compounds.

MO 038
Toxicity of PAHs in the marine environment: using passive dosing to approximate the toxicity in seawater and sediments
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The acute toxicity of several PAHs at aqueous solubility to the marine crustacean Artemia franciscana nauplii was tested using both solvent spiking and passive dosing, and the results compared. The results from the toxicity tests with passive dosing were then used to establish a chemical activity-mortality relationship for PAH toxicity to Artemia franciscana nauplii. Passive dosing was then directed at reproducing the composition of a PAH mixture, measured for a coastal area of Spain (Bay of Algeciras) as part a monitoring program, in toxicity tests. HPLC analysis was used to confirm the reproducibility of the dissolved exposure concentrations for the individual PAHs and mixtures in the passive dosing tests, as well as to determine the salting-out for the synthetic seawater used. Finally, equilibrium sampling with silicone coated vials was used to measure free concentrations and chemical activities of PAHs in field sediments from this area, and the measured chemical activities in the sediment related to the chemical activity toxicity relationship obtained above for Artemia franciscana nauplii, with the aim of predicting sediment PAH toxicity. This study shows that passive dosing is a good method for toxicity testing in sea-water, maintaining constant exposure concentration over time and also being able to faithfully reproduce real mixtures of hydrophobic organic pollutants such as PAHs in sea-water toxicity tests.

MO 039
Microscale passive dosing ensures defined and stable gradients of hydrophobic organic chemicals
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The environmental chemodynamics of hydrophobic organic chemicals (HOCs) are often rate limited by their diffusive mass transfer through stagnant boundary layers (SBL). This then results in concentration gradients on the microscale, which are crucial for both diffusive mass transfer phenomena and the chemotaxis of motile cells and organisms that are able to sense chemical signals. For studying both processes, practical experimental systems are needed to generate well defined and stable gradients of HOCs. At the same time the system should allow for visualisation of cell behaviour and quantitative data analysis. In the present study passive dosing on microscope slides was employed to generate diffusive gradients by partitioning between a loaded and clean polymer. Several passive dosing formats were considered among which silicone O-rings seemed to be best suited when placed in a Dunn chemotaxis chamber. Thereby an outer clean ring serves as a sink and a loaded inner ring as source, imposing a highly stable gradient on the culture medium over the distance of 1mm between the rings. This setup offers the possibility to study chemotaxis of prokaryotic and eukaryotic cells exposed to a gradient of chemical activity and to simultaneously investigate diffusive mass transfer phenomena.

MO 040
Integration of partition based dosing into a Skeletonema costatum microplate toxicity test
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The microplate toxicity test is a widely used, high throughput method to determine the toxicity of compounds and effluents to algae. One of the potential limitations of the microplate test is the underestimation of toxicity to hydrophobic compounds. This is due to the binding of hydrophobic chemicals to the test vessel, which will result in a decrease in the concentration of the substance during the test. Many of the contaminants classed as priority pollutants by the US EPA are hydrophobic. However, similar limitations have been overcome in other 96 well plate based techniques using a Poly(dimethylsiloxane) (PDMS) based carrier for the contaminants. This system allows the release of the compounds at a steady rate into the aqueous solution, a process called Partition Based Dosing (PBD). Through the use of PBD a predetermined concentration can be maintained in the test medium throughout the exposure. Such an approach, used with the microplate algal test, would allow for the toxicity testing of hydrophobic compounds much more accurately than is currently possible. Current guidelines suggest testing hydrophobic compounds by preparing Water Accommodated Fractions (WAF), by stirring and phase separation. The toxicity of three hydrophobic compounds to Skeletonema costatum were tested with both PBD and WAF procedures. Differences in measured concentration of chemicals and dose response curves between these procedures are discussed.

MO 042
Passive dosing of triclosan in a large-scale experiment exposing biofilm in water channels
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Passive dosing is an approach to establish and maintain constant freely dissolved concentrations of a compound during an experiment, e.g. in bioassays. However, up to now, passive dosing has been mainly applied in small-scale systems, such as multi-well plates but not applied to model

ecosystems in larger scales. Within a mesocosm-study looking for possible effects of the bactericide triclosan on structure and function of biofilm communities (microalgae + bacteria) passive dosing by silicon rods was successfully used to ensure stable concentrations for a period of 10 days. The experimental system consisted of glass channels filled with 3.5 litres of river water and natural biofilm grown on artificial substrate.
The passive dosing system consisted of pieces (2.52 cm) of a flexible silicon cord. Amounts of triclosan necessary to reach intended water concentrations in the water channels (2 µg/L to 150 µg/L) were estimated assuming equilibrium partitioning between the silicon rods, water and biofilm. Equilibrium partitioning coefficients were obtained from literature, and exchange kinetics were studied in a pre-experiment. In the main experiment, quick achievement of equilibrium was supported by adding stock solutions of triclosan to the water channels at the beginning of the experiment.
Water concentrations were measured throughout the experiment by micro-liquid-liquid extraction and gas chromatography-mass spectrometry in order to verify stable triclosan concentrations. Concentrations of triclosan in the biofilm and glass beads (artificial substratum < 63 µm) were analysed at the end of the experiment (day 12).
Equilibrium was reached within 2 days, and concentrations in the water phase stayed largely constant for the remaining period of the experiment. Concentrations were slightly higher (up to a factor of 2.8) than estimated by equilibrium partitioning calculations. Amounts extracted from glass beads were low (highest value: 0.21 µg g-1 dw), indicating losses to glass surfaces of the test device to be negligible. At all concentration levels, more than 84 % of the triclosan were stored within the silicon rods. High proportions of substance in the passive dosing system help to ensure a quick and efficient mass transfer to the water phase and biofilm, and are a key requirement for maintaining constant concentrations.
The experiment successfully demonstrated that the passive dosing technology can be utilized to maintain constant exposure in large-scale systems.

MO 043
Passive dosing beyond toxicity testing: a new analytical tool
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The main feature of passive dosing is control of the freely dissolved concentrations by partitioning from a pre-loaded polymer. In the present study we apply this for the development of a new analytical tool to study speciation and binding of hydrophobic organic compounds (HOCs) in aqueous solutions. Further, passive dosing was applied to several medium constituents covering a wide range of interactions with HOCs. The first step of the new method is to control the freely dissolved concentration of HOCs and the second is to measure the total HOCs concentration in the equilibrated aqueous solution (=Ctotal). The measured concentration in the solution can then be combined with the corresponding equilibrium partitioning concentration in pure water (= Cwater) to determine: 1) speciation properties of aqueous samples (ff= Cwater/Ctotal) [1] 2) partitioning of HOCs with dissolved phases e.g. cyclodextrin, humic acids, micelles for determination of KDOC and KD values 3) the enhanced capacity (E) of solutions for HOCs (E= Ctotal/Cwater) and 4) salting-out effects (Setschenow constants). Application of passive dosing for speciation and binding studies has a number of advantages: 1) it is a precise and simple method, 2) it requires no phase separation step and no mass balance assumption and 3) it is possible to dose mixtures at defined and environmentally relevant free concentrations.

EC05 - Environmental fate and exposure of Pharmaceuticals and Personal Care Products (PPCPs)

MO 046
Optimization of a GC-QqQ-MS method for the analysis of siloxanes in water samples. Occurrence of siloxane in real urban wastewater samples.
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Siloxanes, and more concretely cyclic volatile methylsiloxane (cVMS) and linear methylsiloxanes (lVMS), are volatile and semivolatile organosilicon compounds which find a wide range of applications in industrial and consumer products. As an example, several of these compounds are commonly added to personal-care products (PCPs) formulations. These compounds have been used and disposed in the environment during the last decades and during the recent years have attracted considerable attention because of their persistence, bioaccumulative properties and moderate to high toxicity concern in humans. In addition, these compounds have been related to long-term toxicity [1,2] and narcotic effects [3] to several aquatic species. In addition, siloxanes have been detected in environmental samples from urban areas and also in remote areas such as the Arctic Ocean [4]. Therefore, there is a need of fast, reliable and sensitive methods for the ultratrace analysis of siloxanes in environmental samples.
The present work presents a new analytical method for the analysis of three cVMS and three lVMS based on liquid-liquid extraction followed by GC-QqQ-MS in SRM acquisition mode. The new analytical approach has presented excellent repeteabilities, offering good recoveries rates in different types of waters and achieving instrumental limits of detections ranging from 340 fg to 6.0 pg. The method was developed and validated by spiking experiments using different types of high purity water. The major contamination sources were identified and minimized in an accurate blank study, which involved the standard and sample storage, the solvent purity determination and the instrumental blanks analysis. The quality parameters of the method are presented. The method was applied to spiked and real urban wastewater samples and the results are presented.
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MO 047
Optimization and validation of the chromatographic conditions for the trace analysis of pharmaceuticals used in aquaculture by means of liquid chromatography
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In recent years, there have been a lot of scientific reports contradicting the myth that pharmaceutical residues used in both human and veterinary medicine are not to be treated as pollutants and do not contaminate the environment. Hence, it is crucial to develop sensitive and reliable analytical methods for the trace analysis of these pollutants at low concentration level [ng/L] in the environment.
One of the major groups of contaminations are pharmaceuticals used in aquaculture. This is due to the fact that aquatic organisms are usually treated using therapeutic agents added to feed or directly to fresh water baths. There is a risk that these drugs will bioaccumulate in the water, sediment and animal tissue.
This paper presents the results of optimization and validation of the analysis of antibacterial (fluoroquinolones), anthelmintic (benzimidazoles) and antiparasite (nitroimidazoles) pharmaceuticals that are employed in aquaculture. These methods have been established using the technique of liquid chromatography. The aim of the research was to select the appropriate parameters of analysis with the highest sensitivity and accuracy. Factors that were optimized were as follows: the composition, elution program and flow rate of the mobile phase, the type of column packing, time of analysis and detection parameters. The next step was to carry out validation of these optimized chromatographic conditions. Validation parameters - such as standard deviation, variation coefficient and accuracy - were found to be within the norm for environmental samples.

MO 048
Trace analysis of β-blockers and β-andenomimetics in natural water samples by SPE-GC technique with application of different types of detectors
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One of the most relevant topics in today's environmental analytical chemistry is water quality control. In this situation, the concentrations of pharmaceuticals in aquatic system should be monitored. β-Blockers and β-adrenomimetics occur at surface water and wastewater at very low concentrations. Matrices of these samples are variable and complicated, therefore purification and concentration steps using solid-phase extraction (SPE) are necessary. The sensitivity of the used analytical methods can be improved by the application of the higher sensitive techniques of detection. Nowadays gas chromatography is one of the most selective and sensitive analytical method, but transformation of the polar analytes into volatile derivatives is required. In this work several derivatization agents (MSTFA, BSTFA, TMSI, HFBI, MTBSTFA, MBTFA, PFPA, TFAA, PFPOH, HMDS, CMDMCS) were used to prepare the appropriate to GC analysis derivatives of six β-blockers (acebutolol, atenolol, metoprolol, nadolol, propranalol, pindolol) and two β-adrenomimetics (salbutamol, terbutaline). Different kinds of detectors (e.g. MS, ECD) in GC system were tested to choose the most suitable one for trace analysis of these compounds using GC method. The results of these investigations will be discussed. Additionally, the application of the proposed procedure to analysis of natural surface water samples will be shown.
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MO 049
Trace analysis of pharmaceuticals in aqueous samples
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Among the anthropogenic substances that may be harmful to the environment, increasing attention is drawn by residues of pharmaceuticals and their metabolites. The environmental consequences of the presence of pharmaceutical residues depend on qualitative and quantitative composition of a 'cocktail of pharmaceuticals', which penetrated into the natural matrices and the possibility of an interaction between these substances. One of the contemporary trends of environmental chemistry is elaboration of new analytical procedures which allow to determine a wide range of analytes present at low concentrations in environmental samples during the single analysis.
In this study, the occurrence of pharmaceutical residues in water samples was examined. Investigated compounds include frequently used non-steroidal anti-inflammatory drugs (NSAIDs): ibuprofen, ketoprofen, flurbiprofen, diclofenac, diflunisal, indomethacin, aminopyrine naproxen and hormones: estrone, β-estradiol, estriol, 17α-ethynylestradiol and diethylstilbestrol. The analytical method consisted of pre-concentration and isolation of analytes using solid phase extraction, derivatisation using the mixture 99% BSTFA /1% TMCS and analysis by GC-FID and GC-MS. This procedure was applied to investigation of surface and wastewater samples collected in Poland. Up to now, only limited data about the presence of these compounds in Polish water samples are available. Therefore this study increase knowledge on this subject.
Acknowledgement: Financial support was provided by the Polish Ministry of Research and Higher Education under grant N N204 260237 (2009-2012)

MO 050
Development and validation of analytical method for determining the drug diclofenac, nimesulide and acetaminophen in surface waters from São Carlos, SP, Brazil
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Over the last 10 years there has been increasing interest in the study of drugs, which made the monitoring of waste gained great importance due to the fact that many of these substances are frequently found in surface waters of the cities, in concentrations ranging from μgL^{-1} to ngL^{-1}. In the last few years, the occurrence of pharmacologically active compounds in the aquatic environment was recognized as one of the emerging issues in Environmental Chemistry. Recent surveys indicate that between 50 and 90% of a dose of the drug is excreted unchanged and persists in the environment, reaching water sources by passing through sewers. Diclofenac, nimesulide and acetaminophen are drugs widely consumed in Brazil and about 60% of medical prescriptions have one of the three drugs as the main agent for treatment of diseases. HPLC-DAD was used to analyze these three substances in reversed phase, using a C_{18} column (250 x 4.6 mm, 5 mm), coupled with pre-column C_{18} (12.5 x 4.6 mm , 5 mm), flow rate 1 mL min-1, injection volume of 20 µL, excitation at 280 nm and emission at 300 nm. A gradient mobile phase made up of methanol and water free of organics was used, both acidified with trifluoroacetic acid. Samples were pretreated with C_{18} adsorbent (AgilentSampliQ OPT), 3 mL / 60 mg, conditioned with 3.0 mL of hexane, 1.5 mL of acetone and 3.0 mL of methanol. Then, 200 mL of filtered sample were percolated through the cartridge, which was dried for 10 minutes. The analytes were eluted with 10 mL of methanol. The extract was dried under gentle stream of nitrogen and reconstituted with 500 µL of methanol. After half an hour, 20 µL of sample were injected into HPLC-DAD. Table 1 presents the linear equations, the detection limits (LOD), the quantification limits (LOQ) and the correlation coefficient (R^2) for each studied drug.
Drug t_R (min) Linear equation R^2 LOD* LOQ*

Diclofenac 14.74 y = 12.706x + 2.5405 0.994 0.5 1.1
Nimesulide 13.64 y = 2.1x - 1.6 0.992 0.5 1.1
Acetaminophen 9.41 y = 5.0357x - 2.0714 0.991 0.8 1.2
* μgL^{-1}
Table 1 - Values of t_R, LOD, LOQ and R^2
The proposed method was adequate for the separation and quantification of the drugs studied, and the validation parameters were considered satisfactory for acceptance of the proposed method.
Acknowledgement - The autors thanks CAPES and FAPESP

MO 051
Optimization of chromatographic conditions to the analysis of sulfonamide residues using experimental design
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Sulfonamide is an antibiotic class used in human and animal drugs. The presence of their residues in environmental and feed matrices is a great concern, because it can cause bacterial resistance, alergic reactions and intoxications, which prejudices the effectiveness of medical treatments and causes environmental and health problems. In this study, chromatographic conditions were optimized to the analysis of the sulfonamides: sulfathiazole (STZ), sulfamethazine (SMZ) e sulfadimethoxine (SDM), by using experimental design. The separation was performed on a HPLC-DAD, using a Luna C18 (100 x 4.6 mm, 5 µm) column. The injection volume was 17 µL and the wavelength was 270 nm. A complete factorial design with a central point was built to scan the significant variables, considering: flow rate, column temperature and proportion of ethanol and acetic acid in the mobile phase. The analyzed responses were the resolution (R_S) between the peaks and the ratio between the resolution and the analysis time (R_S/t). The only variable that had significant effect was the proportion of ethanol, that was negative for the separation between STZ and SMZ, and positive for SMZ and SDM. Thus, gradient elution was necessary to separate the three analytes. The gradient optimization was performed, finding out good separation using as initial condition 0:100 ethanol:water, which was ramped linearly to 20:40 at 5 min, to 40:60 at 8 min and to 50:50 at 14 min, then ramped over 1 min to 0:100. A second factorial design was built to evaluate the influence of the following variables: flow rate, temperature and proportion of acetic acid in the mobile phase. Choosing as answers the peak's height (H) and the ratio between the peak's area and width (A/w), all the variables had negative effects, that means that the best levels were: flow rate of 0.5 mL min_{-1}, temperature of 20 ºC and mobile phase without acetic acid. Therefore, the chromatographic conditions were optimized to the analysis of the sulfonamides using relatively low flow rate and mobile phase composed by water and ethanol, which is an renewable organic solvent less toxic than other ones traditionally used in HPLC. The linearity of the equipment to the analytes was over 0.999 for concentrations ranging from 5 to 500 µg L-1, with quantification limits under 10 µg L^{-1}. Thus, this is a greener chromatographic method, which can be used to analyze sulfonamide residues in environmental and feed matrices, after a suitable sample preparation.

MO 052
Analysis of phosphodiesterase type V inhibitors: distribution, occurrence and fate in surface water and suspended solids
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The presence emerging contaminants like pharmaceuticals, endocrine disruptors, and illicit drugs in waters has been continuously reported in recent years [1]. The consumed amount of drugs, like stimulating drugs, is usually higher than legally prescribed quantities. Among these compounds, a class of drugs, which is often illegally acquired, includes the active ingridients of Viagra, Levitra and Cialis, namely sildenafil, vardenafil and tadalafil [2]. They have been used in therapies of erectile dysfunction acting as a selective inhibitors of phosphodiesterase type V (PDE-V) [1-3]. This work was focused on developing a method to analyze PDE-V inhibitors: sildenafil, vardenafil and tadalafil, two main metabolites (demethylsildenafil and desethylvardenafil) and also some analogues such as, hydrohomosildenafil, homosildenafil, thiosildenafil (which have been already detected as adulterants in herbal products) and norneosildenafil, which has never been detected in environmental samples. Surface water like, wastewater (influent and effluent samples) and river water were analyzed along with suspended solids to investigate the distribution of these compounds in the environment.

MO 053
Optimization of SPE conditions for anti-cancer drugs and antidepressants isolation
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Among the various compounds considered as emerging pollutants, pharmaceuticals at ng/l levels are of particular concern because of both their ubiquity in the aquatic environment and their health effects. The number of either prescribed substances, especially antibiotics and antidepressants or non-prescribed drugs,. All mentioned compounds are bioactive - they are synthesized for specific intended effects on living beings. To estimate environmental risks it is required to collect data connected with documenting contamination of the aquatic environment by these molecules. Different analytical methods are used to detect these substances in human organisms and environment. In environmental studies solid-phase extraction is extensively used to extract pharmaceutical products from aqueous matrices.
In this work analytical procedure involving solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) has been developed for determination of 7 compounds including anti-cancer drugs (flutamide, melphalan, chlorambucil) and antidepressants (doxepin, desipramine, domipramine, imipramine). Each condition affecting the efficiency of the SPE step, for example: sample pH, nature of the adsorbent in the cartridge, and the elution strength of solvents were optimized before application of the optimized procedure to environmental samples. Five different sorbents (e.g., Oasis HLB, Strata-X, Lichrolut C18, and Lichrolut EN, XAD-2 resins) have been assessed for pre-concentration as well as for clean-up of pharmaceuticals in water samples.
A sensitive, specific and highly reproducible method for the separation at the trace level has been obtained. The developed method was applied to analysis of these compounds in environmental samples.
Acknowledgement: Financial support was provided by the Polish Ministry of Research and Higher Education under grant N N204 260237.

MO 054
Application of solid-phase microextraction coupled with gas chromatography for the analysis of tricyclic antidepressants and anti-cancer drugs
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In recent years, toxicant poisoning and environmental pollution by pharmaceuticals have received great attention. The appropriate control of these problems and prevention of related health hazards has become an important issue. Therefore, there is a need for an accurate and precise method for determining these compounds in various matrices. Currently, SPE is the most popular, well-established sample-preparation technique, with which the best sensitivity is obtained. Alternative techniques (e.g., Solid-Phase Microextraction, SPME) should be applied more often because of several advantages that they have over SPE in terms of speed, ease of sample handling and minimizing solvent use.
In this study SPME coupled with gas chromatography and gas chromatography-mass spectrometry has been used for the determination of four tricyclic antidepressants (doxepin, desipramine, domipramine, imipramine) and three anti-cancer drugs (flutamide, melphalan, chlorambucil). In SPME sampling extraction is based on the distribution equilibrium between the SPME fiber and the sample matrix. The conditions of the greatest effect on extraction efficiency such as nature of stationary phase, extraction time, temperature, rate of stirring, salt concentration (to induce a salting out effect which can enhance extraction efficiency in conventional SPME) and desorption time were optimized for all compounds. A polydimethylsiloxane-divinylbenzene (60-µm film thickness) fiber was selected after the assessment of different types of coating. The obtained SPME procedure was combined with derivatization (99% BSTFA /1% TMCS) of analytes adsorbed on SPME fiber and GC-FID or GC-MS analysis. Under optimal conditions the method was validated and good analytical performance parameters were obtained. The method has also been shown to be a useful tool for environmental application.
Acknowledgement: Financial support was provided by the Polish Ministry of Research and Higher Education under grant N N204 260237.

MO 055
Membrane assisted solvent extraction combined with large volume injection gas chromatography for the analysis of musk compounds in environmental water samples
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Nowadays, Personal Care Products (PCPs) are ubiquitous in the environment due to the wide use of soaps, household detergents, perfumes or fragrances. Consequently, the analysis of PCPs and their possible impact on ecosystems and biota have become in an emerging area of research. Among other compounds, synthetic musks are common compounds in fragrances and they are largely being studied due to their persistence and/or their toxicity.
In recent years, many analytical procedures have been developed for the analysis of musk fragrances in several environmental matrices such as waste-waters, sediments, sludge or biota in which both extraction and detection procedures have been optimised. The extraction and preconcentration step take an important role since the determination of sub-ppb quantities of these compounds is usually required. Liquid-liquid extraction (LLE) or solid phase extraction (SPE) have been commonly used for these purposes. However, the current tendency is to find environmentally friendly solvent-less approaches such as solid-phase micro-extraction (SPME), stir-bar sorptive extraction (SBSE) or membrane assisted solvent extraction (MASE). Although SPME and SBSE have been already proposed for the analysis of synthetic musk in environmental matrices the later has not been taken into account as alternative extraction method.
The aim of this work is the development of an analytical approach to determine synthetic musk compounds in water samples by means of membrane assisted solvent extraction coupled to large volume injection gas chromatography. For this purpose, on the one hand, the optimisation of MASE has been carried out studying the significant variables which can affect the extraction efficiency such as extraction solvent, sample volume, pH adjustment, MeOH and NaCl addition or the type of non-porous membrane. On the other hand, factors affecting large volume injection have been also optimised in order to obtain the best sensitivity. The performance of the analytical method was validated attending to detection limits, accuracy and precision. The optimised method has been applied in real water samples to analyse these priority pollutants.
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MO 056
Development of an analytical method for analyzing parabens and UV filters in water by LC-MS/MS
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The presence of pharmaceuticals and personal care products (PPCPs) in environmental samples is a topic of increasing interest.
Among PPCPs, triclosan and triclocarban are antimicrobial compounds used in soap, toothpaste, deodorants and other consumer products.
Another group of PPCPs are UV filters. These sunscreen agents are chemical compounds which are used in a variety of cosmetics (sunscreen creams and lotions).
Another group of PPCPs is made up of parabens which are the most common preservatives and bactericides in pharmaceuticals and food products. Methyl paraben and propyl paraben are the most widely used and are normally used together due to their synergistic preservative effects.
The focus of this poster is the development of a method for determining a set of personal care products from different groups in a single analysis. Parabens, UV filters and two antimicrobial agents have been determined in water samples using solid-phase-extraction followed by ultra high performance liquid chromatography-tandem mass spectrometry.
The presentation could be divided into 3 main parts:
- Development of extraction procedure and optimization of the different parameters (choice of the cartridge for solid phase extraction and choice of the solvent for elution)
- Evaluation of the analytical method in term of performance of the method (LOQ) and repro-

ductibility (recovery, relative standard deviation).
- Analysis of real samples and presentation of the first results obtained.

MO 057

Extraction and detection of Ionophores in the aquatic environment

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Ionophores are the most heavily applied sup-group of the two sub-groups of anticoccidial agents, which are antiparacitic compounds used extensively worldwide as prophylactic chemotherapeutics and growth promoters in livestock production. As an example, the yearly consumptions of active compounds are more than 10 tonnes in Denmark and for the Republic of Korea more than 800 tonnes (Hansen *et al.* 2009a, Kim *et al.* 2008).
Ionophores are antibiotic drugs that form lipid soluble complexes with, primarily, alkali cations that inhibit or kill pathogenic parasites in livestock. Several reports have revealed that ionophores are emerging environmental contaminants in agricultural run-off waters, surface waters, sediments, and ground waters, due to their continuously increased and constant application as feed additives in modern livestock production (Dolliver *et al.* 2008; Hansen *et al.* 2009a and 2009b). Recent investigations has further reported that metabolites of certain veterinary drugs such as antibacterial agents (i.e. tetracyclines) and antiparacitics (i.e. ionophores) posses an environmental effects of similar level as their parent compounds on the soil bacterial community (Halling-Sørensen *et al.* 2002; Hansen *et al.* 2009c).
The focus of the present study is on the recent advances of a new analytical method for sampling, extraction and detection of ionophores in liquid matrices. The hyphenated method consists of an integrated clean-up with solid phase extraction followed by high-performance liquid chromatography tandem *in space* mass spectrometry.

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MO 058

Detection of Chitosan Oligosaccharide by MALDI-TOF MS

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Because of their increasing use in the pharmaceuticals and cosmetics industry, there is a need to study the fate of soluble, bioactive chitosan oligosaccharides and their degradation products in water. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a fast but highly selective way of detecting oligomers like chitosan.
In this study, the parameters in the MALDI-TOF MS analysis of chitosan oligosaccharide lactate were optimized to improve sensitivity of the method. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix. The spots prepared by vacuum drying gave more intense and more reproducible analyte ion signals. Compared to the spots prepared by drop and dry and by quick and dirty techniques, vacuum dried spots were thinner, less crystalline, more evenly spread and with fewer hot spots.
Similar to that of other polymers, the presence of cations is necessary in the formation of ionized chitosan, mostly as sodium adducts. For better signal, however, the amount of salt was optimized by partial desaltation using Amberlite mixed-bed ion exchange resin. Fractionation by size exclusion chromatography improved the signal of the higher molecular weight chitosan.

MO 059

Comparison of online pre-concentration and direct injection volumes for the quantification of pharmaceuticals and personal care products in water with Orbitrap™ high resolution mass spectrometry

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There is a growing environmental concern regarding the health impact of trace levels of pharmaceuticals and personal care products (PPCPs) in groundwater. For this reason, the US EPA has recently developed an LC/MS/MS method for the quantification of dozens of PPCPs in environmental water sources. Prior to LC/MS/MS analyses, EPA Method 1694 calls for the solid phase extraction (SPE) of up to 1 L of water to achieve low ng/L limits of quantitation (LOQ). Instead of processing 1 L of water by the manual, time-consuming process of SPE, we present the alternative approach of on-line pre-concentration in series with LC/MS/MS using smaller volumes of water (0.5 mL) to achieve ng/L quantitation limits. For comparison, the same compounds were analyzed using a 5 uL direct injection volume without any pre-concentration. The mass spectrometer used was a High Resolution Accurate Mass (HRAM) Exactive Orbitrap instrument operated at a resolution of 50,000 and a Mass Accuracy of < 5ppm. The High Resolution in combination with the High Mass Accuracy giving the advantage to separate interfering matrix or other compound with very similar m/z values.

MO 060

Quantitative determination of endocrine disrupting chemicals and pharmaceutically active compounds in Singapore's marine environment

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The discharge of endocrine disrupting chemicals (EDCs) and pharmaceutically active compounds (PhAC's) into the environment via household, municipal and industrial wastes is a pressing public health issue. Many of these emerging environmental contaminants are high production volume (HPV) chemicals and thus may attain sufficiently high environmental concentrations to cause toxic effects. The objective of this study is to provide robust analytical techniques for accurate multi-residue analysis of several EDCs and PhACs in complex environmental and biological matrices such as seawater, sediments and tissues of marine organisms. Here we present development of analytical methods for the quantitative determination of several EDCs and PhAC's in surface waters, sediments and biota from Singapore's marine environment. Target analytes include a comprehensive list of brominated flame retardants (BFRs), perfluoroalkyl chemicals (PFCs), synthetic musks, plasticizers, pesticides, as well as common pharmaceuticals such as Atorvastatin, Carbamazepine, Diltiazem, Dilantin, Risperidone, Ibuprofen, Sulfamethazine, Sulfamethoxazole, Paroxetine, Cimetidine, Naproxen and Fluoxetine. We conducted a comprehensive field survey of surface waters, sediments and organisms at various locations from Singapore's coastal marine environment. Seawater samples were filtered/extracted using a filtration apparatus containing glass fibre filters and solid-phase extraction (SPE) disks in order to determine freely dissolved and particulate bound chemical concentrations. Marine sediments and tissue samples were extracted using accelerated solvent extraction (ASE) and further cleaned-up using gel permeation chromatography (GPC), SPE and/or Florisil chromatography. Identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) or gas chromatography mass spectrometry (GC-MS). 13C or deuterated mass-labeled standards were used as internal standards to correct for matrix effects. Results of analyte recovery tests, procedural blanks and replicate sample analyses demonstrate generally good performance of the methods. The significance of the occurrence, levels and patterns of various EDCs and PhACs in Singapore's marine environment are discussed.

MO 061

Comparison of sorbents used in solid phase extraction to multi-residues analysis of pharmaceuticals in water samples

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Following the increasing consumption, and thus the production and excretion of pharmaceuticals, there is a high risk of penetration of these compounds to aquatic and terrestrial ecosystems. Consequently, the quality of drinking water may be endangered, so the monitoring of wastewater effluents, surface and tap water is required. The polar, water-soluble pharmaceuticals are present in trace amounts, and in the case of surface water or wastewater, they are accompanied by complex organic matter. Nowadays, modern analytical methods combine sample enrichment (solid-phase extraction, SPE), derivatization and GC analysis for trace-level detection.
The main aim of our study was to develop SPE-derivatization-GC method for the determination of 13 pharmaceuticals including non-steroidal anti-inflammatory drugs (ibuprofen, ketoprofen, flurbiprofen, diclofenac, diflunisal, indomethacin, aminopyrine and naproxen) and hormones (estrone, β-estradiol, estriol, 17α-ethynylestradiol and diethylstilbestrol). During this study special attention was taken into SPE step. Water samples were spiked with a known amount of analytes and subjected to SPE procedure with application of different types of sorbents (Oasis HLB, Strata X, Oasis MCX, Lichrolut EN, C18) and different conditions of conditioning, loading, washing and eluting steps (e.g. methanol and ethyl acetate were tested as an eluent). Recoveries and LODs values of individual target compounds were investigated. The optimized SPE procedure was combined with derivatization step and GC analysis giving the method suitability in surface and wastewater monitoring.

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MO 062

Investigating the presence of pharmaceuticals and metabolites in environmental water and wastewater by UHPLC-MS/MS with triple quadrupole and time of flight mass analyzers

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Pharmaceutical residues are a matter of concern because of their wide consumption and potential negative effect on water quality and living organisms. After human and veterinary consumption, pharmaceuticals are excreted mainly as the parent compound, although many of them are also partially metabolized. Consequently, both parents and metabolites enter into urban wastewater. Most of them are not completely removed during wastewater treatments and they can finally arrive at surface and ground waters.
In this study, a UHPLC-MS/MS (QqQ) multi-class method for the determination of 47 pharmaceuticals in environmental and wastewater samples has been developed. The target list of analytes included analgesic and anti-inflammatories, cholesterol lowering statin drugs and lipid regulators, antidepressants, anti-ulcer agents, psychiatric drugs, cardiovasculars and a high number of antibiotics. Quantification and confirmation was simultaneously carried out by acquisition of two SRM transitions and the compliance of retention times and Q/q ratios.
In parallel, a wide-scope, multiclass, screening of organic contaminants based on UHPLC-QTOF MS has been developed and applied to the water samples. Around 1100 organic contaminants were investigated, including many pharmaceuticals and metabolites, but also drugs of abuse, pesticides, mycotoxins, hormones, UV-filter agents, colorants, preservants and phenols. The QTOF instrument was used under MSE mode, which allowed to obtain full spectrum acquisition data at low and high collision energies. Data at LE and HE were processed using specialized software in a targeted mode. The presence of the ion (typically the (de)protonated molecule) measured at its accurate mass (nw-XIC of 0.02 Da) at the expected retention time (if available) was evaluated. Additionally, the presence of CID fragments or a characteristic isotopic peak was assessed for the reliable identification of the compounds detected.
The developed methodology was applied for monitoring pharmaceuticals in surface and effluent wastewaters from the Spanish Mediterranean region. Using triple quadrupole target method, analgesics and anti-inflammatories, lipid regulators and quinolone antibiotics were the most detected groups. QTOF MS allowed detecting additional pharmaceuticals such as angiotensin II receptor antagonists and the diuretic furosemide, which did not form a part of the list of target analytes in the QqQ method. Drugs of abuse and pesticides were also identified.

MO 063

Determination of selected pharmaceuticals in biota from Swiss surface water using liquid chromatography-tandem mass spectrometry

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Since numerous pharmaceuticals have been detected in the influent and effluent of waste water treatment plants or in waste water treatment plants themselves and surface water they have become an emerging issue in environmental chemistry. In most cases water samples have been collected and concentrated by solid phase extraction prior chemical analysis. However, the current project analyzed residues of polar to lipophilic pharmaceuticals from different trophic levels.
The determined pharmaceuticals in fish and macrobenthon included the beta blocker Atenolol, the antihistamine Diphenhydramine, the calcium channel blocker Diltiazem, the anticonvulsant Carbamazepine, the antidepressant Fluoxetine and its metabolite Norfluoxetine, the analgetics Ibuprofen and Diclofenac. Mefenamic acid, a non-steriodal anti-inflammatory drug and Sulfamethoxazole which is a sulphonamide bacteriostatic antibiotic were also analysed.
Trace analysis of compounds was performed in liquid chromatography coupled to mass spectroscopy (LC-MS). Identification and quantification of pharmaceuticals in biota were arranged in

MS/MS mode. MRM transitions were classified in a different elution time window to increase measurement sensitivity.
Residue data from fish and macrobenthon analysis confirmed four substances (Norfluoxetine most important active metabolite of widely used antidepressant Fluoxetine, Fluoxetine itself, Diphenhydramine, Carbamazepine) to be present in biota and fish. The identified concentrations of analytes were ordinary higher in liver than in muscle tissue.

MO 064

The occurence of selected antibiotics in the southern Baltic Sea

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In the course of the last decade pharmaceuticals have become recognised as relevant environmental contaminants. Surface waters receive continuous inputs of pharmaceuticals, but the substances also undergo various chemical, physical and biological processes. Seas can be seen as the final sink of the most persistent compounds. However, knowledge on their behaviour and fate in marine environment, including the Baltic Sea is still very limited. Special attention should be paid to antibiotic residues. These bioactive compounds may strongly affect bacterial populations and induce biological responses in nontarget organisms due to prolonged exposures, potentially disrupting ecosystem processes. Hence, it is essential to assess the impact of antibiotics on marine ecosystems. One of the main targets in this issue is identification of their concentration levels in the environmental samples. This study report first measurements and identification of selected antibiotic classes occurring in water and sediment samples collected from the southern Baltic Sea.

MO 065

Survey of pharmaceuticals and personal care products in the Pearl River and risk characterization

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Ten non-steroidal anti-inflammatory drugs (NSAIDs), two blood lipid regulators (BLRs) and two anti-epileptic drugs (AEDs) were analysed in the Pearl River system (i.e., Liuxi, Zhujiang and Shijing Rivers) and four sewage effluents during the dry and wet seasons, and the environmental risks they posed were assessed. Eight pharmaceuticals were detected in the rivers and effluents, including five NSAIDs (i.e., salicylic acid, ibuprofen, diclofenac, mefenamic acid and naproxen), two BLRs (i.e., clofibric acid and gemfibrozil) and one AED (i.e., carbamazepine). The median concentrations for the eight pharmaceuticals ranged from 11.2 to 102 ng/L. Seasonal variations were not obvious for most pharmaceuticals in the three rivers except for salicylic acid and clofibric acid in the Zhujiang River, and diclofenac in the Zhujiang and Shijing Rivers. However, spatially considerable variations in the concentrations were observed for the eight pharmaceuticals in all three rivers. For most of pharmaceuticals, the effluents from the four wastewater treatment plants and Shijing River water were found to be the major discharge sources for Zhujiang River, but with additional discharge sources from some small urban streams in the wet season. Diclofenac in Shijing River was the only pharmaceutical that had a risk quotient (RQ) greater than 1, indicating a high risk to aquatic organisms in the river. The other pharmaceuticals all had RQs less than 1, indicating minimal or medium risks to aquatic organisms. Although higher RQs were calculated for the mixture of the pharmaceuticals in each river, the risk rating remained the same for the three rivers with the RQ being more than 1 only in Shijing River.
Keywords"ð Pharmaceuticals Occurrence Surface water Risk quotient River

MO 066

Pharmaceuticals, sources and risk analysis

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The occurrence of several pharmaceutical compounds in waters has raised concern, since they are found in surface water (ng/l-ug/l) as well as in drinking water (ng/l). The current project will highlight and summarize the effort of the past couple of years. The emission from larger hospitals to smaller care institutions and nursing homes are investigated and compared with emission from households. As a final prioritization effort, the emission data are ranked based on environmental hazards. The study will highlight the emission loads of the different sources and draw conclusions for the best removal options in terms of costs and removal efficiency. The effect of the emission load of pharmaceuticals in the ecosystem depends on the dilution factor to the receiving water. Next to this, the type of pharmaceutical determines the hazard and from this, the actual risk. The study highlights that routes of the emission need to be examined to not only predict better the actual environmental concentrations and measure these, but also to indicate the best removal options.

MO 067

Occurrence and bioaccumulation of organic UV filters and UV light stabilizers in Japanese rivers and lakes

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We established methods of simultaneous determination of eight organic UV filters and 10 UV light stabilizers in water, sediment and biota. We applied these methods to 26 rivers and three lakes with BOD concentrations in the range of 0.6-80 mg/L. The total contaminants identified in dissolved water samples ranged from N.D. to 4,928 ng/L. Their profiles in dissolved water samples were much different among streams contaminated by domestic wastewater, sewage treatment plant effluent, rivers and lakes contaminated and little contaminated rivers. Though concentrations of UV filters and UV light stabilizers in sediment samples ranged from 1 to 6,643 mg/kg (organic carbon basis), their profiles were little different among all samples. UV-328 and UV-234 accounted for 70 to 80% of the total contaminants identified. Octocrylene, 2-ethyl hexyl-4-methoxycinnamte, benzophenone and UV-326 were also ubiquitous in sediment samples. We also measured their concentrations in aquatic biota at various trophic levels. The contaminants were detected in all biota samples at concentrations in the range of 38 -116,000 ng/g (lipid weight basis). Measuring carbon and nitrogen stable isotope ratios in biota revealed that UV326, UV-327 and UV-328 were not bioaccumulative in aquatic food webs.

MO 068

Occurrence of pharmaceuticals in the settleable particulate material (SPM) of urban and non-urban freshwaters

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Pharmaceuticals are a diverse group of anthropogenic chemicals continuously released to the environment, commonly detected in surface waters as concentrations ranging from ng/L to low µg/L. Besides those dissolved in discharges, wastewater treatment plants (WWTP) are also important sources of settleable particulate material (SPM), heading to sediments with suspended solids originating naturally. So far there is only little information about the occurrence and consequences of pharmaceuticals in sediment systems. The objective of this work was to study if pharmaceuticals are detected from SPM next to WWTPs or even in rural areas, thus being subtle for sedimentation.
SPM samples were collected from ten sites in Finland, grouped into reference, rural and wastewater effluent sites. SPM collectors made of stainless steel were placed to ca. 35 cm above lake or river bottom for about two months in summer. After extraction, a set of 18 pharmaceuticals was analysed with LC-MS/MS.
Several pharmaceuticals (from 8 to 13) were detected in SPM accumulated in sites next to WWTPs. Concentration of citalopram was notably high (300-1350 ng/g dw). Also bisoprolol and ciprofloxacin were detected at high concentrations (6-325 ng/g dw and 9-390 ng/g dw, respectively). On the other hand, none were detected above LOD from reference or rural sites. There is no previous information about the presence of pharmaceuticals in SPM. Our results show that pharmaceuticals are sorbed to particles in WWTP and nearby, eventually ending up into sediments. These results also indicate that pharmaceuticals are not markedly contaminating the sediment in rural areas. In urban habitats however, depending on persistence and bioavailability, sediment-bound pharmaceuticals may affect benthic invertebrates and habitats. However, more data are needed for the fate and ecotoxicity assessment of risks to the benthic biota.

MO 069

The presence and fate of pharmaceuticals and personal care products (PPCPs) in wastewater and Charleston Harbor, South Carolina, USA

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Pharmaceuticals and personal care products (PPCPs) are considered to be a group of emerging contaminants that includes over-the-counter, prescription, and veterinary drugs, as well as chemicals found in cosmetics and common household products. These chemicals may be introduced into aquatic environments by non-point and point sources including domestic wastewater treatment plant effluents. This study assessed seasonal and regional trends of PPCPs detected in samples from two local wastewater treatment plants (WWTPs) and surface water from the Charleston Harbor over the period of twelve months. After modifying EPA Method 1694 to include hormones, analysis using HPLC/MS/MS revealed that of the 19 target compounds examined, 11 were quantified above method reporting limits in wastewater influent, 9 in effluent, and 7 in surface water samples. Concentrations were reduced by > 90% for most chemicals in effluent compared to influent, though concentrations of some PPCPs in effluent were higher than those in influent. Differences in effluent concentrations and estimated removal between WWTPs are believed to be related to variation in general operating parameters and/or the anoxic basin employed by one of the facilities. Overall, future monitoring of PPCPs may aid in minimizing potential negative impacts of increasing urban/industrial development in coastal regions.

MO 070

Broad screening of organic chemicals in Dutch groundwater and their occurrence in relation to land use and hydrology

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Groundwater is considered a valuable non-renewable resource for drinking water. It is therefore important to monitor groundwater quality, assess the vulnerability of groundwater wells, and identify anthropogenic activities that can result in groundwater contamination. In this study high resolution mass spectrometry (hybrid linear ion trap (LTQ) FT Orbitrap MS) was applied to screen for a broad range of more than 600 known and unidentified (polar) organic chemicals. Nearly 400 chemicals were observed, of which 82 could be identified and more than 313 could not be identified.
The results obtained were interpreted in relation to the source characteristics and land use. Groundwater that was affected by landfills showed the highest total MS response (ion counts) and most individual chemicals and was therefore considered most contaminated, while river bank filtrated water was generally more contaminated than phreatic groundwater, and groundwater from (semi-)confined aquifers was most pristine. Additionally, industrial chemicals were more frequently observed in river bank filtrated water and pesticides were more frequently observed in water originating from rural areas. Finally, the applied broad screening technique illustrated that only 42 ± 25% of the ion counts came from known chemicals. Screening for both known and unidentified chemicals provides more information on the over-all water quality and reveals that there is a 'blind spot' considering the total pool of organic contaminants in the environment.

MO 071

Using GIS to establish an emission type based sampling scheme with passive samplers in Luxembourg

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Waste Water Treatment Plants (WWTP) have been identified as a major source for pharmaceuticals in river networks in recent years. WWTPs remain neglected as a source for pesticides although a substantial body of evidence shows that especially for chronic exposure WWTPs have a significant impact. Monitoring xenobiotics is a cumbersome and costly endeavour if the dynamics of the emissions are to be taken into account. The other aspect is the identification of locations where the exposure is expected to be the highest and most frequent. This poster presents an approach which used GIS and WWTP information to identify typical immission situations in Luxembourgish rivers. The following five immission situations were considered: Rivers impacted by high WWTP discharge, subdivided in those with more urban connections to the WWTPs and those with farms owning lots of agricultural land. The latter are more prone to emissions from left-over spilling of pesticides and spraying equipment cleaning. The second source group targets runoff from surfaces with low- and high infiltration soils and impermeable urban surfaces as categories. All Luxembourgish water bodies have been analyzed with a GIS system in order to isolate monitoring locations that would best correspond to one of the categories defined above.

Twelve sampling locations have been monitored in the main pesticide application seasons with 14 day passive sampler exposure summing up to 4 months monitoring at each spot. The results served to establish a ranking of most detected pesticides and pharmaceuticals and to verify the soundness of the allocated emission sources. In addition the mean exposure has been compared to ecotoxicological assessment methods such as SPEAR, toxic unit as well as pesticide mixture evaluation. The poster discusses the combination of GIS and passive samplers as a reliable monitoring approach.

MO 072

Determination and distribution of triclosan and methyl triclosan in estuarine settings

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Triclosan (TCS), an antibacterial agent, and methyl triclosan (Me-TCS), TCS degradation product, are sewage-derived contaminants. A method for the determination of both compounds in sediment and water samples has been optimized. For solid samples, extraction and cleanup were integrated into the same step using pressurized liquid extraction with in-cell-clean-up. Sediments (4 g) were homogenized with sodium sulfate anhydrous and introduced in a 11 ml stainless-steel extraction cells containing 1g of Florisil. The extraction was performed using dichloromethane at 100 ºC, 1500 psi and 3 static extraction cycles of 5 min each. For water samples, stir bar sorptive extraction-liquid desorption (SBSE-LD) has been used. Stir bars coated with polydimethylsiloxane (PDMS) were placed in a flask containing the water samples (100 ml) and stirred for 24 hours at room temperature. Then, the bars are sonicated during 30 min using acetonitrile to release the analytes. Me-TCS and a silylated derivative of TCS were determined by gas chromatography -mass spectrometry (GC-MS). Recovery experiments in water and sediments were performed and the results ranged from 65-144%. Limits of quantitation (LOQs) were 15 ppt for TCS and 10 ppt for Me-TCS, in water samples, and 0.1 ppb for TCS and 0.2 ppb for Me-TCS, in solid samples. The method was validated by carrying out a sampling in the estuary of Guadalete River (SW Spain). TCS and Me-TCS concentrations up to 9.6 ppb in sediments and 310 ppt in water were measured. Their distribution in sediments was affected by the presence of wastewater discharges, where maximum concentrations were detected. Highest values were reached in the water column during low tides as the water volume in the estuary becomes lower.

MO 073

One year monitoring of pharmaceuticals in aquatic systems: acquisition of data concerning fate and behaviour in surface and groundwaters

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Occurrence of pharmaceutical products in aquatic systems has been highlighted for few years, showing a potential sanitary and environmental impact on water quality. The present work deals with monitoring of 70 stations located in Loire -Brittany river basin, including surface waters and groundwaters. 40 pharmaceuticals and veterinary products including metabolites and owning to the main therapeutic groups were monitored in Summer 2009 and Winter 2009-2010 in order to document seasonal variability and assess contamination level in French aquatic systems. Two rivers were particularly monitored, with monthly sampling of 4 stations spreaded over the river from upstream to downstream of WWTP discharge, to better understand seasonal variations, fate of pharmaceuticals and impact of WWTP effluent introduction in the river.
Results gave a global overview of the pharmaceutical contamination. Frequencies of detection depending on the type of water and the land uses in impacted river basin allow to determine "key pollutants"that can be used as tracers of pharmaceutical pollution, considering on the other hand their persistency or their high reactivity in aquatic systems.
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MO 074

A multi-component snapshot of new pharmaceuticals and pesticides in the river Meuse

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The river Meuse serves as drinking water source for over six million people in France, Belgium and the Netherlands. In 2009, over 500 million m3 surface water from the Meuse was abstracted for this purpose. Rapid improvements in chemical and bio analytical techniques have led to the discovery in surface waters of all kinds of so-called emerging contaminants at (sub) ng/L to µg/L concentrations, including pharmaceuticals, illicit drugs, sweeteners, perfluorinated compounds, and various steroid hormones. Drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during purification processes. Pharmaceuticals and pesticides, both developed to be biologically active, are important classes of contaminants present in the river Meuse.
In this study, sensitive and specific multi-component methods on UPLC-MS/MS were developed for the analysis of new pharmaceuticals and pesticides. The methods were applied to analyse water samples from 15 locations along the Dutch catchment of the Meuse. In this way, a snapshot was made of its chemical water quality with respect to pharmaceuticals and pesticides. The results were used to estimate the contribution of specific emission sources (industry, WWTP, agriculture) in the catchment. Multiplication of measured concentrations with water discharge data was performed to assess the (bruto) loads of pharmaceuticals and pesticides transported to the North Sea and the contribution of activities in the Dutch catchment to these loads.

MO 075

First reports of pharmaceuticals and personal care products from Ushuaia, Tierra del Fuego, Argentina

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Ushuaia is commonly regarded as the southernmost city in the world, is located in a wide bay on the southern coast of Isla Grande de Tierra del Fuego, bounded on the north by the Martial mountain range and on the south by the Beagle Channel. Ushuaia has developed on the coastal areas adjacent to Ushuaia and Golondrina Bays and currently has approximately 64.000 inhabitants. For 20 years, the entire coastal area has been receiving input from sewage, industrial and port, producing visible changes, some of them partially quantified. In particular is concerned generally poor and inadequate functioning of the sewage collection system and dispersant, located at the western end of the Ushuaia Peninsula, which has led to the discharge of untreated sewage along the coast. We study the drainage channels that cross the city, which receive the discharge of all types of wastewater. The samples were characterized by measuring physical and chemical parameters: dissolved oxygen, conductivity, salinity, turbidity, pH, temperature, total hydrocarbons, BOD, COD, soluble phosphorus, ammonia, nitrates, fat and oils, fecal and total coliforms, algal, cladocerans and fish acute toxicity, and chronic toxicity to Daphnia magna. Analytical determination of Pharmaceuticals and Personal Care Products compounds in surface water were performed by manual solid-phase microextraction with unbonded fibers of polydimethylsiloxane at a stationary phase of 100 and 7 µm thickness and one of 85 µm of polyacrylate that were injected into a GC-MS. The main substances determined were: Personal Care: Benzyl cyanide, Benzyl oleate, Tetramethyl octacosanol, Decylhydroxylamine, Acids: Myristic, Hexanoic, Decanoic decyl ester, Cyclopentaneundecanoic, Hexadecanoic methyl ester, Didecil ether, Naphthalenone, benzophenone, Ethanol Tetradecyloxy, Musklactone, Isopropyl Myristate, Octadecane, Ethanol dodecyloxy, Benzeneacetonitrile, Biphenyl Diethyl. Pharmaceutical: Di-Tert-Butyl Benzoquinone, Oleamide, Nandrolone, Acenaphthene, Urea, Oxabicyclo-Octane, Tetradecanoic Acid Methyl, Serinol, Oxonanone, Cyclohexanone, Dodecane chloro, Phenol dimethylethyl, Dodecanamine dimethyl, Indolizine. Others: Trichlorophenol, Diphenyl, Octamethylcyclotetrasiloxane, Trybutyl phosphate, Eicosanol, Ethanediol monoformate, Naphthalene Ethenyl, Aminoheptadecane. Many of the detected compounds are included as those with High Production Volume since their environmental impact could be relevant and should be assessed.

MO 076

Occurrence and fate of selected pharmaceuticals in biotic and abiotic compartments from water bodies directly impacted by reclaimed water irrigation.

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Municipal wastewater has been recognized as one of the main routes bringing pharmaceuticals and personal care products (PPCPs) into the environment. Fish, however, are a sensitive indicator of what substances may be entering our water systems. In this study, we have developed methods for the analysis of 11 pharmaceuticals representing multiple therapeutic classes in fish tissues, sediments and pond water directly affected by reclaimed water. 1 g of fish tissue and 2 g of sediment were extracted by methylene chloride using accelerated solvent extraction followed by mixed-mode cation exchange cleanup and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Compared to the previously reported methods, developed protocol offers cleaner extracts giving lower method detection limits (MDLs) for 10 out of 11 selected pharmaceuticals. A simple SPE method was used to concentrate and cleanup all 11 pharmaceuticals in reclaimed water and thesurface waters from where fish were collected. During the period of a month, 9 out of 11 selected pharmaceuticals were continuously detected in reclaimed water, but only caffeine, diphenhydramine and carbamazepine were detected in fish tissue and pond water samples. Analysis of the pond sediments and some bottom feeders confirmed that pharmaceuticals such as fluoxetine and diltiazem were quickly removed from surface waters due to the adsorption to sediments where they seem to be persistent. To teh best of our knowlegde this is the first report of the potential accumulation of caffeine in fish tissue.

MO 077

National substance flow analyses for the assessment of illicit drug abuse using a sewer epidemiology approach

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Sewer epidemiology is an emerging field that relies on the measured concentrations of select active pharmaceutical ingredients (APIs) in wastewater influents to estimate the prevalence of illicit drug abuse within the wastewater contributing community. However, for certain illicit drugs of abuse the most suitable marker compound may not only originate from illicit abuse of the drug but also from multiple licit sources. In particular, the suggested API markers for heroin, amphetamine, and methamphetamine abuse are, respectively, morphine, unchanged amphetamine and unchanged methamphetamine, all of which have multiple licit sources that could lead to these APIs being released to sewers and hence be present in wastewater influents. Hence, recognizing that from a sewer epidemiology perspective the main interest lies in quantifying the fraction of such APIs that originates exclusively due to illicit drug use, it is important to compare the relative contributions of such a source to those that are expected to be realized through all licit sources. Further, by conducting such an analysis on a national level for many different countries, one can assess whether the sewer epidemiology approach for tracking the abuse of heroin, amphetamine and methamphetamine is more feasible in certain countries than others. The primary goal of this communication is to precisely address such concerns. Results obtained suggest that in certain countries sewer epidemiology can be readily used to monitor the abuse of such drugs, whereas in other countries consumption patterns of licit sources for relevant APIs are such that sewer epidemiologist are cautioned on exclusively relying on such a methodology. For example, when considering a substance flow analysis for morphine, which is the marker for heroin use, the results obtained suggests that in most European countries (with the exception of Scandinavian countries), a dominant or significant fraction of the sewer morphine load is expected to originate due to the populations' abuse of heroin. In contrast, in North American countries, the majority is expected to primarily originate due to the consumption of medicinal drugs that are metabolized to morphine.

MO 078

Persistency assessment and aerobic biodegradation of selected cyclic sesquiterpenes present in essential oils

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Sesquiterpenes are ubiquitous in essential oils but an assessment of their environmental behaviour is still required for their use as components of natural fragrance ingredients and oral care flavours. Persistency plays a key role in hazard and risk assessment, but the current knowledge on the biodegradation of sesquiterpenes in the aquatic environment is limited. This could have important consequences for PBT/vPvB assessments of essential oils since most of the sesquiterpene components, especially hydrocarbons, have a logKow of > 4.5 and are identified as potentially bioaccumulating according to REACH screening criteria. This work describes the results of a persistency screening assessment for a number of representative sesquiterpenes present in essential oils. Some of these natural sesquiterpenes contain structural fragments, such as quaternary carbon atoms and fused or bridged ring systems, which are often associated with poor biodegradability in xenobiotic organic chemicals. Current biodegradation prediction models (BIOWIN, BioHCwin and CATALOGIC) were found to be of limited use since most of the sesquiterpenes studied were outside the structural domain of the models. Aerobic biodegradation was measured in a standard

or prolonged OECD 301F ready biodegradability test. Ten out of eleven sesquiterpenes tested achieved significant ultimate biodegradation (ranging from 51% to 88% BOD). Germacrene D achieved 24% ultimate biodegradation and specific analysis at the end of the test indicated complete primary degradation. Given that the shape of the biodegradation curves indicates poor bioavailability and ready biodegradability tests are very stringent, it is expected that all the sesquiterpenes tested in this study would be degraded under environmental conditions. This evidence of non-persistence may be used to read-across to other natural sesquiterpenes within the same carbon skeleton family which are expected to follow similar biodegradation pathways.

MO 079

A tiered procedure for assessing the formation of biotransformation products of pharmaceuticals and biocides during activated sludge treatment

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Upon partial degradation of polar organic micropollutants during activated sludge treatment, transformation products (TPs) may be formed that enter the aquatic environment in the treated effluent. However, TPs are rarely considered in environmental risk assessments of wastewater-relevant compound classes such as pharmaceuticals and biocides. Here, we suggest and evaluate against field data a tiered procedure, including a fast and inexpensive initial screening step based on HR-MS/MS and a subsequent confirmatory quantitative analysis, that should facilitate consideration of TPs formed during activated sludge treatment in the exposure assessment of micropollutants. At the first tier, potential biotransformation product structures of seven pharmaceuticals (atenolol, bezafibrate, ketoprofen, metoprolol, ranitidine, valsartan, and venlafaxine) and one biocide (carbendazim) were assembled using computer-based biotransformation pathway prediction and known human metabolites, and screened for in sludge-seeded batch reactors using high-resolution tandem mass spectrometry (HR-MS/MS). The 12 TPs found to form in the batch experiments were then searched for in the effluents of two full-scale, municipal wastewater treatment plants (WWTPs) to confirm the environmental representativeness of this first tier. At the second tier, experiments with the same sludge-seeded batch reactors were carried out to acquire kinetic data for major TPs that were then used as input parameters into a cascaded steady-state completely-stirred tank reactor (CSTR) model for predicting TP effluent concentrations. Predicted effluent concentrations of four parent compounds and their three major TPs were corroborated by comparison to 3-day average influent and secondary effluent mass flows from one municipal WWTP. CSTR model-predicted secondary effluent mass flows agreed within a factor of two with measured mass flows and confidence intervals of predicted and measured mass flows overlapped in all cases. The observed agreement suggests that the combination of batch-determined transformation kinetics with a simple WWTP model may be suitable for estimating aquatic exposure to TPs formed during activated sludge treatment. Overall, we recommend the tiered procedure as a realistic and cost-effective possibility to include consideration of TPs of wastewater-relevant compounds into exposure assessment in the context of chemical risk assessment.

MO 080

Pharmaceutical P450 metabolism: read-across from human ADME to environmental Transformation Products (TPs)

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The potential risk of transformation products from wastewater treatment and subsequent water-sediment release environment is a current topic of interest given the advances in analytical chemistry and increase detection of pharmaceuticals and their metabolites in the environment. TPs are presumed to be less toxic than parent based on known P450 mechanisms of transformation and its role is detoxification. They are generally not considered in the risk assessment unless there is a need to mitigate a high risk quotient based on the parent compound. The current trigger for TP identification is linked to the outcome of the OECD 308 based on its presence > 10% dose observed during the study.

This poster presents the learnings from identification of TPs from water-sediment studies and compares findings to what is seen in published human absorption, disposition, metabolism and excretion (ADME) studies. Case studies are provided to highlight the potential use of reading- across from human ADME studies to environmental transformation products as a means of assessing their relative risk to parent compound. It is the intent that such data will support constructing a knowledge base around environmental microbial transformations and further discussions around how best to approach environmental transformation products in the environmental risk assessment and further clarify when identification and follow on testing is needed.

MO 081

Sediment-water test systems to evaluate the environmental fate of pharmaceuticals - how representative are the results?

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Sediment-water tests are an established tool to assess the persistence of pharmaceuticals and other organic micropollutants in aquatic systems. To assess the environmental fate of a number of pharmaceuticals, especially some acidic compounds (e.g., ibuprofen, naproxen) and beta-blockers (metoprolol, propranolol), we carried out a series of such tests with river sediment and synthetic river water. We compared sediment from two different rivers (Roter Main, Germany; Säva Brook, Sweden), sediment from several locations within one river, and sediment sampled at two different points in time. Tests were carried out at a concentration of 100-200 µg L-1 over approx. 30 days; samples were filtered and subsequently analyzed by UPLC-qTOF-MS.

All test compounds dissipated from the test systems, with dissipation half-lives between 2.5 and 50 days. Dissipation half-lives in tests with sediment sampled from the same location at two different times were generally varying up to approximately 45 %. Tests with sediment from different locations within one river provided similar dissipation times for ibuprofen and furosemide, while substantial differences were observed for naproxen and the two beta-blockers. When comparing the two rivers, dissipation half-lives for naproxen were in the same range, while substantial differences were observed for furosemide and ibuprofen. Especially the results for ibuprofen are remarkable - this compound is generally know as rapidly biodegradable, and the half-lives with sediment from Roter Main were generally shorter than 5 days. In contrast, the tests with sediment from Säva brook resulted in a half-live of 45 days.

In general, the results of this study allow the assessment of inherent uncertainties and limitations of such laboratory tests. Not only are the results depending on the specific set-up of the tests, but also on the nature of the specific sediment, the sampling location and other variables. We did not observe any systematic patterns in the test results, they seem to be depending on the specific combination of sediment and test substance. Variations of dissipation half-lives of up to one order of magnitude were observed, highlighting the need for a critical evaluation of such test results and also the need for further improvements in the design of such tests.

MO 082

Partitioning of sulfadiazine to soil colloids in the water and soil/biosolid interface

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In animal husbandry, a broad range of veterinary pharmaceuticals is used for therapeutic purposes or as growth promoters. A frequently antimicrobial used in e.g. pig production is sulfadiazine which belongs to the large group of sulphonamides antimicrobials that is used to treat bacterial infections and as growth promotors. In recent years the fate of sulfadiazine in the environment has been widely studied. Several papers have indicated a build up of non-extractable residues in soil. Formation of non-extractable residues has been identified as a major route of dissipation in soil. However, a general understanding of the mechanisms regulating the behaviour is still to be understood. Especially, partitioning and transport at the soil/water interface in the soil environment are important factors to describe. To elaborate, a sorption test system was applied based on Escher and Schwarzenbach (1996). Sorption at the soil colloid/water interface and the dependence of pH and ionic strength were main focus areas.

With the proposed test system we are able to describe a partitioning scenario in a well-defined theoretical manner. Effects of pH (2 - 12) and ion strength (cation concentrations in the range of 0.001-1 mol/L) on the distribution of sulfadiazine in the soil colloid/water interface were studied to give an in-depth understanding of the dissipation in the soil environment.

Furthermore, the results of biosolid amendment to soil are investigated to get an understanding of the changes in the interface environment when adding organic material, hereby mimicking soil management on micro scale.

MO 083

On a way to understand the fate and behaviour of sulfonamides in the environment

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Thanks to their low cost and their broad spectrum of activity in preventing or treating bacterial infections, sulfonamides (SAs) are one of the oldest groups of veterinary chemotherapeutic agents, having been used extensively for more than fifty years. As these compounds are not completely metabolized in animals, a high proportion of them are excreted unchanged in feces and urine. Therefore, they are released either directly to the environment in aquacultures and by grazing animals or indirectly during the application of manure or slurry. Once released to the environment, SAs distribute themselves among different environmental compartments, along with their degradation products, and can be transported to surface water and groundwater. The physicochemical properties, the dosage applied and the nature of the environmental components with which they interact, govern the whole process. In surface waters, these substances may partition to sediments and/or undergo abiotic (photodegradation and/or hydrolysis) and biotic degradation. SAs are recognized as quite persistent, non-biodegradable compounds, which explains the fact that in the last ten years they have been regularly detected in aquatic and terrestrial environments. However, the knowledge on the behavior and fate of these rather polar pollutants in the environment is still limited, especially from a process-oriented point of view. Understanding the effects of individual processes is crucial for analyzing potential risks caused by the presence of pharmaceuticals in the environment.

Therefore, in the present study, the assessment of abiotic hydrolytic transformations in aquatic systems at pH values normally found in the environment (pH 4 - 9) and sorption of sulfonamides onto sediments was determined. Described experiments were performed according to the existing OECD guidelines. HPLC-UV method was used for the hydrolysis and sorption experiments and LC-MS/MS technique for the determination of potential degradation products. The mechanism of sulfonamides sorption onto selected natural soils differing in their organic content, cation exchange capacity and particle size distribution was investigated in detail. Isotherms were employed to describe sorption. The influence of external factors on sorption, such as ionic strength and pH, were also determined.

Obtained results play a crucial role not only in description of the mobility and transport of those compounds but also their stability in the environment.

MO 084

Sorption of sulfaguanidine to soils differentiated in their physicochemical properties

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Sulfonamides are one of the most commonly used drugs in veterinary pharmacotherapy. Their relatively low cost and broad spectrum of antimicrobial activity made them usual choice in animal breeding (i.e. food additives). However the use on the large scale results in real danger for various environmental components. Studies reported that even 30% of antibiotics used in recent years in the European Union in animal husbandry were applied as non-therapeutic agents (e.g. as growth promoters).

Sulfaguanidine is one of the widely used sulfonamides and it is typically utilized as food additive in pastures. By the grazing animals or manure application, this compound can spread into the natural environment by uncontrolled way. Natural components such as soil and sediments are in the major risk, therefore it is important to determine the mobility and the possible interactions of sulfoguanidine in these matrices. Such studies allow to appraise the possibility of infiltration of sulfoguanidine into the groundwater or run off with surface water, and eventually assess the risk to human health and the natural environment.

The aim of this study was to determine the sorption of sulfaguanidine in soils differentiated in their physicochemical properties such as: pH, organic matter content and cation exchange capacity. The strength and the extent of the sorption phenomena were determined by sorption coefficient, isotherms and sorption kinetics. To assess the sorption type, the results were tested by Langmuir and Freundlich model. The influence of external factors, on sorption of sulfaguanidine, like solution pH and ionic strength were also determined. The degree of sorption of this compound to soils was quantitatively assessed using HPLC technique with UV/Vis detection.

MO 085

Influence of non-hydrophobic factors on the sorption of ionizable xenobiotics to solids

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It is well known that xenobiotics sorp to solid phases like soil and sediment, depending on their inherent properties and environmental conditions. Traditionally it was accepted, that the hydrophobicity of the chemical, i.e. the log K_{OW}, as well as the solid's content of organic carbon (OC) were the parameters describing the extent of sorption. Realizing that ionizable chemicals like weak acids and bases not always sorb according to their hydrophobicity, a correcting factor has been suggested. Correcting the hydrophobic sorption according to the Henderson-Hasselbalch equation has recently shown to improve the predicted sorption of weak acids significantly, however, weak bases do still show discrepancies compared with experimental data. In this investigation it was studied how a range of electrostatic parameters have influence on the sorption of weak bases to solid phases. Besides log K_{OW}, pH and OC content of the solids, this investigation also included parameters like clay, silt and sand content, cation exchange capacity, zeta potential and other properties of the solids, and the impact on the sorption of weak bases to solids. Weak bases with pK_a-values differing about half a unit in the range 4-9, resulting in ionization within an environmental relevant pH range, are selected for the study. Literature is searched for data on distribution coefficients (log K_D) where also information about the experimental conditions regarding electrostatic parameters was reported. Taking the above mentioned parameters into consideration, predictions and regressions of the distribution to solids shall be improved.

MO 086

The photodegradation mechanism of clofibric acid by UV photolysis

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Environmental antibiotic contamination presents the potential to select for resistance factors that can be transferred between bacteria, contributing to the establishment of multidrug-resistant variants. In the present study we have investigated the antibiotic sensitivity pattern of bacterial strains isolated from a treatment plant that receives waste-water from 90 different Indian bulk drug producers. In the treated effluent from this plant, we have previously reported very high levels of antibiotics, predominantly fluoroquinolones, at concentrations up to 1,000,000 times greater than those normally observed in treated sewage effluents. The taxonomic identities of all isolates were determined by 16S rRNA gene sequencing, followed by screening their sensitivity to 39 antibiotics belonging to 11 different classes. All isolates were resistant to, at least, 4 antibiotics, thus conferring a 100% multi-drug resistant population, and the majority were resistant to more than 20 antibiotics. Our study suggests that the waste-water treatment plant studied is potentially serving as an enrichment site for multidrug-resistant bacteria, which could pose a major public health issue in the future.

MO 087

Direct and indirect photolysis of the sulfonamide antibiotics sulfapyridine and sulfamethoxazole in Canadian rural wastewaters and surface receiving waters

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The direct and indirect photolysis of the sulfonamide antibiotic drugs sulfapyridine and sulfamethoxazole were measured in both controlled laboratory settings and in field waters at Dead Horse Creek, Manitoba, a rural watershed impacted by high nutrient and organic contaminants from episodic pulse releases of effluent from sewage lagoons of the small communities in its watershed. Both drugs degraded quickly in pure water in laboratory when exposed to light simulating natural sunlight, with half-lives ranging from 30 min to several hours depending on the intensity of light used. Abiotic and biotransformation processes for both analytes were insignificant over the time scale of the studies. The absorbance spectra differed for both compounds depending on protonation state, indicating that speciation may affect direct photolysis rates. Measured quantum yields for each species were consistent with previously published literature for sulfamethoxazole. Indirect photolysis increased dissipation rates in field waters, due to the influence of high levels of nitrate and dissolved organic material in wastewaters and surface waters. These results show that natural attenuation by photolytical processes is a major sink of sulfonamide drugs in impacted aquatic systems.

MO 088

Investigation of the photodegradation of PDE-V inhibitors and analogues under simulated sunlight by ESI-QqToF mass spectrometry

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The presence of emerging contaminants in receiving waters is not longer restricted to the environmental assessment of potentially toxic substances, personal care products and prescription drugs. Recent publications are increasingly covering the field of illicit drugs and lately the forensic evaluation of designing illegal analogues of lifestyle drugs like the phosphodiesterase type V inhibitors Viagra (sildenafil), Levitra (vardenafil) and Cialis (tadalafil). Recently, the presence of all three erectile dysfunction treatment drugs has been reported in wastewaters at very low concentrations. In these systems the fate of drug molecules depends largely on their susceptibility to biodegradation and/or adsorption. In the environment, the contaminants undergo various physical or chemical processes classified into abiotic (photolysis, hydrolysis) and biotic (biodegradation) reactions. Thus, changes in the chemical structure lead to the formation of new transformation products (TP), which may persist in the environment or be further degraded. In the present study the photodegradability of Sildenafil, vardenafil, tadalafil and their main human metabolites was evaluated. The reaction was performed under simulated solar radiation using a SUNTEST CPS system equipped with a high intensity xenon lamp. Solutions of each compound in both HPLC grade water and synthetic river water were irradiated for a period of 40 hours. Aliquots of 1 mL of the exposed solutions were withdrawn at regular intervals and analyzed by UPLC-(+)ESI-QqTOF-MS system . sildenafil, desmethylsildenafil, vardenafil, and desethylvardenafil degraded rather easily with half lives in the order of 2 hours for RW. On the other hand, tadalafil degraded with higher half lives (4 hours). The degradation pathway of vardenafil and its metabolite was similar to that of sildenafil and its human metabolite Five TPs were detected for both sildenafil and vardenafil TP393, TP451, TP491, TP382 and TP479. Tadalafil, showed less products than the other compounds investigated with the identified products being TP262, TP428 and TP272. The metabolite demethylsildenafil was also transformed and the main products were TP448, TP450 and TP462.

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MO 089

Removal of diclofenac in conventional drinking-water-treatment processes

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Pharmaceuticals have been widely found in wastewater, surface water and groundwater. This implies a potential for indirect human exposure to pharmaceuticals via drinking water supplies. The conventional methods of water treatment seem to be insufficient to remove these contaminants which vary widely within and among classes of compounds. In this study, jar test experiments were performed with water prepared in laboratory (color of 20HU, turbidity of 70NTU, pH 7.0, diclofenac (DCF) 1mg L^{-1}) in order to verify the removal of DCF by coagulation with aluminum sulfate, flocculation, sedimentation, filtration in bench-scale sand filters (clarification) and disinfection (chlorination) . The concentrations of DCF in the studied water samples were determined on a high-performance liquid chromatographic (HPLC) analysis and UV detection, using a C18 bonded silica column (250 x 4.6mm, 5µm). The low rate was 0.8mL min^{-1}, injection volume of 20µL, temperature was 25°C and the wavelength was 270nm. An isocratic mobile phase of acetonitrile (65%) and ultrapure water (35%) was used, both acidified with 0.05% trifluoroacetic acid. The DCF was extracted with C18-E adsorbent (phenomenex), 500mg/6mL, conditioned with 5.0mL of methanol and 5.0mL of ultrapure water. Then, 100mL of water sample were percolated through the adsorbent. Calibration functions, quantification and detection limit, intra- and inter-day reproducibility and accuracy were estimated. The method was successfully applied to determine the drug in water samples of 3 to 1,000µg L^{-1}. Removal of total organic carbon (TOC) was also investigated. Results of study indicate wide variability in the effectiveness of each treatment stage. The average percent removal of diclofenac was calculated from water samples collected after filtration and disinfection. The clarification stage not removed diclofenac. The disinfection of the filtered water in bench-scale sand with 5mg L^{-1} Cl_2, 0.5 and 24hours accounted for 36.12% and 97% removal, respectively. However, TOC was not removed by clarification and desinfection. The identification of DCF e TOC in finished water indicates incomplete degradation or removal of DCF through the conventional treatment process used in this study and that drinking water can be a source of human exposure to drugs.

MO 090

Removal of pharmaceuticals during dune filtration simulated by batch reactor experiments

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Dunea, the drinking water company for the Dutch city The Hague and its surroundings, aims to produce drinking water of irreproachable quality, particularly with respect to organic micropollutants. Surface water from the river Meuse is infiltrated and abstracted in the dune areas along the coast. Advanced treatment technologies such as membrane filtration or advanced oxidation are options for additional removal of organic micropollutants. However, the necessity of their application to ensure safe drinking water is still under debate. In addition, most of these techniques have high energy demands. The removal achieved by already existing infrastructure and natural treatment (dune filtration) processes is therefore of first interest and more fundamental knowledge about the underlying removal mechanism is required. In this study, dune infiltration was simulated by batch reactor experiments. The removal of pharmaceuticals was investigated under oxic and anoxic conditions.

Laboratory scale batch reactor experiments were conducted to investigate the removal of 14 different pharmaceuticals during dune filtration. 14 pharmaceuticals were spiked to batch reactors filled with dune sand and water under oxic and anoxic conditions (oxygen concentration below 0.5 mg/L). Samples were taken directly after spiking, after contact times of 10 days and 30 days. Analysis of pharmaceuticals was performed by direct injection of glass filtered samples on UPLC-MS/MS.

Experiments showed that higher removals were achieved under oxic conditions than under anoxic conditions. Ketoprofen and naproxen were almost completely removed under oxic conditions whereas carbamazepine and phenazone were persistent both under oxic and anoxic conditions. The average removal of tested pharmaceuticals was 77%. 12 pharmaceuticals were removed for more than 50% after 30 days under oxic conditions. Under oxic conditions, the removal efficiency was highest between day 0 and 10, whereas under anoxic conditions, the removal efficiency was highest between day 10 and day 30.

MO 091

Removal of fragrance compounds from waste water by ozonation and identification of oxidation by-products

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A large number of persistent organic pollutants can be found in the effluents of conventional activated sludge waste water treatment plants. Waste water treatment using ozone has been proven as an effective treatment process for a wide spectrum of emerging aqueous organic pollutants. Ozone removes organic contaminants either by direct reaction, or through the formation of OH radicals. However in most cases the pollutants are not removed by complete mineralization, but they are oxidized to by-products. In this study, ozonation of musk and related fragrance compounds in water in the presence and absence of an OH radical scavenger, tert-butanol, was investigated. Mass spectrometrical non-target screening by GC-MS was performed for the identification of oxidation by-products. Oxidation by-products were found, identified and verified in the case of HHCB, while no oxidation products for the fragrance compounds AHTN, OTNE, MX and MK were clearly identified. HHCB-Lactone was identified as a main oxidation product of HHCB. Comparison of the mass spectrum and retention time with a HHCB-Lactone standard verified the finding. In kinetic studies, the required ozone doses needed for the removal of the parent compounds were determined and reaction rate constants were calculated. The reaction was considered pseudo first order as the ozone concentration (5 mg L-1) remained constant as

the reaction proceeded. Rate constants for the ozonation of the fragrances in waste water were in the range of 1-100 M-1s-1.

MO 092

Persistence of a human pharmaceuticals cocktail during drinking-water disinfection by chlorine.

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Human pharmaceutical products (HPPs), so called emerging contaminants, are continuously released into the environment. As numerous studies reported their presence in surface water at the µg/L level and their persistence behaviour to natural attenuation in resource water, the chronic human exposure in mixture at sub-ng/L via DW may hence constitute a potential threat to human beings.

Waste water and drinking water (DW) treatments processes contribute to the decrease of HPPs concentration levels in DW resources and tap water. Nevertheless, few works have already reported the persistence and/or degradation of HPPs during the disinfection process by chlorine, the ultimate step of classic DW supply before entering to the DW distribution network.

In this study, 20 HPPs were selected among the 3000 HPPs released on the market. These 20 target compounds, owning to 11 therapeutic classes (antibiotics, non-steroidal anti-inflammatory drugs, beta-blockers, anti-epileptic, anti-anxiety, antineoplastic, etc.), were chosen particularly upon their already confirmed presence in DW resources and analytical feasibility.

Lab-scale chlorination experiments were performed with the aim to assess persitence of a 20 HPPs mixture upon chlorination at pH 7. Samples of ultrapure water were spiked at 1 µg/L with a mixture of HPPs and 1 mg/L of hypochlorous acid. Chlorination was performed during 18 hours, while the determination of HPPs concentrations in samples was carried out every hour with one single multi-residues method by SPE-UPLC/ESI/MS/MS reaching the ng/L quantification limits.

The results about degradation kinetics of each HPPs will be presented and crossed with results from previous study investigating the occurrence of the 20 target HPPs in real samples of DW. Emphasizing resistant and non resistant HPPs to disinfection by chlorine, these observations will hence contribute to discuss current and future needs as well to improve DW treatment processes to eliminate persistent HPPs from resource water as to focus further efforts on the behaviour of non persistent HPPs during chlorination, possibly involving the formation of unwanted by-products.

MO 093

Oxidation of synthetic phenolic antioxidants during water chlorination

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Antioxidants are substances which prolong the shell-life of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity and colour changes. The most frequent synthetic antioxidants used are the phenolic antioxidants: butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ). These compounds have been detected in river, ground and wastewater samples. Levels of these compounds were typically in the 10- 2000 ng L^{-1} range, depending on the sample nature. Moreover, their degradation products should also be evaluated since they may pose an environmental or human health risk.

Thus, the aim of this work was to study the chlorination of phenolic antioxidants and some metabolites, bearing in mind that, according to the European Federation of Chlor-alkali Producers, 98% of the DWTPs in Europe use chlorination as one of the main disinfection steps in drinking water production and is sometimes used for tertiary treatment of wastewater. Gas chromatography-mass spectrometry was used to follow the time course of the antioxidants and by-products, and also used in the identification of the by-products. Under strong chlorination conditions (10 mg L^{-1} chlorine, 24 h), five of the target compounds were significantly degraded, while only BHT-Q (2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione) and BHT-CHO (3,5-di-*tert*-butyl-4-hydroxybenzaldehyde) were stable. The addition of bromide to the sample was negligible, but for BHA, where an increase in the degradation rate was observed. Moreover, the degradation kinetics were investigated at different concentrations of chlorine and pH of sample by means of a factorial experimental design. It was observed that the pH of the sample was a significant factor for BHT and BHA, and chlorine concentration was significant for BHT, resulting in increased degradation kinetics as they are increased. The degradation of these compounds has revealed two main processes: hydroxylation and oxidation of the aromatic system. The by-products were investigated in real samples (surface and waste water) by SPE-GC-MS.

MO 094

Generation, assessment and affinity purification of a broad-specificity high titre antiserum to penicillins using 6-amino-penicillanic acid as a hapten

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Penicillins belong to the group of β-lactam antibiotics and are most widely used in veterinary medicine for treatment of bacterial infection, or to serve preventive and prophylactic purposes. They are also provided illegally as supplements in feed stuffs to promote growth of food-producing animals. Therefore, control of penicillin residues in milk is a major issue since the have been reported to cause allergic reactions and carcinogenicity. In addition, continuous presence of the antibiotics in heavily consumed foods is thought to promote of the spread of bacterial resistance to the valuable antibiotics used in human medicine.

The aim of this study was to determine optimal conditions for penicillin determination in milk by means of an enzyme-linked immunosorbent assay (ELISA). A polyclonal antiserum to penicillins was generated in sheep using maleimide-caproic acid-6-aminopeniccillanic acid derivative coupled to thiolated KLH as immunogen. Plate coating antigen conjugates of 6-aminopenicillanic acid or ampicillin and gelatine were prepared using cyanuric chloride as a linking bridge. Indirect competitive assays were developed to assess the antiserum, potential performance of the assays. However, in order to improve specificity and detection limits and reduce assay time (direct competitive assays), the polyclonal antibody was purified by means of antigen-based affinity chromatography in which bound antibodies were eluted with a mixture of acetonitrile and propionic acid from Sepharose 4B-hapten gels. ELISA tests were used to monitor the absorption and elution and assess the degree of structural and functional purity of the isolated antibody preparations. Finally, the affinity-purified antibody was labelled with horseradish peroxidase using cyanuric chloride as a bridging molecule.

ELISA assessment of the labelled antibody showed very high antibody activity (high titre) and coupling of over 80% of the enzyme. The direct competitive assay developed for detection of penicillins was applied in serum and centrifuged milk, with minimum detection value in the region of 10 ng/mL and a dynamic range of 10 ng/mL to 25 µg/mL. Assessment of the competitive ELISA response to various penicillins and other groups of antibiotics showed high degree of response to all tested penicillins (100%) but no detectable cross reactivity with any of the commonly used non-penicillin compounds including tetracycline, sulphonamides, macrolides, cephalosporines and fluoroquinolones.

EH02 - Monitoring and modeling stressed ecosystems to support ecosystem-based management

MO 098

Urban environmental zoning depending on industrial loading for environmental monitoring and spatial planning

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Perm is a large industrial city situated in the western Ural Mountains, Russia, where several dozens of oil-refining, petrochemical and metal-working enterprises, power facilities, mechanical etc. are located. During a long period of time the urban environment has been exposed to complex anthropogenic pollution. Abiotic factors, in particular the composition of air, soil, environmental waters, have led to significant urban ecosystem degradation.

The Perm citizens, as an integral part of the urban environment, are also exposed to anthropogenic environmental pollution.

In order to develop the General Plan and improve the whole urban spatial planning system, the city area was divided into nine zones wherein the levels of anthropogenic loading appear to be the highest and most critical. These are the main principles of urban spatial planning aimed at reducing environmental loading:

- optimal restriction of production facilities, i.e. sources of adverse impact, to well-defined industrial areas;
- meeting the standards of sanitary protection zones around industrial hubs;
- locating production facilities outside dwellings areas or performing the conversion of industrial enterprises having hazardous impact on the urban system;
- locating new production facilities within the city area provided that they utilize exclusively low-waste technologies meeting the highest technological and equipment standards;
- making maximum use of the natural potential of green spaces, the city forests and other areas for natural environmental self-cleaning and restoration.

The General Plan includes a green belt composed of minor river valleys reorganized into recreation zones around the central part of the city. It also stipulates that sanitation and environmental rehabilitation measures including water bodies' treatment, soil reclamation, and flora improvement should be taken within a number of city districts.

It was determined that the pollution levels in industrial zones could be also reduced by thickening barrier vegetation with the density of such green belts will be from 800 to 1000 trees and 1500 - 2200 bushes per hectare depending on the composition peculiarities of a given territory.

In conclusion, urban environmental zoning depending on industrial loading for environmental monitoring and spatial planning allowed the identification of priority zones that require restoration and the development of the most efficient measures aimed at environmental improvement.

MO 099

Heavy metals and their chemical forms in eroded soils

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Matrix population models are some of the most widely used models by population biologists. They are easy to construct and understand, but are they a good choice for use by ecological risk assessment of chemicals? Here, I discuss the strengths and weaknesses of matrix models as a tool for ecological risk assessment using models developed for a number of organisms exposed to pesticides. Results of this study indicate that matrix models are a good choice for an initial screening of toxicant effects because multiple effects (lethal and sublethal) can be easily incorporated into these models, they are easy to develop, use, and to understand. Therefore, matrix models should be the first choice for an initial risk assessment.

MO 100

An integrated modeling framework for decision support in ecosystem-based management: case study Lofoten/Barents Sea

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Ecosystem based management is today the foundation of international regulatory frameworks for managing the oceans and seas. The approach enables sustainable use of marine goods and services while maintaining good environmental status and preventing subsequent deterioration. Standard methods for regulatory risk assessment are based on precautionary principles and worst case assumptions. Although these methods are useful for environmental management and risk mitigation for the petroleum industry, they have limited value in ecosystem-based management approaches where comparative risk/benefit assessments are essential. To support ecosystem based-management, models should be developed that simulate in more realistic detail the marine biosphere and focus on the prediction of actual potential impacts instead of worst-case risks. Today we lack holistic models capable of supporting ecosystem based management decision-making.

To resolve this problem, an international consortium of petroleum industry partners has established a project to develop an integrated modeling framework for ecosystem based impact assessments of the marine environment. An integrated modeling framework will be developed including an ecosystem and ecotoxicology module, which integrates existing ecosystem models for adult fish, fish eggs & juveniles, phyto- and zoo- plankton, chemical fate and ecotoxicology. Development of the integrated modeling framework involves experts in ecology, ecotoxicology, risk assessment and modeling from 16 institutes and 8 nations.

The framework is initially being developed for applications in the Lofoten/Barents Sea to address the combined effects of fisheries and petroleum activities. The region is rich in petroleum resources and is also characterized as a key spawning and egg and larval drift areas North Atlantic commercial fish species. Here we describe the model system and developmental process and show how such approaches are of value toward providing stakeholders with more quantitative information on the environmental risks and benefits of petroleum development activities in potentially contentious areas.

MO 101

Combined use of environmental data and biomarkers in Liza aurata from a eutrophic and metal-contaminated coastal lagoon (Óbidos lagoon, Portugal)

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An investigative biomonitoring study was carried out in a eutrophic coastal system with a moderate contamination by metals (ð'bidos lagoon, Portugal). The selected approach combined the evaluation of field exposure levels (metals and nutrients) with metals accumulation and oxidative stress responses in gills of the fish Liza aurata. Two contrasting seasons (winter and summer) were considered at three sites: Barrosa (BB) and Bom-Sucesso (BS) inner branches; Middle lagoon (ML). Data on water column pointed to a higher metals and nutrients availability at BB site that was reflected in the higher metal contents in gills, particularly in winter. Similarly, oxidative stress responses demonstrated a pro-oxidant challenge at BB (winter and summer), being substantiated by a general stress index (IBR). Gills' metals load was higher in summer than winter, explained by the increased environmental concentrations in combination with elevated metabolic rates. Catalase (CAT), glutathione-S-transferase (GST), total glutathione (GSHt) and lipid peroxidation (LPO) increases observed in winter at BB were related with metal accumulation. Conversely, summer enhancements of GPx, GR, GST and GSHt were associated with other stressors. Inter-site differences on the basis of IBR were more accentuated in winter. The applied approach combining biomarkers in gills and environmental data demonstrated to be useful in the environmental health assessment, particularly under a moderate contamination scenario. Gills can be considered as a "mirror-door"in the context of water contamination assessment, based on its confirmation as an important route of entry for contaminants and the currently demonstrated ability to reflect environmental health status.

MO 102

Benthic fauna indicates chemical sediment quality

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The European Water Framework Directive (WFD) defined a suite of chemical and biological quality elements and yardsticks to demonstrate Good Ecological Status (GES). The yardsticks are designed such that in case of deviations from GES, they also indicate potential causative factors. In the Netherlands, recently a new biological yardstick was developed to indicate chemical sediment quality in type R8 tidal fresh water systems (lowland river estuaries) by evaluating local assemblages of taxa of benthic fauna communities. Further insights into the R8 yardstick and its meaning were obtained by applying two techniques. Firstly, the concept of toxic pressure quantification for mixtures was applied, replacing the originally used per-compound concentration data. The toxic pressure concept quantifies which fraction of the species is likely affected by a local mixture of toxicants. Secondly, focus was put on the abundance of separate taxa in relation to a potential suite of stressors, among which the toxic pressure of chemicals. The results showed that the acute toxic pressure of the local mixtures in the study area varied between 0 and 42%. Chronic toxic pressure varied between 0 and 76%. These findings imply that the local toxicity of mixtures is likely to affect local species composition. Furthermore, it was demonstrated that toxic pressure is highly significantly related to variations in abundance in 74% of taxa. It was observed that the earlier proposed R8 classification in groups of indicator taxa, based on multi-variate patterns in response to toxicant exposure is largely, but not fully reflected in the taxa specific sensitivity towards toxicant mixtures. Finally it is concluded that increasing sediment toxicity is related to increasing effects, both on taxon richness and abundance.

MO 103

Consistency in diatom response to metal-contaminated environments

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Diatoms play a key role in the functioning of streams, and their sensitivity to many environmental factors has led to the development of numerous diatom-based indices used in water quality assessment. Although diatom-based monitoring of metal contamination is not currently included in water quality monitoring programs, the effects of metals on diatom communities have been studied in many polluted watersheds as well as in laboratory experiments, underlying their high potential for metal contamination assessment. Here, we built large database of river diatoms (comprising more than 600 taxa) that were exposed to various loads of heavy metals in the water was investigated. The samples were collected during field surveys carried out in 6 different countries (France, Spain, Switzerland, Canada, Vietnam, China). After taxonomy harmonization, the patterns in diatom community structure were investigated for 202 samples, all collected from hard substrates in rivers with circumneutral water pH. As the sites were contaminated by a mixture of different metals (mainly Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn) with various loads, metal concentrations were converted into a single score after Clements et al. (2000) in order to classify sites according to potential metal toxicity. Metal contamination proved to be a strong driver of the community structure, and enabled for the identification of tolerant species like *Eolimna minima*, *Surirella angusta, Cocconeis placentula* var. *euglypta* or *Pinnularia parvulissima*. Traits were also tested: diatom cell size and the occurrence of diatom deformities were found to be good indicators of high metal contamination. This work provides a basis for further use of diatoms as indicators of metal pollution.

Clements WH, Carlisle DM, Lazorchak JM, Johnson PC, 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications 10: 626-38.

MO 104

Background concentrations of POPs in the Arctic: a risk assessment

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Persistent organic pollutants (POPs) are present in all oceans and the Arctic Ocean in particular accumulates POPs due to northward sea and air currents. Although there are extensive databases that contain field measurements of POP concentrations in Arctic species, they represent a haphazard collection of measurements in various tissues and species. This hampers a standardized quantification of the risk of POPs for Arctic ecosystems. In this study we quantify, for the first time, the ecological risk caused by background concentrations of all POPs measured in the Arctic Ocean between 1985 and 2010. A bioaccumulation model was used to standardize >49.000 measured concentrations in different matrices of various species to lipid tissue concentrations of 96 POPs (PCBs, HCHs, HCB, DDTs and PAHs) in 26 species ranging from northern shrimp (Pandalus borealis) to the polar bear (Ursus maritimus). After cross-validation of predicted tissue concentrations, they were combined with QSAR estimates of no observed effect concentrations to predict the risk quotients (RQs) for all 96 chemicals in all 26 species. The RQs of all substances in all species decreased over time (between -2.48 [GREEKX] 10-7 y-1and -9.46 [GREEKX] 10-7 y-1) with the exception of PAHs which increased (between 1.36 [GREEKX] 10-7 y-1 and 5.32 [GREEKX] 10-6 y-1). The highest RQ was found for fluoranthene in polar bear (Ursus maritimus) in 2010 (1.37 [GREEKX] 10-4). Assuming concentration-addition toxicity of the 96 chemicals, the total RQ increased on average twofold between 1985 and 2010 with current day total RQs varying between 2.48 [GREEKX] 10-4 for fish larvae and 9.29 [GREEKX] 10-4 for polar bear.

MO 105

Using river metabolism monitoring to quantify sediment budgets in river stretches

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Sediments play an important role in river ecosystems as a food source and pollutant mediator: they can figure as pollutant sinks but can also transfer these pollutants to the food web if the sediments serve as subsidy and the pollutants are bioavailable. Hence it is important to quantify the dynamics of sediment budgets and their quality during the seasons. As rivers are hydrologically dynamic and sediment deposits very patchy, representative samplings are an unresolved challenge in sediment research. This poster presents a method relying on the well established river metabolism introduced by Odum, which allow for the calculation of Gross Primary Production (GPP) and Ecosystem Respiration (ER) in river stretches from continuous oxygen recordings. In addition sediments were re-suspended in a dozen spots in these 200 m long stretches and analyzed further in the laboratory. The main parameter investigated was the 24h Biological Oxygen Demand (BOD) of the sediment samples which is related to organic carbon content and other parameters reflecting the lability of the organic matter (chlorophylls, sugars, phospholipids). This poster shows the dynamics of metabolism parameters and sediment characteristics over one vegetative season in two differently polluted Luxembourgish rivers and proposes a method to quantify the sediment budgets by relating BOD and ER measurements.

MO 106

Effects of ocean acidification on marine bacterial communities

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Decreased pH in our oceans is of great concern and effects have been shown on marine calcifying organisms. Still, more effects are to be expected on other organisms and parameters as well. Additionally, different organisms interact with each other in complex ways and the loss of a few species might have drastic effects on many organisms indirectly. This dictates the need to evaluate effects also on the community level. Long-term effects of lowered pH on marine periphyton communities were studied in a flow-through microcosm system. The periphyton was established from the indigenous microbiota in natural seawater from the Gullmar fjord on the Swedish west coast. Acidification to pH 7.7±0.07 was achieved with a pH-stat set up regulating the intermittent inflow of carbon dioxide. pH of the control aquaria fluctuated between 8.4 and 8.1 according to the actual pH of the fjord water. Several ecotoxicological endpoints (genetic profiling, trace elements profiles, leucine incorporation and bacterial catabolic profiling) were used to assess a broad range of effects on periphyton (see contribution by Eriksson et al., same session). This poster reports on the impact of acidification on total bacterial activity (incorporation of radiolabeled leucin), community genetic structure (PCR-TGGE) and on community function (catabolic profiles using Ecolog plates). Acidification led to an increased catabolic activity with respect to 12 out of the 31 carbon sources in total, four of them were affected severely. This indicates that lowered pH induce a shift in community structure and function.

MO 107

Fish liver structural changes as biomarkers to assess stream water quality in Jales Mine area (Portugal) - preliminary data

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Water ecosystems are suffering a worldwide increasing pressure caused by anthropogenic activities, including mineral extraction and mineral processing, which affect direct or indirectly all aquatic organisms. In some cases the abandon of mine activities led to serious environmental problems linked to tailings pollution, like it occurred in Jales Mine in 1992. In 2006 the tailing area was re-qualified in order to solve serious environmental and public health problems. We have been monitoring the local ecosystem using fish, and particularly hepatic changes/lesions, before and after the mitigation project, continuing a previous study published by our group (Carrola et al., 2009).

The principal aim of the current approach is to maintain an assessment of the stream closed to the abandoned Jales Mine using wild fish, namely Chondrostoma polylepis duriensis and Salmo trutta fario. For such purpose, we continued to survey liver histopathology, now integrating other bioindicators such as macroinvertebrates.

Fish were caught in September 2010, by electroshocking, in the same points used in the previous monitoring: upstream, the reference site (FB) and downstream the Jales Mine tailing zone, namely Peliteira-Tinhela (PT), Murça (MU) and Reboredo (RE).

Two main types of histopathological lesions were observed, namely hepatocytic necrosis and

lymphocytic hepatitis. Just in one fish, there was slight vacuolation of liver cells. No severely vacuolated hepatocytes, basophilic cell foci, bile duct hyperplasia, epithelial dysplasia and fibrotic adventitial sleeve, were observed, contrary to those reported in the 2009 study.
We concluded that in 2010 the liver histopathology disclosed no substantial differences among upstream and downstream fish. Moreover, the fish presented lesser quantitative as well as qualitative hepatic lesions when compared with the previous study performed in the same points, thus suggesting an improvement in water quality after the environmental re-qualification project.
Reference: Carrola J et al. (2009). Bull. Environ. Contam. Toxicol. 83: 35-41.
Acknowledgements: FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).
Key Words: biomonitoring, liver, mine, fish.

MO 108

Monitoring predatory mite populations in the field: validation of assessment tools

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Predatory mites provide for an important component of plant protection services in various agro-ecosystems. Adequate monitoring of predatory mite populations is therefore key to exploit this service optimally. We have validated and cross compared three monitoring tools that are currently widely used for use in orchard and vineyard systems. In all cases leaves were detached before counting started. We compared direct counting on detached leaves and counting following extraction of the mites from the detached leaves. The extraction was performed through Berlese extraction and through washing of the leaves. Following extraction the solution containing the mites was transferred to a counting device that could be placed under the binocular microscope. The three techniques were found to differ importantly in their accuracy and their sensitivity to uncontrolled variation in operator handling.

MO 109

Degree of population level effect of multiple stress depends on exposure interval in relation to life history and seasonal phenology

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Predatory mites provide for an important component of plant protection services in various agro-ecosystems. Depending on species, crop and region typical phenologies differ. We investigated how, following exposure to insecticidal stressors, the capacity to return to levels of undisturbed populations might relate to the underlying life history and the resulting seasonal phenology. We studied two agroecosystems, both typical for the southwest of France. Amblyseius andersoni is a predatory mite abundant in orchards. It overwinters as adult on the weeds under the trees and returns to the trees in spring when the buds open. Typhlodromus pyri is a predatory mite common in vineyards. It overwinters on the vines, and starts populating the green part of the plant when the buds break. In both cases populations are first composed of adult females only, which is followed by a phase where larvae dominate the age distribution. As the season progresses the density per leaf increases and the age distribution stabiles and the density of mites per leaf reaches a plateau. Because apple trees have an earlier development than vine, the mite populations on these crops also have a different seasonal phenology. Effects of insecticidal stressors were found to vary with life history characteristics of the species involved and with the timing of the exposure.

MO 110

Microtox - Transformation, bioaccumulation and toxicity of organic micropollutants in the Yangtze Three Gorges Reservoir

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The Three Gorges Dam is one of the great projects that enduringly change Chinas nature. Its Reservoir covers a total area of ca. 1085 km2 of the Yangtze river in a total length of 663 km. The creation of the reservoir caused the flooding of former urban, industrial and agricultural areas. This caused a release of substantial amounts of organic and inorganic pollutants into the reservoir. Beyond contaminants and nutrients (e.g. nitrate, phosphate) enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore. The Three Gorges Reservoir and its surrounding area is the source of food and water for plenty of people. Based on these risks for the population and to preserve this newly created ecosystem is it of importance to develop techniques and procedures that sustainably limit the emission of contaminants into the reservoir.
The recent project is undertaken within the joint research framework "Sustainable Management of the Newly Created Ecosystem at the Three Gorges Dam (YANGTZE-PROJECT)"(www.yangtze-project.de) financed by the Federal Bureau of Education and Science (BMBF) as part of the research cluster "Pollutants / Water / Sediment - Impacts of Transformation and Transportation Processes on the Yangtze Water Quality". The joint project is carried out in cooperation with numerous German and Chinese partners.
Aim of the presented project is to elucidate the fate (Module 1), potential of bioaccumulation (Module 2) and ecotoxicological properties of major Yangtze pollutants to indigenous organisms (Module 3).Overall objective is to develop a monitoring strategy to evaluate the degree of pollution and identify pollution hot spots in the Three Gorges Reservoir. Beyond may these findings serve as a starting point for a subsequent project on management strategies to reduce the pollution in temporary Yangtze flooding areas.

MO 111

Long-term effects of lowered pH on marine periphyton communities

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The phenomenon of Ocean Acidification has been identified as a potential threat to several marine organisms and might lead to adverse disturbances of marine ecosystems. Although the knowledge about effects of rising acidity in the oceans is increasing for some species, the knowledge of these effects on the community level of biological complexity is very scarce. Still, community-level effect indicators are needed in order to predict direct and indirect effects of Ocean Acidification on marine ecosystems. In a community the organisms live in their realized niche with important ecological interactions (e.g. competition, grazing and predation) present. This gives community approaches in ecotoxicological tests high ecological relevance. Since lowered pH might eliminate species or strains that are sensitive to such stress, and select for the ones that are more competitive under this condition, a community approach have the potential to detect any pH-induced change in community structure or function. We have used marine periphyton communities in a long-term study of effects of increased partial pressure of CO2 in the water and the accompanied lowering of pH. Periphyton was allowed to colonize and grow on glass substrata for 3.5 weeks in flow-through microcosms. The pH in the microcosms was either that of the incoming natural surface water or was manipulated by bubbling of CO2 down to approximately 7.7. We used Pulse Amplitude Modulation (PAM) to detect effects on photosynthetic electron transport and estimate induced community tolerance to low pH. In addition, we used confocal microscopy to trace effects on biofilm architecture and biofilm thickness. We have also estimated microbial diversity and composition of trace elements in periphyton using PCR-TGGE and X-ray fluorescence respectively. First results indicate that long-term effects of lowered pH change the capacity of communities to tolerate further changes in pH, making them more sensitive to pH-stress. However, no long-term effects on photosynthetic electron transport or trace element composition was detected.

MO 112

Linking the individual to community levels: a place for arthropod molting enzymes in ecotoxicology?

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Chitobiase is one of two molting enzymes (the other being chitinase) used to cleave chitin polymers that comprise the exoskeleton of aquatic arthropods. Following molting this enzyme is released into the water and can be quantified by a simple fluorescence assay. The standing activity and it's rate of production has been used as a measures of: 1) secondary production rates; 2) moulting rates; and 3) developmental biomass for entire crustacean zooplankton communities under laboratory and field conditions; and 4) responses to some contaminants in zooplankton and crustaceans. Yet, despite its relative methodological ease, chitobiase has only been used sporadically as an ecotoxicological tool to monitor and quantify changes in secondary production as a result of exposure to a stressor(s). In this paper we reviewed all the available literature pertaining to the use of chitobiase as a measure of stressor response and make the following recommendations for future research: 1) current methodologies be further validated, e.g., quality assurance and quality control measures be adopted, enzyme storage conditions and times be experimentally confirmed; 2) that the variation in activity relative to life history stage and/or body size for different taxonomic groups be characterized in order to examine the impact of stressors in laboratory-based studies and the utility of the approach as a field-based measure of community-level processes; and 3) the response of selected organisms be characterized for model stressors to confirm the utility the observed responses relative to more traditional endpoints, e.g., reproduction and growth. Overall, the use of chitobiase has enormous potential as a tool in ecotoxicology, but extensive groundwork and validation is still required before it can be widely adopted.

MO 113

Characterization of kidney histology in tilapia (Oreochromis niloticus) as biomarker tool for fish health assessment - preliminary data

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Human activities have resulted in environmental pollution worldwide, by releasing different kind of chemical compounds. These pressure affects water availability and quality, in most cases unsuited for the desired use. But have also serious effects on all living organisms, including animal and human health.
Biological monitorization make use of histological biomarkers, among others, integrated in a multidisciplinary approach, as a complement to classical physical and chemical monitoring parameters. Thus, several organs can be used for this purpose like fish kidney and normal histology knowledge is essential. Field and laboratory studies have established a causal relationship between fish pathology and levels of pollution.
Fish kidney can be divided in two main regions, the anterior (or head kidney), composed of hematopoietic tissue, lymphoid tissue and cells with endocrine function, and the posterior (or trunk kidney), composed of renal parenchymal tissue, whose functional unit is the nephron, which plays an essential excretory function.
This work is centered on the study of tilapia kidney ultrastucture and histology (anterior and posterior regions) to apply that data for future studies, as a complement to other organs histopathology.
After being euthanized with 2-phenoxyethanol, tissue samples were collected from the anterior and posterior part of the kidney of 7 male fishes, and fixed with formaldehyde. Then sections were cut (5 μm) and stained with H&E. After that, slides were examined by light microscopy.
Preliminary data show that the anterior kidney region of tilapia is constituted mainly by interrenal cromaffin and lymphoid tissue, as well as melanomacrophagic centers (MMCs) while in the posterior region we found renal corpuscles with well developed Malpighi glomeruli, Bowman's capsule with two layer (visceral and parietal) and Bowman's space, and a conspicuous system of tubules (proximal, distal and collecting) as found in other fish species.
We also observed differences in cellular organization of the kidney, related with age and degree of sexual maturation of fishes.
This study was focused in histological characterization of tilapia kidney and may constitute a basic support for future studies for assessment of histopathological lesions/alterations in fish kidney exposed to chemical compounds.

MO 114

The flood-prone transport of historical contaminants to the Elbe Estuary: modelling their deposition and monitoring their effects on the ecosystem

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High discharge conditions in the Elbe river lead to resuspension of highly contaminated sediments in the up-stream part of the Elbe catchment, the most contaminated areas being in the Czech Republic, in the catchments of the tributaries Mulde, Saale (about 300 km upstream of Hamburg), and probably in the groyne fields in between. While we are currently modeling the contaminant transport along the major river (see Moshenberg, SETAC-Europe 2009), the area of the Elbe estuary still is a big challenge: The tidal impact along with dredging activities of the river and a large turbidity cloud, drifting back and forth in the estuary, all lead to a complex sedimentation pattern in an area where nature reserves are in close proximity to the second largest harbor in Europe.

This poster describes the results of several event-based sampling surveys carried out over 2 years, describing the ecotoxicological and chemical effects of flood-induced transport of contaminants to the estuary from the Hamburg area to the mouth of the river. Applying a hydrodynamic 3-dimensional model that has been developed by the Federal Waterways Engineering and Research Institute over years for the Elbe estuary, we interpret those data on the basis of projected transport pathways and sedimentation patterns of relevant contaminants in the tidal Elbe area comprising nature reserves, harbor basins, beaches, fishing grounds and urban environments. Together with assessment of contaminant loads, carried downstream by flood events, and climate change projections, temporal development of the contamination pattern in the Elbe will be discussed.

MO 115

Assessing the impact of watershed land use on aquatic biodiversity over large spatial scales using benthic macroinvertebrates.

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Data from the United States Geologic Survey (USGS) North American Water Quality Assessment (NAWQA) and the United States Environmental Protection Agency (EPA) Wadeable Streams Assessment (WSA) were used in an attempt to identify correlations between land use parameters and a measure of aquatic biodiversity at large spatial scales. Data from the USGS NAWQA program included multiple years of sampling at single sites, ranging from 1993 to 2008. Data from the EPA WSA study included many more sites but only from a single year. Results from the assessment show correlations between measures of aquatic biodiversity and forest land use (positive correlation) and urban land use (negative), but fail to show a correlation between agricultural land use and aquatic biodiversity. This outcome agrees with other studies on the impact of anthropogenic land use and water quality, indicating a much stronger influence of urban systems. Though the influence of urban land use appears to be much more pronounced than agricultural land use, this does not mean that agriculture does not impact aquatic biodiversity. Instead it suggests that under specific conditions and proper management practices agricultural land use has a lower threat potential than urban land use. This gives hope to the prospect of reducing the impact of urban land use through use of management practices similar to those currently employed in the agriculture sector.

MO 116

High frequency aquatic ecosystem biomonitoring

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In world threaten by multiple anthropogenic impacts, innovative approaches to lake-ecosystem monitoring and assessment of lake ecosystem functions are required for a correct management of water resources, the biodiversity that they harbor and the services that they deliver. We developed an innovative and potentially ground-braking tool for high frequency monitoring of lake phytoplankton. We have designed a lake monitoring platform for the characterization and counting of algal cells (based on scanning flow-cytometry), coupled with measurement of the physical water environment (multiparameter probe). The aims of our technology are to monitor automatically and with high frequency the vertical profile of the water column for phytoplankton diversity, composition and abundance, functional traits, and physico-chemical parameters. The platform performed its first monitoring program in full functional mode in May 2010 in Lake Lugano (Switzerland). Data were collected with a frequency of 2 vertical profiles of the water column per day. Automated data were coupled by fortnightly independent measurements of physico-chemical characteristics of the water column and phytoplankton for an assessment of the actual capabilities of our technology. Results of our automated platform were comparable with traditional monitoring data. Our description of phytoplankton diversity patterns offered levels of statistical reproducibility, depth resolution and high frequency that are unachievable with traditional limological campaigns. Additionally, our functional classification of organisms and the real-time measurement of morphological traits hold the potential of increasing our ability to explain the organization and predict the reorganizations of the community under applied stressors. Our automated time-series of high frequency data offers novel opportunities for tracking, understanding and modeling changes in aquatic biodiversity and its drivers at the community level. Identification of drivers of community diversity and the effects of diversity on ecosystem functions is expected to lead to a better understanding of the adaptive potential and resilience of an aquatic ecosystem to change.

EP04 - Emission of chemicals from consumer goods - from emissions to effects

MO 123

Origin, source, exposure and toxicology of Non-Aroclor PCB11

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Despite being a member of one of the most regulated compounds in the world, PCB11 (3,3'-dichlorobiphenyl) is a virtually uncontrolled global pollutant. It was not produced in significant quantities as a component of commercial mixtures of polychlorinated biphenyls (PCBs) and therefore was not subject to the scientific attention paid to many PCB congeners. However, the compound has been reported in air and wastewater around the world. Here we summarize our current knowledge of the sources, environmental history, and toxicologic effects of PCB11.
We recently reported that PCB11 is produced inadvertently during the manufacturing of commercial paint pigments and we have hypothesized that this production explains the presence of this compound in air and water around the world, including urban centers like Chicago and Philadelphia and remote regions of the Great Lakes and in the Arctic. Although the presence of PCB11 in the environment was only recently detected, the history of environmental exposure dates back at least 70 years. Using dated sediment cores from the Great Lakes, we have found that North American environmental exposure to PCB11 began to increase in the 1940s through the 50s and accelerated until the early 1980s before falling back to 1950s levels where they remain now.
Because PCB 11 is not among the PCBs commercially produced, the hazards associated with this long period of environmental exposure are poorly known. Our laboratories are currently evaluating the toxicity of PCB11 and initial studies show that PCB11 is a weak endocrine disrupter and demonstrates little cyto- and geno-toxicity in cultured mammalian cells. However, its putative primary hydroxylated metabolite 3,3'-dichlorobiphenyl-4-ol induced significant clonogenic cell killing as well as increased reactive oxygen species in exponentially growing immortalized human prostate epithelial cells. In addition, treatment with superoxide dismutases and catalases after 4-OH-PCB11 exposure significantly protected the cells from PCB toxicity. These results strongly supported the hypothesis that exposure to a metabolite of PCB11 can inhibit human cell proliferation and cause cytotoxicity by increasing the reactive oxygen species that can be mitigated with antioxidant treatments following PCBs exposure. Additional studies evaluating the toxicity of PCB11 and its metabolic progeny are ongoing.

MO 124

PAH release from rubber granulates of artificial turf fields: preliminary hazard assessment for the athletes

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Nowadays synthetic turf areas, made by an infill of rubber granulates from used tyres or virgin rubber which contain compounds such as PAHs and heavy metals, are widespread in almost all sport facilities.
We collected different samples of rubber granulates from nine synthetic fields where the levels of 16 EPA's priority PAHs have been evaluated by Soxhlet extraction and HPLC analysis.
Then, a sequence of proofs was carried out at 60°C, a temperature that these rubber granulates can easily reach in the environment, to see whether PAHs release occurs and at which concentrations and to estimate the "hazard"by breathing for an athlete who trains on these fields.
The results showed high concentrations of PAHs in all samples of rubber granulates exceeding the threshold values imposed by the "Italian National Amateur League"(LND). The sequence of proof at 60°C, from the same granules, shows that every time this temperature is reached there is a little release of PAH compounds that does not decrease over time, suggesting a chronic contamination related to these areas. Through the airways, after attendance at these surfaces, it was estimated that an athlete who weights 70kg inhales from 0.02 to 0.149 µg/kg body weight (b.w.) of BaPeq per day, an amount considerably higher than the virtually safe dose.
The main conclusion we can draw from this preliminary study, which will be validated by further investigations both in field and in laboratory, is that synthetic fields release PAHs at any rise in temperature of the surface and this does not make them completely safe for public health. In fact, theoretical estimates made by extrapolating data obtained in the laboratory showed that the toxicity equivalent (TEQ) of the evaporated of the different granules is hardly negligible and weighs deeply on the total load of PAHs a man takes daily via different routes.

MO 125

Intense sweeteners in the environment- is there a reason for concern for wildlife effects?

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Intense sweeteners such as aspartame, acesulfame K, saccharin and sucralose are produced and used in large quantities in all major markets of the world as low calorie sugar substitutes. Many of these sweeteners pass through the body either unaltered or as modified compounds and are ultimately released to the environment predominantly through the sewage system. Some of these sweeteners have been detected in various environmental compartments and has raised concern about potential biological effects in non-target species living in areas receiving discharges from anthropological activities. Existing data on fate and effects of intense sweeteners in the environment and novel experimental data for the accumulation and genomic (transcriptional) effects of the intense sweetener sucralose will be presented to elucidate whether these compounds may cause adverse effects to aquatic animals such as plants, algae, crustaceans and fish. The bioaccumulation studies, which were performed with the algae Pseudokirchneriella subcapitata, the crustacean Daphnia magna and zebrafish (Danio rerio), were all conducted as semi-static exposure studies over a period of 48 hours with two concentrations of sucralose (10 and 100 mg/L), with analytical determination of sucralose in both water and biota. Chronic toxicity tests show that sucralsoe at concentrations of 1-100 mg/L did not adversely affect the growth of neither the aquatic plant Lemna gibba nor the survival, growth or reproduction of the mysid shrimp Americamysis bahia. Additionally, the hepatic transcriptional response in Zebrafish (Danio rerio) after 48h waterborne exposure to 1-100 mg/L sucralose was determined by microarray-assisted analysis as a pre-screening tool for identifying potential biological effects of the compound in non-target organisms. A combination of data from literature and the experimental approaches will be used to assess the risk posed by sucralose and other intense sweeteners to aquatic organism under relevant exposure scenarios.

MO 126

Alkylphenols and Alkylphenol ethoxylates in consumer goods and their contribution to a Wastewater Treatment Plant in Stockholm, Sweden

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Stockholm has been a case study in several Substance Flow Analyses (SFA:s) published. There is a research tradition in the Stockholm studies with a lot of empiric data of the content in goods and emission factors to be able to estimate the inflow, stock and emissions of the substances. The SFA research started with heavy metals and has been followed by organic substances. Knowledge of the sources is necessary for the waste water treatment works to be able to mitigate pollutants before they reach the sewers.
Alkylphenols and alkylphenol ethoxylates are widely used in various applications that are partly under environmental restrictions within Europe. The study set out to analyze the most important sources of this large group of organic compounds in an urban wastewater system using SFA methodology. The study covered the technosphere in Stockholm, Sweden allowing a comparison of 13 groups of goods' emissions to wastewater. The groups were: textile and leather, cleaning products (e.g. cleaning agents for households and industry, washing powder), personal care products, paint and lacquers, engineering industry (degreasing products, lubricants), glue, concrete, plastics, agriculture products, laboratory chemicals, photographic chemicals, paper and others. Several different data sources were used; national authorities (Statistics Sweden and Swedish Chemical Agency), trade associations, businesses and non-governmental organizations (NGOs). Earlier measurements of the content of alkylphenols and alkylphenolethoxylates in textiles were the most important data sources to the contribution from textiles. The main body of data was taken from the Product Register at the Swedish Chemical Agency. 78 different CAS numbers for alkylphenols and alkylphenol ethoxylates were included. It was found that the groups of textiles and cleaning agents were the major sources to wastewater, while the group's personal care products and paint and lacquers give smaller contributions. The content of alkylphenol ethoxylates

in goods, especially in textiles, is a most significant source and is probably valid for other urban areas as well.

MO 128

Leaching of halogen free flame retardants (HFFRs) from polymers

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Information about the production, distribution and consumption of flame retardants in electric and electronics (E&E) equipment (plastics) is well described. However, there is a knowledge gap in the amount of flame retardants leaching from plastics of electric and electronic (E&E) equipment to the environment. In the EU funded project ENFIRO halogen free flame retardants (HFFR) are studied that are viable alternatives to specific commercial brominated flame retardant (BFR). Leaching experiments on BFRs from different types of plastics has been described in literature, however, limited information on leaching of HFFRs is available. Six of the fifteen selected HFFRs that are studied in ENFIRO are metal based flame retardants. Metal based flame retardants are stable in the plastic (polymer) product, but are dissociate in the environment. Monitoring of the fate of metal-based HFFRs in the environment is therefore difficult. Therefore, leaching experiments of HFFRs from plastics is an alternative method that may contribute to the exposure and risks assessment and understanding of the fate of HFFRs in the environment. The current study shows the leaching properties of different HFFRs from polymers in comparison with BFRs. Thereby, the influences of pellets vs. moulded plates and pH on the leaching properties are taken into account. Two types of leaching protocols were tested. The first is the TLCP protocol from the US EPA that use worse-case leaching conditions (low pH) to simulate a municipal waste landfill, to study if waste has a characteristic toxicity and therefore is hazardous. The second protocol is DIN 38414-S4 that determines the leaching by water, and has been widely used for regulatory purposes in Europe. The results show that no differences in leaching properties between the DIN and TCLP methods for two metal-based PBT pellets were found. Higher rate coefficients of HFFRs leaching from PBT pellets than from PBT moulded plates were found, which is probably a results of the differences in surface:volume ratio. Important aspects for further study are the influence of steady state conditions vs. flow through leaching systems, and the influence of leaching conditions (e.g. salinity, humic acids) to simulate different environmental conditions.

MO 130

Measurement of release rate of plastic additives from consumer products using Micro chamber method

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To determine the release rate of SVOC during service life of products, we measured emission rate of plasticizers from PVC products and flame retardants from home appliances by a microchamber method newly developed for SVOC. The microchamber method which has two processes of emission test and thermal desorption test, was elaborated in Japan and published in the JIS A 1904 "Determination of the emission of semi volatile organic compounds for building products -- Micro chamber method"in 2008. In Europe, there is no acceptable method for measuring SVOCs; therefore, the JIS A 1904 is considered to be incorporated as ISO regulations.
First, we not only used product samples received from industrial companies, but also prepared pressed plastic sheets containing plasticizers and flame retardants for standard test samples. Second, release tests were conducted focusing on parameters of thickness of sheets, concentration of chemicals and temperature, input flow rate and duration of emission test. Then, we analyzed and quantified the release rate of DEHP, DINP, DIDP, decaBDE, TPP, BDP and antimony trioxide from the samples of products and sheets.
As a result, the release rate of plasticizers was limitedly influenced by the thickness of sheets, concentration of chemicals and input flow rate, while the release rate became increased at a higher temperature due to an increase in a vapor pressure of the compound. The release rates of flame retardants except TPP fell below the lower limit of detection in all samples at high temperature at 60°C and duration of two weeks. Based on these results, we examined several procedures to estimate the release rate of plastic additives from consumer products.

MO 131

Missing links in the regulatory chain controlling emissions of hazardous chemicals from articles

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It is widely acknowledged that the management of risks associated with chemicals in articles, including consumer products, need to be improved. From a chemical and ecotoxicological point of view, it is crucial that the articles' whole life cycle is taken into account in this effort, i.e. design and manufacturing, use, and end-of-life including the waste phase.
The purpose of the present study is to empirically analyze to what extent European legislations that include measures to control emissions of chemicals from articles throughout their life cycle are coherent or not. To illustrate this, the regulation of a number of case-study chemicals, used in various consumer products and in high volumes, is scrutinized.
This analysis identifies missing regulatory links between the rules that are relevant for the manufacturing and use phases and the rules applicable to the end-of-life phase. Many consumer products, such as clothes and furniture, are only regulated by REACH and REACH regulates the use of chemicals in articles only to a very limited extent. Product-specific directives, such as the Toys Safety Directive and the RoHS Directive, are therefore important complements in controlling the use of hazardous chemicals in articles. However, with the exception of the RoHS (and WEEE) directives, a clear connection to the rules for waste is missing in the regulatory system. Clear links are also missing between the rules regulating chemical emissions during the articles' life cycle and the maximum environmental concentration limits, set for e.g. sludge and surface water. These legislative gaps will encourage end-of-pipe solutions, rather than actions to manage the source of the problem.
The Waste Directive, the Water Framework Directive and the Sludge Directive all refer to EU environmental and waste policies. These policies are based on the precautionary principle, and state that preventive action should be taken, that environmental damage should be rectified at source and that the polluter should pay. The lack of connection between the rules regulating different phases of an article's life cycle makes these objectives difficult to fulfill. We argue that to obtain a sustainable development it is necessary to minimize the input of hazardous chemicals into articles, so that waste and other end-products, such as ash materials and sludge, can be recovered and used without harming human health or the environment.

MO 132

Source classification framework for an optimized European wide emission control strategy

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European legislation such as the Water Framework Directive (WFD) from 2000 and the Environmental Quality Standards Directive and the Marine Strategy Framework Directive both from 2008 focus on a range of priority substances (PSs) with the aim of obtaining an ecological and chemical healthy environment. This should be obtained through reducing releases or phasing out of discharges said chemicals. In order to appropriately design emission control strategies (ECSs) and monitor releases before and after implementation of various measures it is required to identify pollution sources and releases, and thereby establish an appropriate inventory containing such information. Suited for this purpose a Source Classification Framework (SCF) was developed. It consists of harmonized European classification codes for economic activities and emission processes combined with the CAS# for the PS as well as an urban structure descriptor. It also includes a release profile descriptor and when ever possible the release factor describing the extent of PS release from a given pollution source, i.e. commodity or activity. It has been possible to establish PS emission inventories for a given catchment of an urban environment by testing the approach on a range of the PSs listed on the WFD, and thereby identify potential problematic pollution sources. To the extent published data on release factors allows it, it has also been possible to quantify PS load to the considered catchment and thereby compare with European environmental quality standards for the considered PSs. The developed SCF emphasized the need for further knowledge and research within the area of quantification of PS releases from given commodities and activities. These release factors are required for a more thorough, solid and valid quantification of the PS environmental emission. The SCF also provides a well structured approach for European pollutant source and release classification and management. With further European wide implementation, the SCF has the potential for an optimized ECS in order to obtain good chemical status of European water bodies.

MO 133

Emissions of chemicals from the economy-wide stock of plastic material - a first model iteration for Sweden

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Estimating the size of the problem with release, fate, exposure and effects from the human use of chemical substances of materials and consumer products is daunting. More than 100,000 chemical substances are in commercial use and a reasonable description of their existence in, and release from, plastic polymers, glues, paints, fibres, lubricants etc. comprise a big challenge. In this study, a generic emission model has been developed and applied to estimate emissions of a set of organic chemicals from products. The scope of the estimate is emissions from products containing plastic materials during their average lifetime within the geographical boundaries of Sweden. The customs Combined Nomenclature has been used to divide the products into categories for which the chemical composition, surface area, thickness and accumulated stock in society has been described and estimated using several approaches for approximation in cases where data have been lacking. For this, information from national trade statistics as well as lifecycle assessments and building product declarations was used. Anti-oxidants, flame retardants and plasticisers are among the most interesting use categories of additives that are emitted in significant quantities. Thus for anti-oxidants in plastic material in the economy-wide product stock in Sweden as an example, the emissions in the service-life were estimated to be almost 500 tons/year divided on 32 individual chemical organic substances, stemming from a stock of anti-oxidants of 84000 tons, contained in a stock of plastic material of 24 million tons.
Until now, the method has only been used to estimate emissions of additives from plastic materials, but it is believed to also be applicable to other materials. However, the uncertainties in the estimations are currently large, which is partly due to model uncertainties but to a large extent also due to uncertainties in the input data.

MO 134

From product flow data to organic substance emissions to the environment - the case of PVC flooring

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This case study on PVC flooring investigates the possibilities and barriers to extract and combine Swedish product flow data from Statistics Sweden, in order to derive data detailed enough to populate a developed emission model and execute calculations on total emissions from all PVC flooring in Sweden during one year. A comparison is made, between calculated emissions using either product flow data from Statistics Sweden or from other domestic floor branch sources.
At Statistics Sweden, existing databases, published statistics and available methods were used for identifying and illustrating the possible methods and barriers for extraction and combination of product flow data. For calculations of emissions a combined material flow and diffusive mass transfer model was developed and used.
This case study shows that the data categories and the methods needed for estimating the accumulated product area exist at Statistics Sweden, in principle, but the data is sometimes too aggregated or too protected to make the calculations on a detailed product category level, as in this case for PVC flooring. Using product flow data from the least aggregated level of the Combined Nomenclature, for PVC flooring, generates calculated emissions three times higher than the emissions calculated from the more specific product flow data from The Swedish Flooring Trade Association.
The PVC flooring case study boils down to the facing of a trade off when it comes to national emission assessment of several product categories; using data from Statistics Sweden generates emissions within a reasonable period of time, but with aggregated and in this case, too high values - using branch product flow data generates more accurate emission values but is not a plausible option due to the steep increase in resource demands.

ET06 - Ecotoxicology of amphibians and reptiles

MO 138

Evaluation of the effects of titanium silicate nanoparticles in tadpoles of Pelophylax perezi (Seoane, 1885)

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Due to the growing production and usage of titanium silicate nanoparticles (NP-TiSiO4) in a wide range of products and industrial applications, it is expected soon to be found in the environment. Since these NP tend to form large aggregates when suspended in aqueous media, it is presumed that, when entering in the aquatic environment, they will settle over the sediment. Therefore, benthic species, or organisms that actively interact with the sediment will constitute key receptors of these NP. Accordingly, this work intended to evaluate the effects of NP-TiSiO4 in tadpoles of the frog species Pelophylax perezi (Seoane, 1885), as this life stage uses and compete for sediment as a nutritional resource. To attain this objective, tadpoles at Gosner stage 24-25 were exposed, for 96-h, to a series of five concentrations of the NP-TiSiO4. At the end of the assay, mortality, behavioral shifts, and enzymatic activity of acetyl-cholinesterase (Ache), glutathione S-transferase (GST), lactate dehydrogenase (LDH) and catalase (CAT), were monitored. Mortality never exceeded 20% for the control and for all tested concentrations of NP-TiSiO4. Significant behavioral changes were also not observed. However, the analysis of enzymatic activities revealed a significant activation of CAT and LDH when exposed to the lowest concentration. No significant changes were observed for Ache or GST. Proton nuclear magnetic resonance (1H-NMR) analysis for lactate, glucose and alanine content in NP-TiSiO4 exposed tadpoles will be presented.

MO 139

Salinity and copper interactive effects on tadpoles of the green frog Pelophylax perezi (Seoane, 1885)

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Several works have been focused on metal contamination of freshwater ecosystems and its impact on amphibians. It is expected that in the future, such metal impacted populations of amphibians will have to deal with additional stressors associated with global climate changes, namely increased salinity due to sea level rise. Accordingly, this study intended to assess the interactive effects of salinity and the metal copper on tadpoles of the green frog Pelophylax perezi (Seoane, 1885). To attain this objective, single and combined effects of salinity and copper on survival of larval stages of P. perezi were evaluated in a multifactorial design and enzymatic activities were quantified. Larval stages of the P. perezi were collected in the field and kept in the laboratory for at least 24h in the artificial medium FETAX. Tadpoles (Gosner stage 25-28) were exposed to a gradient of concentrations of Cu2+ (0 to 2.4 mg/l), NaCl (0 to 2 mg/l) and both. Increasing copper concentrations caused an increase in the activity of ChE and catalase, and a decrease in the activity of LDH and GST. When combined with intermediate concentrations of NaCl these trends were attenuated. Results of mortality showed effects at none salinity level. Concentrations of copper above 0.8 mg/l revealed a high mortality rate. Copper combined with intermediate concentrations of NaCl were less lethal than the metal ion alone, indicating the occurrence of antagonistic effects. Biochemical analysis support these results. It is therefore suggested that a moderated increase in salinity would have a shield effect against copper toxicity for tadpoles.

MO 141

Metals of priority concern in early life stages of reptiles: maternal transfer and bioaccumulation

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Environmental contamination with metals is a serious problem worldwide. Cadmium, lead and mercury rank highest among the pollutants of regulatory concern, are known to bioaccumulate, cause adverse effects of priority concern such as carcinogenicity, neurotoxicity, reproductive and developmental toxicity, and are Suspected Endocrine Disrupters. Over one in five reptile species are threatened with extinction both worldwide and at the EU level, and pollution represents a main category of threat. Among the almost 300 publications on the ecotoxicity of metals in reptiles reviewed [1], overall 46 publications reported concentrations of 21 metals in 18 different "transgenerational compartments"for 30 reptile species with focus put on Cd, Hg, and Pb (43 publications) for 23 species with half of them falling into the Lower Risk IUCN threat status category. Information is available for only four out of 151 European reptile species. The present review confirmed that early life stages of reptiles are almost ubiquitously exposed to the metals Cd, Hg, and Pb which were detected in male and female gonadal systems during gametogenesis, fertilization, and egg shell formation, as well as in laid eggs, embryos and hatchlings; were detected in the vast majority of the samples analysed; and were transferred via both maternal tissues and external incubation substrates to the progeny where they accumulated in a dose-dependent manner. Moreover, mean concentration levels of these metals detected in the early developmental stages of reptiles matched those reported for mammals and birds, and fell within the range of reproductive and developmental toxicity thresholds reported for these taxa. However, for Cd, Hg, and Pb in reptiles, no critical metal concentrations in reproductive tissues, and no exposure-effect relationships could be established. Based on the published data set, the threat metals of priority concern might pose upon threatened reptile species can not been assessed. [1] Grillitsch B.; Schiesari L. 2010. The ecotoxicology of metals in reptiles. p. 337-448. Appendix: Metal contamination in reptiles. p. 553-903. In: Sparling DW; Linder G; Bishop CA; Krest S. (Editors). Ecotoxicology of amphibians and reptiles. SETAC, CRC Press, Boca Raton. 916 p.

ET10 - Linking chemical residues with biological responses in wildlife

MO 146

Predicting exposure of bats to soil-associated heavy metals

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Wildlife may be exposed to heavy metals in soils via uptake through the food chain. The accumulated metals may then affect the health of the wildlife species. It is therefore important to develop a better understanding of the potential for wildlife to be exposed to metals via the food change and to assess the subsequent risks. In this study, we developed a spatial model to predict the exposure of insectivorous bats to cadmium, lead, copper and zinc in soils. Data were collected on the distribution of 14 bat species in the UK, the diet of bat species, bioaccumulation factors for invertebrate prey and concentrations of heavy metals in soils across England and Wales. These data were used in a GIS-based exposure model to estimate daily levels of exposure for heavy metals in bats across England and Wales. Our results show that highest exposure occurs around industrial areas, where predicted exposures for several bat species are at a level at which toxicological effects might be expected. As many species of European bats are of conservation concern, this model has the potential to identify populations that might be particularly vulnerable to environmental contaminants. Further work is planned to evaluate the model against experimental monitoring data of bats and to explore in more detail the transfer of metals through different stages of the food chain.

MO 147

Use of MDA as biomarker of lipid peroxidation in roe deer (Capreolus capreolus): the influence of gender

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Oxygen metabolism in aerobic organisms implies the formation of reactive oxygen species (ROS), their production being greatly enhanced by exogenous factors like environment pollutants, drugs, radiation and pathogens. These ROS can oxidize biologically relevant molecules leading to alterations in normal cell and tissue functions, but this oxidation is functionally minimized by a broad spectrum of antioxidant defenses (AD). Therefore, a steady-state rate of ROS production, molecule oxidation, and antioxidant consumption is continuously taking place in aerobic cells and tissues. One of the most studied processes in relation to damage produced by ROS is the lipid peroxidation. Lipid peroxidation is a complex process, which can be considered as a sequence of events initiated by a hydrogen atom abstraction, followed by reaction of oxygen with the subsequently formed radical, and by further free radical chain reactions. Lipid peroxidation is an important process initiated by free radicals, which is involved in the events leading to cell death. In the present study the thio-barbituric assay for malondialdehyde (MDA), biomarker of both primary and secondary decomposition of lipid peroxidation products, was used as indirect measure of endogenous lipid peroxidation in three different organs (liver, kidney and lung) of male and female roe deer (*Capreolus capreolus*) from NW Spain, in order to assess the influence of this variable (gender) in future biomonitoring studies in the wild. The results showed higher MDA levels in male than in female liver (0.292 ± 0.045 and 0.160 ± 0.023 nmol/mg protein, respectively), this difference being statistically significant ($p< 0.05$). This result could be explained according to the fact that in females, the hormone estradiol, like other protective molecules, may act by regenerating endogenous antioxidants present in membranes, thus delaying the appearance of lipid peroxides, like MDA. With respect to the other tissues, kidney and lung samples showed quite similar levels of MDA in both males and females (0.212 ± 0.020 and 0.213 ± 0.020 nmol/mg protein respectively), with no statistical relevance.
The findings are indicative that gender can play an important role in the expression of oxidative stress in the case of hepatic tissue, rendering this variable of interest in biomonitoring programs.

MO 148

Incidence of intersex and reproductive status in the clam Scrobicularia plana from 13 sites in NW France

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The endobenthic clam *Scrobicularia plana* is important for the structure and functioning of estuarine and coastal mudflats, and thus the reproduction success of this species is a topic of interest for environmental conservation. In thirteen estuaries from NW France differing by their degree of contamination (as documented by the data of RNO for Réseau National d'Observation, now ROCCH, Réseau d'Observation de la Contamination Chimique du milieu marin at http://wwz.ifremer.fr/envlit/), intersex and different parameters linked to reproduction (sex-ratio, gonadosomatic index) were examined in *S. plana* collected at the beginning of gametogenesis and at the peak of sexual maturity. Both temporal and intersite differences were shown. Some reports exists showing that the presence of several endocrine disrupting chemicals (EDCs) may be directly linked to the incidence of intersex in *S. plana* and, in other bivalve species, an induction of female-biased sex ratios were observed after exposure to EDCs at the larval stage. Thus, the presence of EDCs in superficial sediments collected in parallel to clams was investigated by using an approach combining quantitative chemical analyses of the main classes of EDCs and a battery of *in vitro* bioassays allowing the quantification of receptor-mediated activities, namely estrogen (ER), androgen (AR) and dioxin (AhR) receptors. In addition, the reproductive success may be impaired indirectly when species used as food are impacted in contaminated sites, limiting food availability and then energy ressources devoted to the reproduction of consumers. This hypothesis was tested concurrently with endocrine disruption by determining hepatosomatic indices and measuring chlorophyll and phaeopigments in superficial sediments, the microphytobenthos of which is a major food source for deposit-feeding bivalves such as *S. plana*.

MO 149

Cellular energy allocation and biomarkers of oxidative stress in the green crab (Carcinus maenas) after exposure to a polycyclic aromatic hydrocarbon (fluoranthene)

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Fluoranthene (FLU) is a priority polycyclic aromatic hydrocarbon that has been detected in aquatic systems around the world, including in sediments from Northern Portuguese estuaries. Several works have shown its ability to induce adverse effects in wild aquatic species. *Carcinus*

maenas is a key inhabitant of European estuarine and coastal systems for which exposure to pollutants has been shown to alter physiological and biochemical responses. Despite this, information on sublethal effects of FLU in *C. maenas* is scarce or unavailable, particularly regarding physiological parameters related to the maintenance and performance of the organisms such as net energy balance and oxidative stress. To assess the effects of FLU exposure in relation to tissue residues accumulation, a 7-day laboratory exposure to concentrations ranging from 2.56 to 100 µg/L was performed. Crabs were caught in a reference estuary, acclimated in filtered seawater (salinity 15‰, temperature 16±1ºC, photoperiod 16hL:8hD), and individually exposed to FLU. At the end of the bioassay the crabs were ice-anaesthetised and samples of tissues were collected for chemical analysis and biochemical determinations, namely: the activity of the enzymes lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), gluthatione-S-transferases (GST), gluthatione peroxidase (GPx), gluthatione reductase (GR), the levels of total gluthationes (GT) and lipid peroxidation (LPO), the content in glycogen, lipids and proteins and the mitochondrial electron transport activity (ETS). Despite low tissue accumulation, significant alterations of several biomarkers were detected. An induction of GST activity was found in the animals exposed to the 3 highest test concentrations. A decreasing trend in LPO levels was also found, with significant differences detected in crabs exposed to 100 µg/L. Available energy reserves were significantly higher in the hepatopancreas than in the muscle. Moreover, the latter were significantly decreased in crabs exposed to FLU. Overall, FLU enhanced C. maenas biotransformation processes (GST activity) and altered muscle energy availability.
This work was supported by nacional (MCTES, Foundation for Science and Technology) and European funds (FEDER) through CRABTHEMES Project (FCOMP-01-0124-FEDER-007383, FCOMPETE Program) and a PhD grant to A. Rodrigues (SFRH/BD/65456/2009).

MO 150

Is the reproduction of Donax trunculus affected by their site of origin contrasted by their level of contamination?

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Coastal zones are extremely fragile due to increasing stress from anthropogenic activities (urbanization, intensive agriculture[3DOTS]). Thereby, mixtures of contaminants are present in these zones.
Since mollusk bivalves are good bioindicators of environmental quality, we used the suspension-feeder bivalve Donax trunculus, largely distributed in West-African, European and Mediterranean coasts. The reproductive cycle of bivalves is regulated by several natural environmental factors but exposure to chemical pollutants can also interfere and may result in advanced or delayed spawning season.
To our knowledge, the gametogenic cycle of D. trunculus has not yet been used as biomonitoring tool in ecotoxicological surveys. The aim of this study was to examine over a year physiological reproductive endpoints (sex-ratio, gametogenic and energy reserve cycles) in D. trunculus originating from two sites differing by their level of contamination. Specimens were collected bimonthly from November 2008 to October 2009 from a polluted site (Radès Méliane) and a comparatively reference site (Sidi Jehmi) in the Gulf of Tunis (Tunisia). Five stages were depicted by histological examination of gonads: indifferentiated, development, mature, spawn and spent. Differences in the gametogenic cycle according to the site of origin of bivalves were observed. The spawning period began in March and was maximum in May in bivalves from both sites, but the percentage of spawning animals was higher in the polluted site (March: 30 %, May: 89.47 %) vs reference site (March: 8.57%, May: 71.42 %). The spawning period was shorter (beginning: March, end: May) in animals from the polluted site comparatively to the reference site (beginning: March, end: October). The sex-ratio was equilibrated in bivalves from both sites excepted in March in the polluted site where the percentage of females (66.6%) was higher than males (33.3%). Energy reserves (Glycogen, lipids) were higher in March and May comparatively to the other periods in bivalves from both sites. Lower energy reserves levels in animals from the polluted site could be explained by the cost of tolerance of bivalves exposed during long time to sublethal levels of contaminants. Indeed, increased defense and damage biomarkers were depicted in animals from the polluted site (Tlili et al., 2010). Therefore, D. trunculus appears as a suitable sentinel species for the assessment of the ecotoxicological risk of contaminants such as endocrine disruptors.

MO 151

Use of biomarkers in Cyprinus carpio as tools in biomonitoring programs: experimental exposition to the pesticide Deltamethrin

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The presence of toxic pollutants in aquatic ecosystems possesses a serious threat to environmental health. Use of biomarkers in fish is considered as a cost effective strategy to obtain information on the state of the aquatic environment and the effect of pollutants on living resources. Biomarkers are defined as changes in biological responses (ranging from molecular through cellular and physiological responses to behavioral responses) which can be related to exposure or to toxic effects of environmental chemicals. With these considerations, the objective of this study was to evaluate the usefulness of a suite of both enzymatic and non-enzymatic biomarkers in *Cyprinus carpio* L. in assessing and monitoring pollution of aquatic environments.
For this purpose, fish were exposed for 30 days to deltamethrin, which was added to the tank water at concentrations of 0, 0.08, 0.4 and 0.8 µg/L. Animals from every tank were sampled at the beginning and at 4, 15 and 30 days of the experience, and their liver and brain were isolated. Subcellular fractions were prepared by differential centrifugation in an appropriate buffer. Ethoxyresorufin-o-deethylase (EROD) and glutathione S-transferase (GST) were the activities measured in liver tissue, whereas Acetylcholinesterase (AChE) activity was measured in brain tissue. Moreover, the effect of pesticide on lipid peroxidation was evaluated, by measuring hepatic malondialdehyde (MDA).
A significant increase of LPO was observed after 15 and 30 day of exposure to the concentration 0.8 µg/L when compared to control group. GST activity significantly increased in fish exposed to both concentrations of 0.08 and 0. 8 µg/L of pesticides, but only after 30 days of exposure, when compared to controls. With regards to both acetylcholinesterase (AChE) and EROD activity, no significant changes in enzyme activity were found in any of the experimental groups when compared to the controls.
The results revealed that response of brain AChE, hepatic EROD, GST and LPO of common carp could be used as a suite of biomarkers in future biomonitoring programs in assessing pollution status and pollution trends in water resources.
Acknowledgements: Special thanks to the Tunisian Ministry of Scientific Research and technology "Research Group: Hydrology and Plonctology"who allowed this study to be carried out. This research was supported by "Agencia Española de Cooperación Internacional para el Desarrollo" (project A/015933/08).

MO 152

Evaluation of the contamination of the Loire estuary by endocrine disruptors

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Estuaries are ecosystems of high productivity, crucial in the life history of fishes, invertebrates, birds, including commercially important species, but anthropogenic activities such as urban effluents are often the main factors responsible for water quality degradation. Complex mixtures of contaminants are present in these zones and in this context; there is a growing interest in the influence of endocrine disruptor compounds (EDCs) on biota physiology. The Loire estuary runs through important urban sites (Nantes, Saint-Nazaire, etc.) with shipping and industrial activities. Moreover, the Loire basin (117,000 km^2) represents more than 1/5 of the French territory and drains a lot of tributaries.
The aim of this research is to evaluate the contamination of the Loire estuary by EDCs. Twelve sites along the Loire estuary (from Saint-Nazaire to Ancenis) were selected because of their typology of contamination (agricultural, urban, and industrial) and of their particular configuration (upstream/downstream of an effluent site close to a wastewater treatment plant). The endocrine activities in sediment were followed using various bio-analytical tools (in vitro tests of hormonal activities on cellular cultures). The presence of PAHs and dioxine-like compounds was estimated using the EROD test. The eel Anguilla anguilla was chosen for her ecological representativeness and economical incidence on fishing activities and was collected on 3 sites along the Loire estuary (upstream, intermediate, and downstream). The in situ effects on fish were evaluated by measuring biological variables at the individual level (size, sex, gonads histology, vitellogenin and aromatase).

MO 153

Identifying Pb exposure sources in waterbirds and adverse effects on porphyrin metabolism using non-invasive faecal sampling

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Waterbird feces (mainly mallard (*Anas platyrhynchos*) and coot (*Fulica atra*)) from four wetlands in Southern Spain were collected in the field or during capture (n = 558 and n = 59, respectively) to study lead (Pb) shot ingestion. Pb and aluminum (Al) concentrations, along with Pb isotope signatures in feces were used to track Pb sources in the birds. The profile and concentrations of porphyrins and biliverdin in feces were then used as biomarkers of adverse effects. Feces with Pb concentrations ≥34 µg/g d.w. showed lower Al levels, together with lower $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios, and higher $^{208}Pb/^{206}Pb$ ratios than feces with <34 µg/g d.w. Isotope signatures and Pb-Al ratios together indicated that Pb shot ingestion caused all the high Pb levels observed, whereas sediment ingestion was linked to lower Pb levels. Coproporphyrin I and protoporphyrin IX were also higher in feces with ≥34 µg/g d.w., indicating measurable disruption in heme synthesis. Non-invasive feces sampling permits the study of the degree of Pb exposure and adverse effects on waterbirds with low effort and minimal disturbance to birds. It also avoids bias associated with capture or hunting. This is the first study of its kind in ducks and coots.

MO 154

Organochlorine pesticides in Razorbill (Alca torda) feathers from Southwestern Mediterranean and their correlations with internal tissues

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The aim of this study was to explore the usefulness of feathers as biomonitoring tool for organochlorine pesticides (OC) in a population of 50 razorbills (Alca torda). It was evaluated the distribution pattern of the compounds and correlations between the concentrations found in feathers and OC levels found in internal tissues (liver, brain, subcutaneous fat and abdominal fat) of the same razorbills. Moreover, additional factors such as age, sex and nutritional conditions of the birds were checked in order to evaluate their influence on the level of pollutants in feathers. Fifteen OC were analyzed in feathers, including α-, β- and δ-HCH, lindane, aldrin, dieldrin, endrin, endosulfan I and II, endosulfan sulfate, p,p'-DDT, DDD, DDE, heptachlor and its epoxide. Mean concentrations obtained in this study were ∑Heptachlor 196.27±174.92 ng/g, ∑Drins 61.14±79.60 ng/g, ∑Endosulfan 114.52±154.44 ng/g, ∑DDT 323.56±372.68 ng/g, ∑HCH 174.99±177.87 ng/g. The higher OC levels found in this study compared with other studies are probably influenced by razorbill diet and migration status. Besides, compound persistence seems to be an influential factor in the capacity of the feather to store and excrete organic compounds. Few significant correlations were found between OC levels in feathers and internal tissues, probably conditioned by the time between the molt and the moment of sample collection. According to the levels found in internal tissues, there is no risk associated to OC concentrations found in feathers in razorbills. Further research is necessary to investigate the correlation between concentrations in feathers, internal tissues, and blood in seabirds. Nevertheless, feathers appear to be a promising tool for OC biomonitoring in seabirds, giving a good estimation of OC levels in a population. Acknowledgements: To MICINN and Fundación Seneca for funding the Projects CGL2004-5959/BOS, CGL-2008-4318/BOS and 08758/PI/08. To Generalitat Valenciana for permission to obtain the samples. Silvia Espín is enjoying a grant from the CEIGRAM.

MO 155

Development of an analytical method for extracting organochlorine pesticides from feathers

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Currently, the search for non-invasive techniques in pollutants biomonitoring is an ethical and moral obligation. Keratin tissues, such as hair, have been used for persistent organochlorine pollutants studies accumulation, suggesting that keratin units could be interesting for biomonitoring these contaminants. In this sense, feather is the most important keratin tissue in birds, animals which have played an important role in documenting human-induced environmental pollution.

Although most organochlorine pesticides (OC) have been banned in developed countries, they are still frequently found in tissues or fluids samples from several species, specially in agricultural regions, where these contaminants have been widely used in agricultural practices. The aim of this study is the development of an extraction method for 15 OC in feathers, including α-, β-and δ-HCH, lindane, aldrin, dieldrin, endrin, endosulfan I and II, endosulfan sulfate, p,p'-DDT, DDD, DDE, heptachlor and its epoxide. The large number of OC to identify and polarity variations pose problems in the recovery process. In order to solve these problems, different mixtures of organic solvent were prepared to find out which one was the most appropriate. In this way, four methods of OC extraction in feathers were evaluated, and it was selected the most appropriate method testing for recovery, linearity and precision. To study these parameters, mallard (Anas platyrhynchos) feathers were spiked with three different levels of OC standard solution. Finally, in the most appropriate method selected, extraction was done with hexane:acetone, clean up via Florisil column chromatography and elution with a petroleum ether:diethyl ether mix. This method presented a range of recovery for the 15 compounds studied between 46.13 and 146.05%. The linearity showed a good correlation between OC concentrations and chromatographic peak area for each compound, with regression coefficient values (R) greater than 0.96. The variation coefficients obtained for the repeatability and reproducibility are indicative of good technique precision, with values below 20%. Therefore, the technique developed in this work is acceptable and can be used as a method for the extraction in feathers of 15 organochlorine pesticides widely used in some areas. Acknowledgement: To MICINN and Fundación Seneca for funding the Projects CGL2004-5959/BOS, CGL-2008-4318/BOS and 08758/PI/08. Silvia Espín is enjoying a grant from the CEIGRAM.

MO 156

PBDEs and legacy POPs in hawk and falcon species from urban environments in British Columbia, Canada, 1999-2009

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Liver tissue from Cooper's Hawks (Accipiter cooperii) and peregrine falcons (Falco peregrinus) found dead, 1999 - 2009, in the urbanized Metro Vancouver area were analyzed and compared to samples from southeast Vancouver Island and the Okanagan Valley regions of British Columbia. Cooper's hawks nest in city parks and along treed streets, while peregrines use high rise buildings, bridges and similar structures. Both species are top predators feeding mainly on birds; populations remain throughout the year in many cities, attracted by pigeons, starlings and common bird-feeder species. Past investigations linked population decline of falcon and accipiter species with exposure to legacy POPs, particularly DDE and dieldrin. Recent studies of salvaged eggs and tissues of carcasses have reported accumulation of toxicologically significant concentrations of both legacy POPs and PBDEs. Carcasses were selected only if body condition was good, and the diagnosis of death was trauma. Hepatic ΣPBDE concentrations in 13 Cooper's hawks from Greater Vancouver ranged widely from 30 to 4820 ng/g ww with a geomean of 450 ng/g ww. Similarly, geomean ΣPCBs were 451 ng/g (range 40.5 to 4280 ng/g), and DDE, 1280 ng/g (range 231 - 10,400 ng/g). By comparison, mean ΣPBDEs in three Cooper's hawk livers from the less urbanized Okanagan Valley were only 17.2 ng/g (range 3.2 -133 ng/g); DDE was higher, 1840 ng/g (range 441 to 3800 ng/g), consistent with heavy past use of DDT in that region. Average values and ranges were similar in peregrine falcon samples. Patterns and trends of the various contaminants will be analyzed and compared to trophic level and carbon source using stable isotopes.

MO 157

Relationship between CALUX bioassay and dioxin-like PCB- TEQs in the eggs of predatory birds from the UK

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Accumulation of polychlorinated biphenyls (PCBs) in predatory birds has been extensively characterised but it is less clear as to the importance of co-planer PCB congeners in contributing to the sum TEQ concentrations in birds that are a result from exposure to PCBs, dioxins, furans and other polyhalogenated compounds. We analysed eggs from three different wild bird species: gannet (Morus bassanus), merlin (Falco columbarius) and peregrine falcon (Falco peregrinus) from various locations in the UK. Dioxin-like PCB congeners were identified and quantified by GC-ECD and Toxic Equivalent Concentrations (PCB-TEQs) were calculated based on the sum of the dioxin-like PCBs and appropriate toxic equivalent factors for birds. We also assessed the total exposure to dioxin-like compounds (CALUX-TEQ) using chemical- activated luciferase gene expression (CALUX) bioassay. There was a significant correlation between CALUX- and PCBs-TEQ values for each individual species and when data for all three species were pooled. PCB-TEQ concentrations represented almost 100% of the CALUX-TEQs in gannet and merlin eggs, but only 10% in peregrine eggs. Overall, CALUX-and PCB-TEQs gannet and merlin eggs were significantly higher than in peregrine eggs. Our results suggest that, in the UK, PCBs account for most of the TEQ concentrations detected by the CALUX assay in gannets and merlin eggs. Peregrine falcons accumulate fewer Ah-receptor ligands (including dioxin-like PCB congeners) in their eggs than gannets or merlins and it is compounds other than PCBs that largely contribute to the total toxicity.

MO 158

Risk assessment of organohalogen exposure in a Eurasian eage owl (Bubo bubo) population from Southeastern Spain

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Exposure to polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine insecticides (OCs) are associated with some toxic mechanisms that impair bird reproduction and have led to population declines in several bird species. Despite the ban of most of these highly persistent compounds, current environmental concentrations may still be important and pose potential risks. According to the NRC requirements, the Eurasian Eagle Owl (*Bubo bubo*) could be considered a suitable sentinel species for monitoring organohalogen compounds in Southeastern Spain. Moreover, because this nocturnal raptor is a territorial year-round and long lived species, it could be especially useful to reflect local pollution. Between 2004 and 2009, 58 unhatched eggs were collected from monitored nests. Eggs were analysed for 7 PBDEs, 22 PCBs and 23 OCs using a GC-MS. Eggshell thickness was measured as effect parameter. Although average concentrations of the compounds analysed were below known threshold levels, about 8% of our samples exceeded the NOAEC for ∑DDTs (3.6 µg/g wet weight) and one egg presented total PCB levels above NOAEC (7 µg/g wet weight). In addition, about 36% of the eggs exceeded the Total TEQs NOAEC (135 pg/g wet weight) and levels in 17% of the samples were above the LOAEC for Total TEQs (400 pg/g wet weight). TEQ concentrations were negatively related to the metabolisable fraction of PCBs (F_{prob},<0.001) which is indicative of hepatic enzymes induction in the birds exposed. These females could be suffering Ah-receptor-related toxic effects, some of which have been related to altered bird reproduction. Eggshell thinning caused by pp'-DDE exposure was also evidenced by a significant correlation between log-transformed pp´-DDE concentrations and eggshell thickness (P= -0.469, p<0.001), finding about 18% of eggshell thinning when pp´-DDE levels were above 8 µg/g wet weight. The persistence of this degree of thinning over a period of time has been related to population declines in other raptor species. Acknowledgments: Supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS), Seneca Foundation (08758/PI/08) and WIMEK. Thanks to Autonomous Community of Murcia Region for permission to sampling. Special thanks to M. León-Ortega, E. Pérez, J.A. Lacalle and J.M. Pérez-García for sampling efforts.

MO 159

Blood cholinesterase characterization of Ciconia ciconia and Ardea cinerea: potential bioindicator of contamination in birds

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One of the most varied and conspicuous group of vertebrates, birds had adapted themselves to the most severe environmental conditions on earth and are usually exposed to xenobiotics in ecosystems. There are a broad range of pesticides, which are used to control pests and disease vectors over large areas as wetlands and agricultural fields, but commonly known to have toxic effects on non-target wildlife. Among them, there are cholinesterase (ChE) inhibiting compounds such as organophosphate (OP) or carbamate (CB) pesticides and metallic elements, that usually are the cause of death of wildlife animals.

This study aimed to characterize blood's ChE and asses its basal activity on the white stork (Ciconia ciconia) and the grey heron (Ardea cinerea), two wading bird species well-known by their foraging habits in shallow-water habitats and possible targets for OP and CB exposure through ingestion and dermal contact. For this, blood samples of both species were taken from healthy individuals inhabiting the Biological Park of Gaia (Parque Biológico de Gaia), Portugal. For each species the ChE activity was measured with three substrates (acetylcholine iodide, propionylthiocholine iodide and S-butyrylthiocholine iodide) and three ChE inhibitors (BW284C51, eserine hemisulphate and iso-OMPA). Both species showed enzimatic activity for all the substrates used, with higher activities when propionylthiocholine iodide was used. The form(s) of ChE activity present in each species will be discussed integrating substrate preferences with specific inhibitors results. These results are crucial for the use of ChE activity as a contamination bioindicator for birds in agricultural areas and environmental monitoring.

MO 160

PFOS, PFOA and PFBS induce embryonal hepatic fatty acid oxidation in chicken eggs

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In this study a new method for toxicity testing is presented which can be used for studies of the effect of environmental pollutants on fatty acid oxidation in avian models. In the current study the method has been used to study the effects the perfluorinated compounds (PFC) perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorobutane sulfonate (PFBS) on the developing chicken embryo. PFCs have many industrial and commercial applications such as water and stain resistant fabric treatments, fire-fighting foams and lubricants and can be found on a global scale in water and wildlife samples. Fish eating birds have a high exposure to PFCs and levels of perfluoroctanesulfonate (PFOS) have been found in levels over 1µg/g in guillemot eggs from the Baltic Sea (Holmström, 2005). More research is needed to evaluate the effects of this exposure. Chicken eggs were incubated at 37.5°C and injected with a water solution of PFOS, PFOA or PFBS on day four of incubation. On day ten of incubation chicken embryos were dissected and the livers were incubated in vitro with media containing tritium labelled palmitic acid. The ß-oxidation of palmitic acid was measured by liquid scintillation counting of the media to measure the amount of tritiated water created by the metabolic processes. All of the tested chemicals showed induction of the hepatic fatty acid oxidation. The highest induction was 65% and was seen for PFOS at 0.3µg/g. The mechanisms behind the induction are not known but could be due to the structural similarity between these PFCs and fatty acids or coupled to effects on membrane permeability. The lowest observed effect level (LOEL) was 0.1µg/g PFOS. Several of the doses in this study are below environmental levels found in bird eggs which indicate that effects of this kind could be present in some bird populations. Birds in the wild are not exposed to single PFCs but mixtures of PFCs and other pollutants. It is not known how mixtures of PFCs affect this system.

Holmström KE, Järnberg U, Bignert A (2005) Temporal Trends of PFOS and PFOA in Guillemot Eggs from the Baltic Sea, 1968-2003. Environmental Science & Technology 39:80-84.

MO 161

Effects of early exposure to BDE-99 on growth, physiology and reproduction in zebra finches and European starlings

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Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that have become ubiquitous in the environment, and BDE99 is one of the most abundant congeners. There is evidence that PBDEs have a wide range of toxicological effects in birds, including negative effects on reproduction, changes in growth, and altered physiology. Concentrations and effects of PBDEs have been most commonly examined in avian species that are top predators, whereas passerine birds have been less frequently studied. In birds, early life stages are the most sensitive to environmental conditions, and exposure to contaminants during the nestling period may have critical effects at concentrations much lower than those required to affect adults. These effects of exposure may not be evident until reproductive maturity, necessitating long term studies to assess fitness implications. In this study, our objective was to use an integrated avian laboratory and field model system to examine the potential long-term effects of early exposure to BDE99 in passerines. The zebra finch (*Taeniopygia guttata*) is a useful passerine model to monitor effects of contaminants under controlled laboratory conditions, as they reach sexual maturity within 90 days, and readily breed in captivity. The European starling (*Sturnus vulgaris*) can be used as an ecological equivalent for comparative in situ investigations. Starlings are widespread, readily use nest boxes, feed on terrestrial invertebrates, and are easy to sample. In both species, we

exposed young for the duration of the nesting cycle to environmentally relevant, sublethal levels of BDE99 (0-250 ng/g bw/day). A preliminary study dosing zebra finch chicks with BDE99 for 21-days during the nestling phase showed that there was a strong dose-dependent relationship for plasma BDE99 levels at 30 days of age. Following dosing, we raised young to sexual maturity. In the zebra finches we monitored female reproductive success, and in the starlings we monitored reproductive development and gonadal growth. For both species we monitored growth, and took blood samples to assess thyroid hormone homeostasis, oxidative stress, immune function, and hematocrit for each treatment group. At our dose levels, few effects on reproduction, growth and physiology were observed.

MO 162

The effect of migration and fasting on fatty acid profiles and organochlorine contaminant burdens in humpback whales

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Humpback whales inhabiting the Southern Ocean are exposed to an as yet uncharacterized level of environmental pollutants, and though not currently considered an at risk species the extreme life history parameters of fasting and migration that characterize this group of mysticetes emphasizes the imminent necessity for toxicokinetic and toxicodynamic analysis. These aforementioned parameters cause the whales fat supplies or blubber to be metabolized and this theoretically forces the release and redistribution of stored toxins such as Persistent Organic Pollutants (POPs), which may result in high circulating levels and adverse health affects. This study focuses specifically on the first generation, or legacy, POPs which encompass the major group of halogenated hydrocarbons, the organochlorines (OCs).
Here we utilize non-destructive skin and blubber samples from free-swimming populations of Southern Ocean humpback whales during two time points of the migration for the purpose of quantifying migration associated OC concentration effects.

MO 163

The toxicological effect of inhalation exposure to nitrocellulose paint thinner fumes (ABRO®, FIAB® and SPRINT®) on selected biochemical and haematological parameters in Wistar albino rats.

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The toxicity effects of the inhalation exposure to fumes of three different brands of nitrocellulose paint thinner (ABRO®, FIAB® and SPRINT®) in wistar albino rats were investigated after 28 days of 4 h daily inhalation exposure. Serum L-alanine aminotransferase (L-ALT), L-aspartate aminotransferase (L-AST) and alkaline phosphatase (ALP) levels after 28 days of inhalation exposure to rats increased ($p \leq 0.05$) significantly when compared to control. Inhalation exposure of rats to different nitrocellulose paint thinner fumes caused a significant ($p \leq 0.05$) increase in haematological parameters (haemoglobin, packed cell volume and white blood cells) when compared to control group. Rats exposed to "SPRINT®"showed the highest levels of Hb, PCV and WBC with respect to other brands (ABRO®, FIAB®). The order of increase was SPRINT® > ABRO® > FIAB®. Inhalation exposure of rats to paint thinner fumes caused a significant ($p \leq 0.05$) increase in selected biochemical parameters (urea, cholesterol, total and direct bilirubin) when compared to control group. Histological examination of the liver tissues of experimental groups showed degenerative changes in the structural integrity of the hepatic cells. The results suggest that continuous exposure to paint thinner fumes may be hepatotoxic and haemaotoxic in rats and may lead to multiple organ toxicity.

ET13 - Moving towards a systems biology approach to predictive ecotoxicology

MO 166

Predictive ecotoxicology: predicting responses and physiological consequences to advance the Environmental Risk Assessment of pharmaceuticals

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Human pharmaceuticals detected in aquatic environments have raised concerns about potential impacts on wildlife. These concerns, exemplified by oestrogens, have led the scientific community to address how to predict such effects and how best to advance the Environmental Risk Assessment (ERA) of pharmaceuticals. To better predict potential effects in non-target environmentally relevant species, one area in which our laboratory (amongst others) has been working is the potential use of non-clinical and clinical pharmacological and toxicological data generated during drug development (read-across). Genetic conservation of receptors and physiological systems between mammals and certain environmentally relevant species (i.e. fish), combined with knowledge of primary and secondary Mode-of-Action (MOA) in mammals, can assist in selection of appropriate aquatic test species, study designs, and endpoints in an approach referred to as 'intelligent testing'. Surprisingly, evidence for the proof of this principle is sparse. In this presentation we build on our previous publications[1,2], concerning how best to use the data available from the human drug development, and to address some of the issues surrounding the validation and equivalence of mammalian to fish read-across.
In order to begin to predict the ecotoxicological relevance of the read across model, we need to understand the broader physiological consequences of these predicted effects at each level of the biological system in order to work towards an effective interpretative model. The work presented here reports the first example of the multilayered (systems) approach that we have taken to predict effects and then test the hypothesis via measured endpoints to advance the ERA beyond the current regulatory requirement, in order to provide evidence to support the proof of principle for an intelligent assessment.

MO 167

Linking DNA damage in sperm with reproductive success in adult zebrafish Danio rerio exposed to cobalt (Co2+)

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Toxicants can damage DNA in gametes and the potential for negative consequences in progeny (embryos) may be related to the capability of DNA repair mechanisms. Mature spermatozoa have little or no capability to repair toxicant-induced damage in DNA, and lesions in DNA that are not repaired during spermatogenesis may directly affect embryo survival. By contrast, oocytes retain DNA repair capabilities, and lesions in DNA of oocytes are therefore less likely to affect progeny. The objective of this study was to investigate toxicant-induced DNA damage in zebrafish (Danio rerio) sperm and determine if induction of DNA repair mechanisms are related to reproductive success. Cobalt was selected as an environmentally relevant genotoxicant and initial acute toxicity trials with larvae resulted in an LC_{50} value of 34 mg.l-1 Co^{2+}. Adult zebrafish were exposed to sub-lethal concentrations of Co^{2+} (0, 5, 10, 15, 20, and 25 $mg.l^{-1}$). Males and females (n=3 f, n=3 m) were separated in the same tanks (n = 5 treatment tanks, n=3 control tanks) and bulk spawned every 4 days (3 pre-exposure and 3 exposure period spawning events). Prior to exposure 88% of the spawned eggs were fertilized and of these 79% survived to 4 days. Exposure to Co^{2+} significantly reduced the percent of fertilized eggs (5.6%, 25 $mg.l^{-1}$), and survival to 4 d post-fertilization (60%, 25 $mg.l^{-1}$). DNA damage was detected in sperm (sub-lethally extracted and analysed with the alkaline gel electrophoresis 'comet' assay) following 13 d exposure (42% tail DNA, 25 $mg.l^{-1}$ Co^{2+}), significantly higher than control (31% tail DNA, dose-dependent increase, $p = 0.0041$, GLM). After 6 d post-exposure, damage dropped in the top concentration (20% tail DNA, 25 $mg.l^{-1}$ Co^{2+}) and there was no significant dose-dependent response ($p=0.1902$, GLM). Expression of DNA repair genes in testes may lead to repair of DNA in developing sperm and reduction in DNA damage in spermatozoa during the recovery period. We are investigating changes in expression of DNA repair genes (e.g. XRCC5, XRCC6, rad51) in testes of fish from this experiment. The results of cobalt-induced damage, and impaired larval development, show transfer of DNA damage from exposed parent to unexposed offspring. This extends the effects of environmental genotoxicants beyond exposed populations to impaired development in progeny, and the potential for long-term transgenerational effects.

MO 168

Conserved toxic responses across phylogenetic divergent lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics

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At military training sites, a variety of pollutants may contaminate the area originating from used munitions. These contaminants, munitions constituents (MCs), include nitroaromatic compounds such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). RDX has been detected in the environment and several studies have reported toxicity to soil invertebrates at high doses. Studies investigating the mechanism of toxicity of RDX have shown that it affects the central nervous system causing seizures in humans and animals. It has the potential to affect many different species, as they can accumulate and be present in many environmental compartments, thus in a long term may also have evolutionary consequences. In relation to this it is important to establish first how phylogenetically distant species may respond to these types of emerging pollutants. Comparative toxicology and comparative genomics can be used to assess the effects of a contaminant on different species. In this paper, we analyzed the effects of RDX on five different species to elucidate if it elicits effects via common pathways among the species examined. We used a genomics and gene network approach to compare and contrast the neurotoxic effects of RDX among five phylogenetically disparate species: rat (Sprague Dawley), the fathead minnow (Pimephales promelas), earthworm (Eisenia fetida), Northern bobwhite (Colinus virginianus), and coral (Acropora formosa). Our results showed that RDX accumulated into the brain of rat, Northern bobwhite and the fathead minnows. RDX impacted neuronal function in rat, Northern bobwhite and earthworm, but apparently not in fathead minnows. Blood-related impacts were observed in most species. The comparison of Gene Ontology terms indicated several biological processes affected by RDX in all species, such as impacts on calcium signaling (involved in seizure response), effects on xenobiotic metabolism, electron transport, and cell signaling pathways. Overall, the meta-analysis using genomic data of the effects of RDX on several species indicated a common and conserved mode of action of the chemical throughout phylogenetically remote organisms.

ET16 - Soil ecotoxicology and quality assessment

MO 170

Uptake and distribution of metals in maize (Zea mays L.) plants grown in municipal solid waste compost-amended soil

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The use of municipal solid waste (MSW) compost is a common practice to improve the physical, chemical and biological properties of impoverished soils by supplying organic matter. Community legislation in the EU considers that biosolids may play an important benefit on Climate Change for its action on carbon sequestration and reducing CO2 and atmospheric pollutant emissions. The disposal of the MSW compost may lead to an increase of metal concentration in soil and in consequences, the productivity of crops may be reduced. Data related to the effects of MSW compost on crop performance and metals distribution in maize plant are relatively scarce. Therefore, a greenhouse experiment was conducted in maize (Zea mays L.), with the following objectives: 1) determine the risk of accumulation of total and available metals in soil, as a result of the use of a MSW compost as soil amendment (50 t ha-1) and 2) identify the ability of uptake and distribution of metals to different parts of the maize plant.
The amendment of MSW compost in soil increased the concentrations of Cu and Zn in soil as compared to the unamended soil; however, the rest of metals (Cd, Cr, Ni, Zn and Hg) showed similar concentrations to those found in the control soil. Root system showed the highest metal concentrations and acted as a barrier for all metals. Generally, the trend of metal accumulation was: roots > leaves > stem > cob > sphates >grain.
This study was funded by the Spanish Project CTM 2010-19779-C02-01

MO 171

Long-term effect of sewage sludge & compost amendment on soil properties: systematic fertilization versus uncontrolled application

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Wastewater treatment processes generate highly biodegradable solid wastes whose final destination is an environmental issue with considerable repercussion for public administrations that intend the sustainable management of the urban wastes. This study aims the advance of knowledge about the effects on environment of the long-term application of sludge and compost in soils in order to enhance their quality and, at the same time, manage this type of wastes in a sustainable way. For that, the analysis of the systematic versus uncontrolled use of sludge and compost as agricultural fertilizers was performed in three regions of Spain. The effects of sludge and compost application were evaluated over some soil physical-chemical properties and functions and also over vegetable growing and pollutants (metals and organics) accumulation.
In general, it was observed that the long-term input of sludge and compost enhances soil properties proportionally to the doses and/or to the frequency of application. The organic amendments increased the organic matter content (and its aromaticity), the soil nitrogen and the microbial activity, improving the mineralization processes of carbon and nitrogen and some enzymatic functions (urease and dehydrogenase). However, there was a maximum dose, from which the soil properties did not show improvement and even decline. Furthermore, it was observed that the repeated and not sufficiently controlled (high doses) sludge and compost amending could be the cause of the imbalance between C and N cycles producing a decrease of the C/N ratio that can involve significant risk for aquifer contamination by nitrates.

MO 172
Dredged sediments use on agricultural land - evaluation using biochemical markers in Eisenia fetida
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High volumes of sediment are removed from Czech rivers, streams and ponds routinely. The subsequent usage of the dredged sediment is an important environmental topic. This material is reach of nutrients and organic matter, but also possibly contains high levels of contaminants. It can be potentially applied as soil improver on agricultural land, but its usage must be regulated based on its contamination or toxicity.
In our study, set of 30 sediments was tested using biochemical markers of oxidative stress and detoxification in earthworm Eisenia fetida. Sediments (samples from rivers, ponds and stockpiles of dredged material) were chemically analyzed and mixed with reference arable soil in 1:3 ratio (v/v) which has been suggested in novel Czech directive (2009) as realistic scenario after deposition on land. Control groups contained just reference soil. Two different experimental designs using solid matrix (avoidance and reproduction toxicity test) were applied to assess lethal and sub-lethal parametres and to obtain earthworm tissue samples for further biochemical analysis. In earthworms, biochemical markers are often used to observe and evaluate toxic effects of organic and anorganic compounds, or their mixtures, but also influences of other stress resources (pH, physical properties of soils). Therefore biomarkers are suitable sensitive tools to assess effects of real solid matrixes and their mixtures on soil invertebrates. In this study, there were established methods for determination a set of biomarkers (GST, GR, GPX, GSH, lipid peroxidation; coefficient of variation ~ 0,1; ≤ 0,2 for GST, resp.) in earthworm Eisenia fetida used as parametres of oxidative stress and detoxication processes. We observed significant effects (both inductions and inhibitions; One-way Anova and Dunnet test, $p<0.05$) on biochemical parametres comparing to physiological levels of control groups. In both used test designs, GST activity indicated the most sensitive responses. Moreover, our measurements were compared to results of chemical analysis (sum of PAHs, PCBs, DDTs, heavy metals) and other toxicity endpoints as mortality, reproduction and avoidance (Pearson correlation, $p<0,05$; e.g. $r = 0,89$ for GSH subset vs. PCBs in chronic test). Our results confirms suitability of biomarkers as sensitive and simple tools in soil ecotoxicology.

MO 174
Differential sensitivity of soil test species to biochar products
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Biochar is a charcoal product derived from the pyrolysis of biomass, destined for agronomic purposes to improve soil characteristics and fertility. Biochar systems may integrate any combination of uses which include biochar as an agricultural amendment, energy production via byproducts of the pyrolysis process, and carbon sequestration due to biochar's high recalcitrance. However, very little is known about biochar's effect on soil communities, particularly invertebrates for which currently there are no data; therefore the aim has been to provide basic information about biochar's effect on invertebrates, using biological endpoints of ecological significance. Laboratory tests used 3 feedstocks and 3 distinct biochar production methods, and a wide range of biochar-soil mixture concentrations covering all potential application rates. Adult survival and reproduction were evaluated for three soil-dwelling invertebrates of distinct orders and trophic levels; Folsomia candida is a collembolan, Enchytraeus cryticus is an annelid commonly known as the white potworm, and Hypoaspis aculeifer is a predatory mesostigmatic mite. Additionally, germination and growth tests were carried out with Lactuca sativa (lettuce) and Lolium perenne (raygrass). Measured responses were survival and reproduction for arthropods and seed germination and biomass for plants. Results show that organism responses are dependent on all factors considered (feedstock, production method, and organism), with important implications for design and feasibility of biochar systems, the clear message being chars can provoke different biological responses depending on feedstock and/or production method, and that sufficient testing and screening should be carried out before field implementation.

MO 175
Effects of different biochars and their corresponding feedstocks on reproduction and survival of the collembolan Folsomia candida
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Biochar is a carbon-rich product of thermal decomposition of biomass in oxygen-starved conditions, also called pyrolysis. There has been growing interest in the use of biochar as an agricultural amendment, due to biochar´s physico-chemical properties which can greatly improve soil fertility under certain conditions and allow carbon sequestration. However, there is little information about ecological effects of biochar, and to date there are no published studies on biochar effects on soil mesofauna. Furthermore, biochars can vary greatly in their physicochemical properties, and organism responses are hypothesized to vary accordingly. Thus the objective of this study was to answer basic questions about biochar´s potential toxic effects on Folsomia candida, a soil-dwelling collembolan commonly used as a test species in ecotoxicological work. Reproduction and survival assays and avoidance tests were carried out in order to understand how different char materials and concentrations can affect physiochemical properties and influence biological responses of organisms.
Reproduction and survival tests of toxicity with 7 feedstocks and their corresponding low (300-350°C) and high (500-550°C) temperature-produced chars showed that adult survival of F. candida was generally unaffected by any of the factors considered. However, reproductive inhibition was evident for some materials at concentrations > 0.2%, and general inhibition was found for concentrations of 7% and greater which is far beyond recommended application rates which are generally under 1%.

MO 176
In-field evaluation of effects of biochar on soil properties and functions
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Biochar is a carbon-rich product of thermal decomposition of biomass in oxygen-starved conditions, also called pyrolysis. There has been growing interest in the use of biochar as an agricultural amendment, due to biochar´s physico-chemical properties which can greatly improve soil fertility and allow carbon sequestration. Experimental plots in a corn field were established in a temperate region (Aurora, NY) in 2007, with treatments of 0, 3, 12, 30 T/ha, and 1 T/ha corn stover biochar applied annually, all with additions of 90% of recommended N fertilizer. Soil functions and physico-chemical and biological properties were investigated at this site during the 2010 growing season.
It was found that important physico-chemical properties in treatment plots including field moisture, pH and electrical conductivity did not differ significantly from control plots. Total soil respiration did not differ between plots, but microbial biomass in 30 T/ha plots was significantly higher with respect to the control as well as other treatments. In-field three-month decomposition rate, mineralization, and faunal feeding activity were generally found to be unaffected by biochar application rate or frequency, all of which suggests that provision of key ecosystem services is neither impaired by biochar addition of up to 30 T/ha, nor by annual application of fresh char.

MO 177
French Bioindicator Program : first results on soil fauna-based tools for soil monitoring, characterization and risk assessment
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The main objectives of the Bioindicator Program is to provide new tools for soil monitoring, characterization and risk assessment, based on soil biological properties. In the first step of this program, research teams have developed and tested their indicator on a few given situations. After selection, the most relevant indicators are now tested and compared on same sites.
This communication presents the first results of the soil fauna working group. Three types of indicator are studied. The IQGS (Global Index of Soil Quality) combine physical, chemical and biological (macro-invertebrates) indicators. A second type deals with measures linked to the structure and/or the diversity of soil invertebrate communities (macro-, meso- and micro-fauna). Last, measures are also realized at organism level (metallothionein coding gene expression in earthworm or metal bioaccumulation in snail or micromammal). These indicators are all tested in 13 sites selected according to their history, their current use and their contamination level.

MO 178
Ciliate communities as a tool to assess soil quality in agroecosystem: investigation on agricultural soils under organic farming
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Ciliated protozoa are ubiquitous eukaryotic microorganisms, which constitutes an essential component of aquatic and soil ecosystems. As single-celled organisms directly exposed to the environment, ciliates are very sensitive to any change in their habitat and fluctuations in their communities can affect the food web and energy transfer within the ecosystem. Thus, the monitoring of the structure of ciliate communities can represent a valuable tool to assess ecosystem quality and functioning. Although the biodiversity and community structure of ciliates has been extensively investigated in freshwater and marine ecosystems, very few studies have been addressed to the analysis of ciliate communities in the soil. The majority of the previous reports mainly regards natural soils such as those of temperate and mangrove forests, and grasslands while, studies on less stable and more potentially "stressed"agricultural soil are very rare and, at least to our knowledge, no studies have been performed on agricultural soil under organic management in Italy.
In our study, ciliates community structures in three fields under organic management located in a hill area (300-550 m asl) of the province of Macerata (Marche) were investigated by means of qualitative (non-flooded method) and quantitative (most probable number) methods. Soil samples were taken twice in autumn and spring. Furthermore, six more sites representative of natural and semi-natural soils, such as forests and grasslands, were sampled for comparison. Physical-chemical as well as pedological data were also recorded. Our preliminary surveys showed a total of 79 species belonging to three classes, 19 orders, 42 genera from all the sites under study. Soil samples showed the dominance of ciliates belonging to genus *Colpoda*, *Gonostomum*, *Oxytricha*, and *Halteria*. The species richness ranged from 37 to 15 and, in agreement with Foissner (1997), is higher in agricultural fields as compared with the natural habitats. This matches the different sites investigated and shows the high bioindicative potential of ciliate species number. This

research represent the first, necessary step toward the development of a molecular profiling assay of ciliate communities in the soil. In fact, the final aim will be that to fruitfully merge taxonomic and molecular data to obtain a more rigorous and detailed picture of the soil ciliate biodiversity.

MO 179

Spatial and temporal distribution of soil microarthropods in in-crop and off-crop habitats

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The vertical niche differentiation of soil organisms is an outstanding trait determining the exposure probability to the application of plant protection products. Recently published "EFSA opinions"pointing at the fate of pesticides in the soil compartment, the relevance of the soil litter layer and the soil ecoregion concept take this feature particularly into account. Several studies on the stratification of soil microarthropods (Collembola, Acari) have been conducted yet. But only few concentrated on the situation in agricultural landscapes. Thus until now there is no well-founded knowledge on their vertical dispersal and seasonal fluctuations in arable fields within the course of a year.

We conducted a field study to increase knowledge on the spatio-temporal dynamics of microarthropod-assemblages in the topsoil of both, in-crop and off-crop habitats. In an arable field and an untreated meadow soil cores (5cm in diameter; 5cm and 10cm in depth respectively) were taken monthly, divided into different layers (0-1cm, 1-3cm and 3-5cm and 5-10cm respectively) and extracted for microarthropods by means of heat extraction in a Macfadyen apparatus. The results contribute to a sound development and evaluation of exposure scenarios.

MO 180

How do climatic changes affect isopods?

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Climate changes have been modifying ecosystems, and these modifications will lead consequently to the adaptation or perish of all the organisms that co-habit in those ecosystems.

Organisms like isopods are important key-species to maintain ecosystems function and stability. Isopods as macrodecomposers play an important role in decomposition processes by the fragmentation of litter material and in the re-cycling of nutrients in soil.

To simulate stress induced by global changes, isopods were exposed to ultraviolet radiation, different temperatures, soil moisture levels and pHs.

Biomarkers (e.g. AChE, LPO, GST, CAT, etc.), energy reserves content (lipids, carbohydrates and proteins), energy consumption and cellular energy allocation (CEA) were determined after exposure.

The present work showed that several sub-individual biomarkers activity can be used as early warnings for natural stressors and that climate changes can be responsible for disrupting physiological processes in isopods. This might also be transposed to the ecosystem services considering that the impairment of physiological processes will lead to a less efficiency role on fragmentation of leaf material, decomposition processes and nutrient cycling in soils.

MO 181

A new guideline for the main investigation on soil contamination in the Netherlands

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The national Dutch government initiated the development of a new guideline for the main investigation on soil contamination as an answer to questions from the market. There was a broad wish to use 'second generation' field techniques and to go back to skilled professionals to perform and assess main investigations. In the project of developing the new guideline approximately twenty representatives of stakeholders were involved. The project was performed by two consultancy firms and coordinated by NEN, the Dutch national standardization institute. The new guideline is not a 'cook book' like the earlier protocols, which were never really followed up. Everyone had good reasons to depart from the strict numbers of drillings, groundwater wells and sample analyses those earlier protocols prescribed. The new guideline describes the process to come to a tailor-made strategy for a main investigation. In this way the strategy of a main investigation can be fitted perfectly to the specific local situation, and the most efficient field techniques can be used. The new guideline, in combination with the formalised website with techniques and examples, is aimed at stimulating the use of 'second generation' techniques. In addition, this approach supports skilled professionals in performing main investigations with quality. The presentation will show how to make a guideline that can be enforced, without giving strict rules for the strategy that should be used. A change of mind is needed, to introduce this new way of working. The presentation will show how we try to reach this.

MO 182

Effects of winter road salting on soil microorganisms at grassland and forest site

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Road salting is used as a dominant way to keep road safety in winter, even in the protected natural areas. In our study, possible effects of winter road salting on soil microorganisms in close road vicinity were investigated. Soil chemical and microbial properties were monitored at forest site in Krkonoše Mountains national park and at grassland site in Kokořínsko protected landscape area in two sampling campaigns (autumn and spring). Effects of road salting on soil chemical properties (Na+ and Cl- levels, pH, base saturation [3DOTS]) were clearly apparent. The most affected plots were 1 and 5 m from the road. At these plots, some changes of microbial parameters were observed in both autumn and spring sampling, which suggested influence of salts. Hence, possible influence on soil biological quality should be considered when assessing the ecological risks of this kind of road treatment, especially in natural protected areas.

MO 183

Contamination, ecotoxicity, microbial and physico-chemical properties of soils in frequent urban roads surrounding

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Intensive traffic has influence on surrounding ecosystems and one of the most important target of this influence is probably soil. In our study, model localities were selected in the surrounding of very busy roads in Prague city. Samples were taken on the roadside and 10-30 meters from the road. Control samples were taken in the Prague city but at significant distance from any busy road (urban background). In the samples, main physico-chemical properties were analysed as well as contamination with heavy metals and organic pollutants (PAHs, PCBs, HCB ...). Microbial parameters of soil were also evaluated. All samples were tested for toxicity using standard soil bioassays with Eisenia fetida, Enchytraeus crypticus, Folsomia candida, Caenorhabditis elegans. The objectives of the study were: 1) to investigate if there are some relationships between contamination of soils and traffic intensity, 2) to investigate the relationships between soil contamination, soil properties, microbial parameters and toxicity in soil bioassays. The results showed increased PAHs at the roadside, but heavy metals at the roadside and further from the road were comparable. No clear relationship between traffic data and contamination was observed. Also effects of soil contamination on soil microbes or model organisms were not apparent. These results show that whole problem (of soil load by traffic and consequent effects on ecosystem) is more complicated. In complex approach, measurement of other factors must be integrated (e.g. local conditions affecting the entry of contaminants to the soil, accumulation properties of soil, bioavailability characterization etc.).

MO 184

Accumulation of metals in vegetation and invertebrates in polluted ecosystems of Flanders

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Soil often acts as the ultimate 'sink' of environmental pollution, because clay minerals and humic materials have a large number of surfaces, chemical groups and organic particles to which pollutants can attach. The concept of soil metal bioavailability refers to metal fractions in chemical forms which can be taken up by different soil organisms and by plants. The ultimate measure of metal bioavailability is the uptake thereof in organisms and vegetation. If a contaminant is found inside an organism it must have been bioavailable while the accumulated concentration can be related to adverse effects. Soil, vegetation and invertebrates were collected over a period of 11 weeks in the region of Antwerp, Belgium. This was done in order to assess the relationship between soil characteristics and the level of bioavailability of metals in contaminated soils. Sampling took place along an historical metal pollution gradient. The following parameters were measured: Soil (pH, CEC, clay %, moisture %, CaCl2, metal concentration in soil mineral fraction and pore water), vegetation (metal concentrations in mono-/dicotyle plants) and invertebrates (metal concentrations in primary, secondary and tertiary consumers). Furthermore, we search for a hierarchical correlation between the species' position in the food web and the level of heavy metal accumulation by performing C/N stable isotope analysis.

MO 185

The effect of natural disturbances on microbial properties in heavy metal polluted soils

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Soil microorganisms play an essential role in the decomposition of organic matter. A large number of studies have indicated that soil microbes from polluted sites reveal heavy metal resistance. In such environment every additional stressor may disturb the structure of microbial community and have adverse consequences on the whole soil ecosystem. The effect of single stressor on the soil microorganisms has been well elaborated, but only limited research has been carried out to test the effect of simultaneously acting diverse stressors.

Aim of the study was to estimate the effect of interactions between long term heavy metal pollution and natural factors (temperature fluctuations and drought stress) on soil microbial communities.

Samples of the O horizons were taken at 5 sites along pollution gradient located near Zn-smelter in Poland. The samples were acclimated under optimal conditions (22oC, 50%WHC) for a week, placed under stressing conditions (temperature fluctuations: 20oC /30oC per 12h, drought) for eight weeks and then incubated again under optimal conditions for six weeks. Microbial properties were measured prior to the stress period, directly after it, two and four weeks after the stress period. Measured were basal respiration rate (RESP), microbial biomass (Cmic), nitrogen mineralization rate and urease activity. Heavy metals and stress period affected RESP, but did not affect Cmic. Drought and elevated temperature period significantly increased RESP, resulting in higher values of metabolic quotient ($p<0,005$). Highly polluted soils exhibited lower RESP. Urease activity increased after the stressing period ($p<0,005$), however the increase was less pronounced in the polluted soils. The stressing period did not affect the contents of NH4+ and NO3- but increased the contents of NO2- ($p<0,005$) in the studied soils. The obtained results indicated that long term heavy metal pollution modifies the reaction of soil microbes to additional stressing factors. We presume that the observed changes in RESP, URE and NO2- after the stressing period were due to changed C and nutrient availability.

MO 186

Zn, Cu, Pb and As accumulation in Brassica oleracea cropped in contaminated soils

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We present a synthesis of the data obtained from the survey, in order to assess the contents of some chemical elements (Zn, Cu, Pb and As) in the soils and in exemplars of horticultural species (Brassica oleracea L. var. oleracea) collected in the area around the tailings of Jales Mine (Vila Pouca de Aguiar, Vila Real, Northern Portugal), taking as a reference exemplars from a location somewhat removed from the mine and therefore less exposed to aeolic dispersion of dust released from it.

In each area we established 5 sampling spots. The soils samples were collected to the depth of 20 cm. The vegetal material sampling fell upon the whole plant and we proceeded to the separation of the different organs (roots, stalks and leaves). The techniques used for chemical analysis involved incineration until everything was reduced to ashes. Then we proceeded to an acid attack over this material, followed by the analysis through Atomic Absorption Spectrophotometry (AAS) to determine Cu and Zn, resorting to the Graphite Chamber to determine Pb and As. The exemplars of Brassica oleracea L. collected in the areas of contaminated soils accumulate heavy elements and arsenic in higher amounts than the ones observed in the exemplars collected in the regulating area. The values accumulated in the plant that we analysed change between 11 to 147 mg kg-1 for Zn, between 1.5 to 5.6 mg kg-1 for Cu, between 2.4 to 7.3 mg kg-1 for Pb and between 1.62 to 11.15 mg kg-1 for As. The contents of Zn, Cu and Pb are within the normal patterns in plants (Kabata-Pendias and Pendias, 1984). The contents of As are above the limits (Adriano, 2001), pointing out to a strong contamination at the biological level.

MO 187

Use of the bait lamina test to assess the effects of lead contamination on the soil organisms of a shooting range

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In Switzerland, assessment of soil pollution is mainly based on chemical analysis of total or soluble content of specific contaminants. Methods assessing soil biological parameters have been proposed to complete the chemical parameter data. Currently, no ecotoxicity tests are employed to assess soil contamination in the field. In order to propose and integrate ecotoxicity tests for soil protection in Switzerland, the use of functional methods such as the bait lamina test is considered for the monitoring of pollution gradient at contaminated sites. In the present work, the bait lamina test was applied at an outdoor shooting range as high lead (Pb) concentrations were found at this site. Changes in overall feeding activity of soil organisms were measured in 4 areas along the Pb gradient of contamination to assess the potential impact of this metal on the soil biocenosis. The bait lamina results were complemented by physico-chemical analyses of soil and by reproduction tests with collembolans on collected field samples. Pb concentrations (pseudo-total content) in soil along the contamination gradient ranged from 40 mg/kg (control area) to 2900 mg/kg for the most contaminated area (target and impact berm vicinity). The overall feeding activity of soil organisms after 46 days for the 4 areas varied between 14% and 20%. The high Pb concentrations found in soil near to the targets and impact berm did not seem to influence the feeding activity of soil organisms when compared to the one observed at the control area. This may be explained by the low Pb bioavailability measured in the analyzed soil samples. In the future, the bait lamina method will be further optimized and additional tests will be conducted at contaminated sites to assess the applicability of this method for the risk characterization of contaminated sites in Switzerland.

MO 188

Quality assessment of soils from S. Domingos Mine (Portugal): chemical, biochemical and ecotoxicological evaluation

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S. Domingos Mine is an abandoned mine located in the Portuguese sector of the Iberian Pyrite Belt. This study presents results obtained for the quality assessment of soils collected in three different locations of the mine area considering: chemical characterization; pseudo-total trace element quantification and their water-leachable fractions (As, Cd, Cr, Cu, Ni, Pb and Zn); ecotoxicity evaluation (plant growth and germination tests with Avena sativa L. and Brassica rapa L., Eisenia fetida mortality, E. fetida avoidance behaviour, luminescent inhibition of Vibrio fischeri and Daphnia magna immobilization); soil enzymatic activities (dehydrogenase, β-glucosidase, cellulase and acid phosphatase) and soil total bacterial DNA quantification. The soils were acid, poor in organic matter and in plant nutrients. Soil A has higher As and Pb content, while soil C has higher Cu and Zn concentrations. B. rapa was more sensitive than A. sativa to this type of soil contamination, and they both allow the classification of soil C as the most toxic. E. fetida mortality was the least sensitive of the direct bioassays used. On the contrary, the behavioural response bioassay, with the same organism, was found to be an extremely sensitive endpoint, allowing the classification of these soils has toxic or with impaired quality, as less than 20% of the organisms were found in the test soil for concentrations above 6.3% (v/v) for soil A and B, and 4.7% for soil C. In the aquatic bioassays, D. magna evidenced as a more sensitive organism to this type of soil contamination, with an EC50 of 3% (v/v) for the leachate from soil C, more toxic than soil B (12% v/v) and soil A (23% v/v), than the luminescent bacteria bioassay, that also allowed the confirmation of the leachate from soil C as considerably toxic. Results from the soils enzymatic activities and from the total bacterial DNA quantification identified soil B as the one containing higher quantity of DNA and higher enzymatic activities. The results from the aquatic bioassays confirmed the soil toxicity categorization obtained with the bioassays testing the whole soil: soil C was found to be the most toxic. Even though, results indicate impaired soil retention function and habitat function for all soils that were evaluated, highlighting the need for a soil intervention at the site.

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MO 189

Landfill impact zone on soils using structural and functional modifications of microbial communities

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An urgent problem is the identification of zones of actual and potential ecological risk from the waste industry. Ecological regulation in Russia uses the scale based on MPC ranking. Effectiveness and informativity of indices used for zoning of the territory adjacent to waste disposal sites are distinct from each other.

We have attempted to perform zoning of the territory adjacent to the landfill (Moscow region), in accordance with variability of the structural and functional parameters of microbial communities. The influence of the landfill as an environmental pollutant on adjacent soils is found to stimulate the soil microbiota development, growth of population, biomass and biological diversity, increased spore fungi biomass, and activation of soil respiration (data received from gas chromatography/mass spectrometry analysis, luminescence microscopy, specimen inoculation on Chapek agar media, CO_2 emission).

The methods applied here determine the impact of the landfill at different distances. Based on the degree of difference in the described indices from the background at different locations down gradient of the landfill, we arranged them from decreasing soil sensitivity to leachate pollution as follows: relative population of soil fungi calculated directly (luminescence microscopy) or by inoculation; total fungi biomass > soil respiration; species diversity of bacterial biomass; total bacterial biomass > spore fraction in the fungal biomass.

To generalize the obtained data and zone the territory, the disturbance index was calculated, representing the deviation of the biotic indices population in the studied samples from the background.

If the total soil pollution by heavy metals at 950 m (in the predominant direction of pollutant migration) is permissible, a 30% difference from the background (conforming with the norms for total indices) can be detected by biological methods only at 1500 m from the landfill, i.e., outside the sanitary protection zone of the landfill (500 m).

MO 190

Detoxifying action of humates towards phosphogypsum

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Phosphogypsum (PG) is a side product in industrial production of phosphoric acid, which is obtained by a treatment of phosphatic raw materials or apatites with a mixture of sulfuric and phosphoric acids. Due to high content of nutrients (calcium, phosphorus and sulfur) it is sometimes used as a fertilizer for poor unfertile soils. But besides nutrients, PG also contains toxic impurities, such as fluorine, strontium and some others. One of contemporary tools to reduce the ecotoxicity of polluted soils and soil-PG mixtures is an application of humic substances (HS). In nature HS are formed in soils, natural waters, bottom sediments, peat and lignite. Nowadays industrial companies produce HS or humates from organic raw materials, mostly lignite and peat. Being the natural organic high-molecular polymers of irregular structure, humates have been showed as substances capable to decrease the toxic effects of pollutants in soils.

The objective of this study was to evaluate the detoxifying ability of a number of humates towards PG applied to model soil mixtures.

Materials and methods. Model soil mixtures (MSM) contained 20% of kaoline, 10% of peat and 70% of sand (ISO 11268-1). PG was carefully mixed with MSM at concentration 3.3 and 7.5%; and humates from peat (Pe-FlexK, Pe-EcoK), coal (BC-EnK, BC-HumNa) and lignosulphonate (OW-LhK) were applied as 0.005 and 0.020% water solutions. Obtained mixtures were incubated during 10 days at 25°C and the ecotoxicity was evaluated using three standardized test-reactions of organisms from different trophical levels and taxonomic groups: microalgae *Scenedesmus quadricauda*, crustaceans *Daphnia magna*, and higher plants *(Sinapis alba*). Toxicometric parameters (EC_{50} and no observed effect level NOEL) and detoxification factor D were calculated.

Results. Analyses of average detoxification factors D_{av} showed that influence of humates depended both on PG concentration and humates' nature. Thus, application of PG at concentration 7.5% was toxic for all the test-cultures at all treatments and humates were not able to decrease the toxicity. At lower rate of PG (3.3%) some humates decreased the toxicity (D_{av} is 0.4-0.8), whether other did not. Among all humates tested the highest detoxifying ability was observed for two samples: Pe-FlexK from peat and BC-EnK from coal. For these humates EC50 and NOEL values increased in 1.3 - 1.8 times for *S. quadricauda*, 1.1 - 1.4 times for *D. magna* and 1.6 - 2 times for *S. alba*.

MO 191

Large Hg pollution of surface waters and sediments due to a chloro-soda industrial plant located in the northeast of Italy

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Since 1979, a chloro-soda industrial plant located in the northeast of Italy discharged an amount of Hg comprised between a minimum of 25 and a maximum of 7200 kg per year. A severe surface water and sediment Hg contamination resulted in the surrounding environment, reaching a contamination peak of 300 g per kg of dry sediment. Research pursued by scientists demonstrated sediment pollution in those rivers and water bodies receiving such industrial wastewaters. Pollution migrated downstream, soon reaching a large lagoon close to the Adriatic Sea. Lagoon mussels and fishes were effected, and probably also those of Adriatic Sea as well. Being the lagoon environment naturally suffering Hg presence, a special analytical technique was necessary to distinguish between anthropogenic pollution vs. natural background Hg. Such technique, called 'Cavelli Method', allowed to precisely identify which lagoon areas were effectively polluted by Hg of industrial origin. After 1989, a higher environmental sensibility produced a drastic reduction of Hg output, ensuing 10-fold lower emissions. This showed the effectiveness of pollution containment policies and regulatory enforcements as well as the feasibility of environmental recovery.

MO 192

Effects assessment of cobalt in the terrestrial environment

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The toxicity of metals in soils is affected by a number of factors such as species sensitivity, soil physicochemical properties, and ageing processes. We conducted an evaluation of Co toxicity to a variety of terrestrial organisms in soils; specific consideration was given to the effect of soil properties and ageing on Co bioavailability and toxicity. In total 127 individual reliable chronic toxicity studies with Co were identified from the literature or from our own empirical studies. Available chronic data included a total of 7 higher plant species (covering both monocotyledons and dicotyledons), 4 invertebrate species (covering both arthropods and non-arthropods) and 3 microbial processes (for both C- and N-cycles). Chronic toxicity data were identified from studies run in 13 separate soils displaying a range of soil properties typical of those found throughout Europe, and after varying equilibration times up to 12 months after spiking with $CoCl_2$. In general, higher plants were found to be most sensitive to Co in soils, while microbial processes were least affected. Laboratory studies showed a 10- to 100-fold variation in chronic toxicity among the various soils tested. Chronic Co toxicity best correlated with the effective CEC (eCEC) of the soil, with toxicity decreasing as a function of increasing eCEC. Linear regression models based on eCEC explained between 59-97% of the variability in Co EC50 for the terrestrial organisms. Ageing of Co-amended soils resulted in decreased Co bioavailability and chronic toxicity. However, the mitigating effect of ageing was noted to decrease with decreasing pH. This observation was confirmed by changes in availability of Co in the soils with time determined by an isotopic dilution procedure. After correction for differences in bioavailability of Co among the available chronic toxicity data, soil-specific Predicted No Effect Concentrations (PNEC) were derived from a species sensitivity distribution. Cobalt PNEC values ranged between 5 and 50 mg Co/kg soil for relevant soil conditions in Europe.

MO 193

Effects assessment of boron in the terrestrial environment

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Boron is a naturally occurring trace element that is essential to a variety of organisms. This poster will describe the available data considered in developing a soil PNEC for boron using the REACH framework. In total, more than 200 reliable individual NOEC or EC10 values were selected for 28 different plant species (covering monocotyledons and dicotyledons), 9 different invertebrate species (including worms, nematodes, collembola, insects and mites) and 2 microbial processes. Plants are generally the most sensitive group to added boron. These data supported the development of a species sensitivity distribution (SSD) and estimation of the HC5 value. Data screening based on relevance was found to be as important as screening for study quality (e.g., Klimisch score) in the evaluation of boric acid and sodium borates. Much of the published research is focused on avoiding B deficiency for crop plants and other publications reported tests in hydroponic or sand/water conditions. Although many of these studies are scientifically valid and perhaps technically stronger than standardized ecotoxicity studies, with longer duration, additional endpoints and testing under field conditions, these data were excluded from the SSD. However, some of these data were still used as supporting evidence in the uncertainty analysis on the HC5 value. Some plant data suggested a narrow range between deficiency and toxicity of boron in soil, with beneficial effects on some species occurring at concentrations associated with adverse effects in others. The bioavailability of many elements varies substantially with soil properties. Limited studies with plants and boron added to soil suggest that added boron will likely be both bioavailable and mobile. Soil properties seem to modify these properties relatively little in the short term and, in general, are less than the variation among species. Bioavailability was therefore not used to modify the effects assessment. An added risk approach was selected because of the narrow range between deficiency and toxicity of B in soil, and the significant difference in solubility between added B and B naturally present in soils.

MO 194
Effects of silver on the terrestrial isopod Porcellionides pruinosus
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Silver is a trace element that occurs at low levels in the environment. However, silver contamination has increased due to anthropogenic inputs, such as industrial activities and sewage treatment plants. As the bioavailable silver can be toxic, studies are required to predict the potential risks in soils. Terrestrial isopods are suitable indicators for assessing the ecotoxicological risk of metals in soils due to their extraordinary capacity to bioaccumulate toxic metals from the environment in their body (i.e. hepatopancreas). In this study, we aim to assess the toxic effects of silver (AgNO3) on the terrestrial isopod Porcellionides pruinosus. For this purpose survival, avoidance behavior and feeding activity endpoints were analyzed. For the survival and avoidance tests, Lufa 2.2 soil was used as exposure medium and lethal effects were observed after 7 and 14 days and avoidance after 48 hours. For the feeding inhibition test, a mixture of finely-ground alder leaves and Ag-contaminated gelatin were selected as a suitable food substrate to expose the isopods for 14 days. The lethal concentration for 50% of the isopods (LC50) was 726 mg Ag kg-1 dry soil after 7 days, which decreased to 398 mg Ag kg-1 soil after 14 days. In the avoidance test, the avoidance concentration for 50% of the animals (AC50) was 8.79 mg Ag kg-1 dry soil. In the exposure to Ag via food, a significant impairment of feeding parameters (e.g. food consumption, assimilation and excretion) was observed during the 14 day exposure, but data still have to be analysed. The avoidance endpoint was more sensitive to Ag exposure than lethality. Feeding impairment might be an indication for ecosystem disruption by Ag contamination.

MO 195
Life table response experiments with two species of Collembola
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Collembola are an integral component of soil ecosystems as decomposers of organic matter or part of the food chain and are vulnerable to effects of soil contamination. Their presence in all types of soil and their importance to soil biology as well as their ease of handling has made them an important and widely used species for ecotoxicological testing. Standard experimental procedures currently focus on reproduction or survival over a short time period starting with half mature juveniles. Both growth and reproduction, as well as survival, of the organisms are essential for maintaining populations, and therefore biological impacts of soil contamination should be investigated from a life-cycle perspective.
Here we present results of life table response experiments performed on two species of Collembola that differ in their reproductive strategy. Cohorts of parthenogenetic *Folsomia candida* and sexually reproducing *Sinella curviseta* were exposed to a heavy metal via soil, soil surface and food on a plaster of Paris mixture in different test setups. The experiments are easily conducted and enable constant observation of the organisms and analysis of additional life-cycle variables to gain insight into the individual-level mechanisms resulting in population-level impacts. Simple population models were used to derive population growth rate as a summary statistic to measure toxicant induced fitness reduction and to link individual performance of the springtails to population dynamics. Outcomes from elasticity analysis on the population growth rate suggest that more attention should be given to the earlier life stages of Collembolans during ecotoxicity testing given the importance of these life stages for population dynamics.
The advantages and limitations of the experimental methods and different exposure routes will be addressed. The results, their implications for testing different life histories and implications for further experimental work are discussed.

MO 196
The toxicity of silver (Ag) to soil-dwelling organisms: plants, earthworms, springtails and soil micro-organisms
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To support the Federal Government of Canada's Chemical Management Plan, soil toxicity tests were conducted to evaluate the inherent toxicity of silver (as silver nitrate) to a suite of terrestrial species, as well as the soil microbial community. Silver was identified as a medium priority inorganic substance requiring further evaluation from a screening assessment perspective, due to uncertainties relative to environmental persistence, bioaccumulation and inherent toxicity. The lethal and sub-lethal toxicity of silver was determined for the earthworm, Eisenia andrei, then collembolan, Folsomia candida, as well as two plant species (Trifolium pratense and Elymus lanceolatus). The tests were conducted according to standardized Environment Canada soil toxicity test protocols, using a field-collected sandy-loam soil. The effect of this substance to the soil microbial community (e.g., microbial activity, biomass and temporal shifts in the soil microbial community) was also investigated using the same soil type. The data derived from this research project will supplement and verify the PBiT (e.g., inherent toxicity) status of this compound, and will support Canada's effort in the screening assessment of this medium priority compound.

MO 197
The use of earthworm metal content in highest tiers of a site environmental risk assessment in the tropics
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The highest tiers of an environmental risk assessment should involve accurate site investigation on the risk of contaminants for specific routes of exposure. The results that will be presented were obtained in an environmental risk assessment carried out in an abandoned lead smelter in Santo Amaro, Brazil, which is an area contaminated predominantly with copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn). In this assessment, metal-contaminated soils were collected along two transects making a gradient of natural metal-contaminated soils, starting from a central point located next to smelter facility and up to 3Km. Aiming to obtain information on the bioavailability and bioaccumulation of metals to earthworms in the study area, five adults of Eisenia andrei were exposed to some of the metal-contaminated soils collected, three reference natural soils and the OECD artificial soil for 6 weeks under laboratory conditions. At the end of this period, the concentrations of Cu, Cd, Pb, and Zn were measured in soil and earthworms (using 69% HNO3 extractions) by AAS-flame. In reference soils, the concentrations of Cu, Cd, Pb, and Zn in earthworms were on average 11 ± 3, 3 ± 0.5, 21 ± 7, and 134 ± 14 mg/kg, respectively. In metal-contaminated soils the highest concentrations of Cu, Cd, Pb, and Zn in earthworms were on average 219 ± 454, 251 ± 34, 1049 ± 235, and 839 ± 338 mg/kg, respectively. Results demonstrated that lead was the most bioavailable metal as expected, followed by Zn. It was also evidenced that there is risk for higher trophic levels up the food chain. In this contribution, the sensitivity of this parameter and its value in the TRIAD within a site ERA scheme are discussed.

MO 198
Higher tier assessment of a metal contaminated area in the tropics: the impact on leaf-litter breakdown
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Litter bag test is considered the most appropriate method available for assessing organic matter breakdown in semi-field or field conditions. As a part of an environmental risk assessment carried out in an abandoned lead smelter in a tropical area from Santo Amaro, Bahia, Brazil, presenting high levels of metal contamination, this test was used in the higher tier assessment, as part of the Ecological LoE of a TRIAD approach. Percentage of remaining mass loss of Schinus terebinthifolius Raddi (Anacardiaceae), a native tree species, was determined in contaminated and reference sites. A total of 192 leaf-litter bags were exposed in October 2009 on the soil surface in all the 12 sampling points considered. Four leaf-litter bags from each plot were collected randomly at each period (15, 43, 83 and 131 days). Treatment of samples, calculations of results and correction factors were carried out according to OECD 2006 draft protocol. When comparing to reference sites, results showed the impairment of leaf-litter decomposition in sites inside the smelter area (most contaminated sites), which may pose a risk to ecosystem functioning. Besides the direct effects of metal contamination, this functional endpoint reflects the damage to the overall ecological status of the site, probably originated by physical characteristics of tails deposits, such as low water holding capacity and low humidity, low vegetation cover and consequently loss of suitable habitat for soil invertebrates and microorganisms. In this contribution, the sensitivity of this parameter and its value in an ERA scheme are discussed.

MO 199
A novel ecotoxicological method: the effect of cadmium on pathogen resistance of Arabidopsis thaliana
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Ecological risk assessment method has not yet been established. Although there is rising public concern that the contaminants in soils might affect ecosystem as well as human health, ecological risk assessment method has not yet been established. The technical barrier to establish risk assessment is to define endpoints that can detect the effect of contaminants in soil quantitatively.
Among soil organisms, plants are responsible for primary productions for all the life on earth. A decrease of plants causes severe repercussions on whole ecosystem. Developing the ecotoxicological methodology using should have a higher priority.
We have been trying to detect the effect of cadmium in various points on Arabidopsis thaliana and found they are susceptible to pathogens. In view of this, we made the assumption that the contaminants in soils may effect on the pathogen resistance of plants and it could cause adverse impacts on ecosystems.
In this study, we try to develop a method to assess the pathogen resistance quantitatively using Arabidopsis thaliana exposed to cadmium. In order to assess pathogen resistance, we inoculated the plant pathogen, Alternaria brassicicola, onto the leaf of cadmium-exposed Arabidopsis thaliana, and then investigated the relationship between cadmium exposure and incidence and extent of the disease. Incidence of the disease was found to be higher in cadmium exposed-plants than in unexposed group. Also there was a dose-dependent increase in necrotic leaf area. These results show that cadmium exposure reduced pathogen resistance of Arabidopsis thaliana. Thereby, we defined "Pathogen resistance factor"to perform a quantitative assessment of effect of cadmium on pathogen resistance of Arabidopsis thaliana. Pathogen resistance factor is expressed as a quotient of the necrotic area ratio to the whole leaf area exposed to cadmium divided by that of unexposed. Pathogen resistance factor decrease to 0.37 and 0.23 by 0.5 and 1 microM cadmium exposure, respectively.
Pathogen resistance factor could be used for quantitative ecotoxicological assessments.

MO 200
Genotoxic effects of Boron contaminated sediments from Cecina basin (Tuscany) on Vicia faba L.
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In this work a Boron contaminated sediment from the Cecina basin in Tuscany (Italy) was considered for its possible genotoxic effect on plants. The greatest part of Cecina basin is located in the geothermal zone of Larderello, where for about a hundred years the mine activities in this area and more recently the geothermoelectric industry spilled in the Possera and Pavone creeks (tributaries of Cecina River) until about 1975. However, contamination is still present in certain areas of the basin with high level of B.
Two samples with different B content were used in phytotoxicity studies: A) with high B concentration of 108 mgkg-1 as total and 43.3 mgkg-1 as bioavailable, B) with 69.8 and 22.8 mgkg-1 respectively as total and bioavailable content.
Previous studies demonstrated that both excess or deficiency of B can affect normal plant growth and as a consequence agricultural production thus the effects of B contaminated sediment were analyzed considering seed germination, root elongation and mitotic division in root tip cells of Vicia faba L., which is commonly used for detecting the genotoxic effects of environmental pollutants.
Seeds of Vicia faba L. were germinated in contaminated sediment and cytological analysis and root tip micronucleus assay (MNC), were performed after 3 days (primary roots) and after 10 days (secondary roots) for genotoxicity evaluation.
The results clearly indicated B toxicity since the length of the primary root and the size of Vicia plants were significantly reduced when compared to control plants. Cytological analysis on primary root tips (3th day) showed no effect on mitotic activity of meristematic centers but a big presence of micronuclei was found (a frequency of 5% of micronuclei in contaminated soils compared to 1% in control). Moreover mitotic anomalies, as chromosome bridges, chromosome lagging and c-metaphases, were found. The analysis on secondary root tips at 10th days from germination confirmed the presence of mitotic anomalies at a higher extent and different behavior of meristematic center was observed since mitotic index (MI) was reduced in root tips from B contaminated soils (MI = 9,3%) when compared to control (MI = 17,7%) but no micronuclei were found. This results indicated the phytotoxicity of B contaminated sediment of polluted area of Cecina. B concentration in soil is a very critical point and more attention should be done on environmental contamination by industrial activity and wastewater pollutants.

MO 201

Earthworms' in situ exposure and evaluation of genotoxicity endpoints to assess the toxicity of uranium mining wastes

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The exploitation of radioactive ore in Portugal lasted from 1908 to 2001, and took place mainly in the centre-north of the country (Carvalho et al. 2009a). The uranium extractive industry generated considerable volumes of wastes often containing high concentrations of toxic metals and radionuclides (Antunes et al. 2008a). Toxicity assays conducted under controlled laboratory conditions, lack the integration of natural fluctuating environmental conditions (Moreira et al. 2005). Consequently, it could be difficult to extrapolate laboratory results to field situations, especially when radioactive environments are considered. By integrating natural fluctuating environmental conditions, in situ assays allow more realistic exposures (Moreira et al. 2005).This study aimed to assess the applicability of the earthworms reproduction assay (OECD 2004) and the evaluation of the geno and citotoxicity endpoints in in situ exposures of Eisenia andrei Bouché to radioactive uranium wastes. In parallel, the bioaccumulation of metals and radionuclides by earthworms was also assessed, to clarify cause-effect relationships. Earthworms were exposed in situ, to a contaminated soil from the Cunha Baixa uranium mine and to LUFA 2.2 soil (control soil). Organisms were sampled from the assay chambers after 0h, 24h, 48 h, 7 days, 14 days and 56 days of exposure. Higher DNA damage, detected by comet assay and flow cytometry, was recorded in earthworms exposed to the contaminated soil. The measurement of cell frequency, median fluorescence intensity (MFI), DNA content and coelomocytes proliferation, by flow cytometry, revealed changes in earthworms immune system and toxicity to coelomocytes. Earthworm's biomass was significantly decreased and the reproduction was also significantly affected in earthworms exposed to the contaminated soil, as cocoon production was completely inhibited in these organisms. Earthworms also showed bioaccumulation of metals and radionuclides. These results suggest that earthworm's reproduction assay and the determination of genotoxicity and cytotoxicity endpoints are applicable to in situ exposures and may constitute powerful tools for environmental risk assessment of radioactive mining areas.

MO 202

Transcriptional regulation of metallothionein and catalase in Eisenia fetida artificially exposed to cadmium in OECD soils with different concentrations of organic matter

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Exposure of earthworms to metal contaminated soils, results in the regulation of the transcription of a set of genes, which are considered to be molecular biomarkers of exposure to metals. Metallothioneins (MTs), are proteins in charge of sequestering and detoxifying non-essential metals, and regulating concentrations of essential ones, in all animals studied to date. Thus, the transcriptional induction of MTs has become a biomarker commonly used to study the quality of soils. Some metals, such as Cd, also regulate the activity of enzymes such as catalase (CAT), that participate in the antioxidant defense against the oxidative stress generated by the exposure. Traditionally, ecotoxicological assays, both acute exposure and sublethal effect assays, are carried out using standard soils, such as OECD soils (1984), with 10% organic matter (OM) content. But real soils can be very different in their OM content, this modulating the bioavailability of contaminants and their derived biological effects. The present work aimed to analyze the transcriptional regulation of MT and CAT by Q-PCR, in earthworms (*Eisenia fetida*) exposed to Cd in OECD soils with different content in OM. With this purpose, earthworms were exposed to 5 and 25 mg Cd/kg in artificial soils with 6, 10 and 14% OM during 3 days. Moreover, we did analyze the differential regulation of studied genes in different body regions along the postclitellar anteroposterior axis. MT was significantly upregulated in a Cd concentration dependent manner, both in soils with 6 and 10% OM, but not in animals in soils with 14% OM. On the other hand, CAT was only significantly downregulated in earthworms in the spiked soils with 10% OM. No significant differences were observed when comparing transcription levels in the anterior and posterior part of the worm bodies, but in the case of CAT in animals exposed to 5 mg Cd/kg only the anterior part showed a significant downregulation. Therefore, the studied gene transcriptional levels point the need to consider concentrations of organic matter in natural soils when trying to interpret results obtained with biomarkers applied on sentinel earthworms.
Funded: Basque Government (ETORTEK BERRILUR IE09-242, INZITOX UE09+/58 and Grant to Consolidated Research Groups GIC07/26-IT-393-07) and BFA (LURTXIP Project). A.I. is recipient of a pre-doctoral fellowship from the Basque Government.

MO 203

Assessment of plant protection products on soil communities under Mediterranean conditions: field evaluations

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Complying with the Sixth Community Environment Action Programme an integrated approach to assess the risk (both fate and effect aspects) of plant protection products on soil communities under Mediterranean conditions was put into practice. Our aim was to contribute to increase knowledge about ecological scenarios under Mediterranean conditions where soil characteristics, climatic conditions and biota are different from those under central and northern Europe. The effect assessment component presented here embraced different levels of environmental complexity: (1) a first-tier comprising laboratory single species tests with terrestrial organisms with 3 model pesticides (chlorothalonil, ethoprophos, azoxystrobin) selected based on the result of acceptance in inclusion in Annex I of Directive 91/414/EEC and as being regularly used in Portuguese model crop systems (potato, maize, onion); (2) and a higher tier comprising field testing of indigenous edaphic communities in a selected study-site located in an intensive crop area and with high irrigation needs neighbouring protected wetlands.
This field study comprised a complete crop cycle with multiple pesticide applications and fertilization under realistic conditions. Sampling was done between Mach and October 2010 in several parcels, both in-crop and off-crop. The organisms were collected using several methodologies (embracing soil macro and mesofauna and soil fauna feeding activity), and samplings were undertaken according to pesticide and fertilizer application. The first data on this field evaluation is presented in this panel. The results from this higher tier (reflecting a realist scenario and thus considered as a reference tier defining protection levels for the lower tier) will be compared with those obtained from the lower tier to evaluate its level of protection towards soil organisms. This analysis will contribute to a more realistic pesticide risk assessment considering the different levels of complexity of agricultural ecosystems.

MO 204

Soil respiration under the fungicide Captan and the bactericide Bronopol revealed different stability of fallow and meadow soil microbial communities

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Due to socio-economical transformations that Poland underwent in the last 20 years, major changes in the land use have taken place in the country. Lowering cost-effectiveness of agricultural production led to a huge increase in the area of abandoned farmlands. Such fallows undergo rapid secondary succession and are examples of unstable ecosystems under changes. The successional changes in plant communities are expected to be reflected in soil microbial communities, offering an interesting system to study stability of communities.
For this reason soil microbial communities from abandoned agricultural lands were compared to communities from rich and well maintained meadows. In this work their resistance and resilience to the bactericide Bronopol and the fungicide Captan was evaluated. Six fallows and six meadows were included in the investigation. Soils were spiked with Captan and Bronopol in laboratory conditions at a rate 5.4 mg a.i. per g dry soil, what corresponds to a concentration approximately 50 times higher than recommended for Captan. Soil respiration rate was measured weekly for six weeks. At the end of the experiment glucose solution was added to soils and active biomass was estimated.
Fallow and meadow soils exhibited opposite reaction to Bronopol in terms of shape and time of changes. The respiration rate of meadow soils increased one day after the amendments and afterwards decreased to levels lower than in respective controls. In contrast, the respiration rate in fallows peaked one week after the treatment and remained elevated in comparison to control throughout whole incubation time.
The shape and timing of response to Captan were similar in meadows and fallows, with an initial increase in the respiration rate followed by a decrease. The communities differed, however, in the intensity of response, which in case of meadows was stronger.
The resilience to the two biocides did not differ significantly, and in 42 days both systems came back to their ground state.
Glucose-active biomass was significantly lower in soils treated with biocides than in respective controls with no difference between the ecosystem types or the pesticides.
In this research the different stability and resistance of soil microbial communities originating from two types of ecosystem was proved. In case of the fungicide only the meadow microbial communities were affected negatively, and in case of the bactericide a significant interaction was found between time and soil origin.

MO 205

Cellulose decomposition in fallow and meadow soils amended with the fungicide Captan and the bactericide Bronopol

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Due to socio-economical transformations that Poland underwent in the last 20 years, major changes in the land use have taken place in the country. Lowering cost-effectiveness of agricultural production led to a huge increase in the area of abandoned farmlands. Such fallows undergo rapid secondary succession and are examples of unstable ecosystems under changes. The successional changes in plant communities in such areas are expected to be reflected in soil microbial communities, offering an interesting system to study stability of communities.
For this reason soil microbial communities from abandoned agricultural lands at the initial stage of plant succession were compared to communities from rich and well maintained meadows. In this work their resistance and resilience to the bactericide Bronopol and the fungicide Captan was evaluated. Six fallows and six meadows were used in the investigation. Soils were spiked with Captan and Bronopol in laboratory conditions at a rate 5.4 mg a.i. per g dry soil, what corresponds to a concentration approximately 50 times higher than recommended for Captan. Following the treatment, cellulose decomposition was measured in laboratory conditions. Amended and control soils were put into Petri dishes and three stripes of cellulose filter paper 10 x 50 mm were placed on the moistured soil surface. Vanishing of the cellulose was measured once a week for 8 weeks by the mean of visual measuring via a transparent template. For each soil treatment

study two replicates were used and readings were done by two people independently.
Cellulose was degraded significantly faster in fallow soils than in meadows, especially in the first weeks. Both pesticides decreased the decomposition rate. Captan inhibited the process almost entirely, which conforms with the commonly accepted fact that fungi are the main cellulose decomposers. Nonetheless, in some soils the cellulose decomposed even when amended with the fungicide. Bronopol slowed down cellulose decomposition at first, but with the time the decomposition rate recovered.
The research showed that the degradation of cellulose is carried out faster and more efficiently in fallows. The bactericide Bronopol did not break this relation. Although soils from both ecosystem types were similarly sensitive to the fungicide Captan.

MO 206
Does the current EU chronic earthworm risk assessment scheme for plant protection products (PPPs) show an appropriate level of protection?
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In Annex VI to the EU Directive 91/414/EEC a trigger of ≥ 5, based on the NOEC of the earthworm reproduction study was set as trigger for the long-term risk assessment of PPPs. The ECPA Soil Organisms ad hoc Team analysed the results of earthworm field studies that were triggered due to failure of this chronic earthworm EU Annex VI trigger of 5 and assessed 17 earthworm field studies that were conducted during the last 5 years in line with the technical recommendations for the update of the ISO earthworm field test guideline (ISO 11268-3) (Kula et al. 2006). In all 17 earthworm field studies comprising 10 fungicides, 4 herbicides and 3 insecticides no unacceptable effects on natural earthworm populations were identified at least 12 months after treatment. This is well in line with the data analysis conducted by Heimbach (1998) and Barber et al (1998). Thus it can be concluded that the current chronic EU earthworm risk assessment for PPPs using the NOEC from the chronic laboratory earthworm study and a maximum PECsoil on the basis of 0 to 5 cm soil layer combined with the EU Annex VI trigger value of 5 is conservative and protective. In fact many unnecessary earthworm field studies are being triggered, confirming the safe use of PPPs for earthworms.

MO 207
Reproduction, behaviour and biochemical responses in Enchytraeus albidus (Oligochaeta) exposed to different pesticides
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Enchytraeus albidus are typical inhabitants of a wide variety of soil types and fulfil important functions such as improvement of the soil pore structure and, indirectly, the degradation of organic matter. These organisms are very sensitive to chemicals and proved to be suitable for environmental risk assessment through the determination of reproduction and survival endpoints (ISO No. 16387, 2003). The avoidance behaviour is also of great ecological importance and can give useful information about the ability of E. albidus to escape from a contaminated soil. To assess the overall quality of the terrestrial environment and better understand the mechanistic bases of chemical action, increasing emphasis has been given to stress responses at lower levels of biological organization. The antioxidant defenses are among the most used biomarkers due to their crucial role in cell homeostasis preventing DNA damage, enzymatic inactivation and lipid peroxidation. Cholinesterases are also important and commonly used biomarkers, namely acetylcholinesterase involved in neuro-muscular function of organisms.
The main goals of this study were: 1) to investigate the effects of different pesticides on survival, reproduction and behaviour of E. albidus; 2) to assess the oxidative stress effects at concentrations that affected survival and reproduction. Additionally, the effects of exposure time (2, 4, 8, 14 and 21 days) on the biomarker responses were investigated. Survival, reproduction and avoidance behaviour assays were performed according to standard guidelines. The biochemical parameters measured in E. albidus whole body were lipid peroxidation (LPO), glutathione redox status, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST) and cholinesterases (ChE).
The results showed effects on survival, reproduction and avoidance behaviour. Reproduction was the most sensitive endpoint. The biochemical responses were affected by the pesticide exposures in the different time points.
This study shows that the determination of effects at different levels of biological organization allow going further on understanding the mechanisms of toxicity that may contribute to mortality and/or reproduction impairment by pesticides.

MO 208
Will juveniles, pre-adults and adults respond in the same way to chemical exposure? The study case of dimethoate.
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In a field situation, organisms are not exposed to a stressor under similar age, growth or moulting stage or even in a determined gender ratio.
A high number of studies focus on the effects of a specific stressor in a specific growth stage (adults or juveniles) mainly due to the fact that juveniles are more sensitive then adults, and that effects on adults will always be related to reproduction and the decrease of the population. Pre-adult or juvenile organisms are not so often used in terrestrial ecotoxicological studies and their response can also be very important and crucial for ecosystem dynamics.
Taking in consideration the three growth stages described before (juveniles, pre-adults and adults), and that to our knowledge no data as been yet published regarding these three stages and their inherent physiological processes, we studied the potential recovery process in all growth stages, regardless the lower or higher chronic effect induced by a specific stressor.
The study consisted in a total of five replicates per age range corresponding to a plastic box with 20 isopods during a 7-day exposure period. Organisms belonged to three different stages (juveniles, pre-adults and adults) were exposed to 10 mg dimethoate/kg soil (concentration below EC50).
Biomarkers (e.g. AChE, LPO, GST, CAT, etc.), energy reserves content (lipids, carbohydrates and proteins), energy consumption and cellular energy allocation (CEA) were determined after exposure.
The present work showed that the response in biomarkers is not equal for all age stages and the detoxification processes do not respond or evolve in the same way.

MO 209
The effect of 1,4-dichlorobenzene on soil organisms
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1,4 - dichlorobenzene is hazardous substance belonging to the group of chlorinated aromatic hydrocarbons which is present in all components of environment. It is a raw material commonly used in industry for example for producing pesticides. Especially the industrial activity, application of sludge on agricultural land or atmospheric deposition are the cause of pollution 1,4-DCB of particular components of environment. Ecotoxicity data for 1,4-DCB for aquatic environment can be found in the literature but there is an absence of information for terrestrial environment. The main aim of this study is to determine sensitivity of soil organisms compared with tested substance 1,4-DCB using a set of contact toxicity tests. The same concentration range of 1,4-DCB 125, 250, 500, 750 a 1000 $mg.kg^{-1}$ was selected for all tests. Toxicity was determined by the help of a set of four terrestrial tests: determination of effects on reproduction and survival of Enchytraeidae (*Enchytraeus crypticus*), further avoidance tests with Enchytraeus crypticus, inhibition of reproduction of Collembola (*Folsomia candida*) and the last one is the determination of effect of pollutants on lettuce seedlings (*Lactuca sativa* L.). This study is in the same time a part of next research which is related to the characterization factors in the methodology CML-IA (CML impact assessment) because 1,4-DCB is one of reference substances used in the methodology CML-IA for LCIA (Life Cycle Impact Assessment) in analysis LCA (Life Cycle Assessment).

MO 210
Effects of petroleum hydrocarbons on photosynthetic plant physiological parameters: Clorophyll Fluorescence and Stomatal Conductance
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Biochemical and physiological changes due to contaminants can be detected earlier than morphological changes. Plant physiological functions such as photosynthesis and stomatal conductance are useful tools to study the effects of environmental stress on plants. Photosynthesis evaluated by the variable chlorophyll a fluorescence and stomatal conductance are considered very sensitive biomarkers when plants have been exposed to pollutants. These functions have been widely applied to study effects caused by water deficit, temperature, nutrient deficiency, etc. Their use in determining effects due to chemicals is more limited and mainly addressed to metals and herbicides.
The purpose of this study was to assess the effects of petroleum hydrocarbons on photosynthesis responses and stomatal conductance. Moreover, their application as non invasive measurement endpoints to assess soil contamination was evaluated. Two hydrocarbon mixtures: diesel fuel and mineral oil, were studied in a dose-response assay with two plant species (Cucumis sativus and Zea mays). The plants were sown on the contaminated soil or germinated in a nursery one week before transplanting to the contaminated soil. The objective was to compare the effects on plants with a fully developed photosynthetic apparatus and plants with a developing photosynthetic apparatus to hydrocarbons stress. In this way, the influence of the hydrocarbon stress on the developing photosynthetic apparatus could be determined. The effects on chlorophyll fluorescence and stomatal conductance were compared with effects on seedling emergence, plant growth and leaf chlorophyll.
The diesel-contaminated soil were more toxic than mineral oil-contaminated soil.
The sown plants showed higher inhibition in all parameters than transplanted plant, indicating the response of the developing photosynthetic apparatus to petroleum hydrocarbon stress. Cucumis sativus had the highest response in all measured parameters with the exception of stomatal conductance, where Zea mays showed the highest inhibition. In general, effects of contaminants were higher on chlorophyll than on PI, a fluorescence parameter that comprises three main energy fluxes in PSII: absorption, trapping and electron transport.
The usefulness of chlorophyll fluorescence and stomatal conductance as endpoints of toxicity will be conditioned by contaminant type, plant specie and the state of plant development.
This work was funded by Madrid Community through EIADES Project.

MO 211
Soil quality assessment using ecotoxicological and enzymatic tests during monitored natural attenuation of hydrocarbon contaminated soils
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Monitored Natural Attenuation (MNA) is one of non destructive techniques that can be applied in cases of hydrocarbon contaminated soils. However, environmental conditions have a great influence on microbial activity and consequently in contaminants bioavailability during the process. The aim of this work was the evaluation of soil quality treated by MNA after a period of 12 months, 15 months and 18 months (T12, T15, and T18) using as biological indicators an ecotoxicological test with a soil organism (collembolan) and an enzymatic test (soil dehydrogenase activity). Soil samples contaminated with crude oil in concentrations of 0.5%, 2.5%, and 5.0% (w/w) were left in the open subject to weather (exposure to sunlight, rainfall, and ambient temperature) in Rio de Janeiro City, Brazil. The ecotoxicological test applied was avoidance behavior with springtails [ISO 17512-2 - Soil quality - Avoidance test for testing the quality of soils and effects of chemicals - Part 2: Test with collembolans (*Folsomia candida*) - draft] that allows a preliminary assessment in a short period of time, with high degree of sensitivity. Adult springtails were exposed simultaneously to the control soil and the test soil. These soils were introduced into each test vessel in opposite sides and after an incubation period of 2 days, the number of springtails was determined in each side of the vessels. The mean number of individuals at the end of the test in the test soil was compared to the mean number of individuals in the control soil using Fisher Test. Control soils were an artificial tropical soil, prepared as ABNT NBR 15537:2007 (Brazilian Standard Rule), and the natural soil without contamination. The enzymatic test was based on the use of Triphenyltetrazolium Chloride (TTC Method). The results showed that samples treated after the period of 12 months were no toxic for the springtail in the avoidance behavior test for all the concentrations, while in T15 and T18 the results for 2.5% and 5.0% concentrations indicated toxicity (avoidance behavior). Simultaneously, it can be noted that in T12 the soil dehydrogenase activity was lower than in 15 and 18 months of treatment. These results

may be related to the increase of rainfall in T15 and T18, influencing the bioavailability of the contaminants.

MO 212

Comparison of bioassay- and chemical analysis in risk assessment of PAH-contaminated remediated soils

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Contaminated sites are a worldwide problem. Many of the sites are polluted with chemicals that are harmful to the environment and humans. In Sweden some of the most contaminated areas are being remediated. Polycyclic aromatic hydrocarbons (PAHs) are common in soil at industrial areas and generic guideline values for soil use after remediation are based on the 16 EPA priority pollutants, which only constitute a small part of the complex cocktail of toxicants in many contaminated areas. The purpose with this study was to elucidate to what extent the actual toxicological risks of soil samples from successful remediation projects could be reflected by these PAHs. In this study we therefore compared chemical analysis (GC-MS) and biological analysis (H4IIE-luc) of a number of remediated PAH-contaminated soil samples. The H4IIE-luc bioassay is an Ah receptor based assay that detects all compounds that activate the Ah-receptor, one mechanism for PAH toxicity. By mass balance analysis i.e. comparison of bioassay derived TEQs with chemical TEQ based on REPs, it is possible to estimate the contribution of instrumentally identified compounds to the observed response in the bioanalysis. Comparison of the results showed that the bioassay-determined toxicity (Bio-TEQ) in the soil samples could not be explained to more than a fraction by chemical analysis of the 16 priority PAHs. This pattern was obvious in all samples but the proportions differed between the soils. Our conclusions are therefore that there is a considerable risk that the current risk assessment method for PAH-contaminated soil in use in Sweden, based on chemical analysis of selected PAHs, is missing toxicologically relevant PAHs and other similar substances. Further chemical identification studies and bioanalytical studies are needed to determine whether these unknown substances pose a risk to humans or the environment. Our results showed the weakness of chemical analysis of a small number of compounds as the basis for classification and risk assessment of remediated soil samples. It is therefore reasonable to include mechanism-specific tests in risk assessment and in the classification of remediated PAH-contaminated soils. This could minimize the risk these soils can pose to humans and the environment and enable greater safety in subsequent reuse of remediated soils.

MO 213

Relationships between cyclodextrin, SEG, and solvent extracted petroleum hydrocarbon concentrations and the results of toxicity tests with plant, earthworm and soil arthropod species

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Two field-collected soils from Saskatchewan (Canada) were amended with commercially-acquired diesel at concentrations ranging from 100 to 30,000 mg/kg soil dry weight; the test soils were artificially aged and weathered by mechanical mixing twice weekly for three weeks prior to use in toxicity tests with plants and soil invertebrates. At the beginning of the tests, soil subsamples were extracted with cyclodextrin, a solvent mixture of hexane and acetone (1:1 v:v), and an enzyme mixture which simulated the gastrointestinal fluids of earthworms (SEG extraction); the exposure concentrations were measured using GC-FID. Toxicity tests with two species each of plants (*Elymus lanceolatus* - Northern Wheatgrass; *Hordeum vulgare*- Barley) and invertebrates (earthworm - *Eisenia andrei*; springtail - *Folsomia candida)* were conducted to determine the biological responses (survival, emergence, growth or reproduction) to a series of diesel concentrations in soil. Multiple regression procedures were applied to the data for the biological responses (ICps), the physico-chemical characteristics of the soils, and the measured concentrations to determine which of the three different extraction methods predicted (correlation) effects.

MO 214

Vicia faba root tip micronucleus test: adaptation of the method to a direct soil exposure

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The Vicia faba root tip micronucleus test is one of the most employed plant genotoxicity assays, and has been used on various types of contaminated materials. This test has been standardized by AFNOR, the French member organization of ISO. However, this test is usually performed with a water extraction step but soil genotoxicity assessment would be more relevant when performed directly in the soil itself. In order to harmonize these protocols, an ISO standard for the V. faba micronucleus test in both liquid phase (exposure of plants to the water extract of the soil) and solid phase (direct exposure of plants to the soil) would be very useful. In this context, we compared two exposure durations (48h and 5 days) for the V. faba micronucleus test with two different well-known genotoxicants, maleic hydrazide and copper sulphate. We concluded that these two durations induced equivalent sensitivity: the micronucleus frequency was significantly increased with 5 µmol maleic hydrazide per kg dry soil and with 2 mmol copper sulphate per kg dry soil with both exposure durations. However, exposing roots to soil to be tested during 48h is more practical.

We also tested three different soils: two 'natural soils' (lufa 2.1 and lufa 2.2) and an artificial soil according to ISO11268-1 and ISO 11268-2 with the genotoxicant maleic hydrazide 10 µmol per kg dry soil, plants were exposed for 48h. We observed with the three soils cytotoxic and genotoxic effects, these ones were not different between the soils.

The number of replicates has been also studied: three and five replicates gave the same accuracy of the results. Then, the normalisation is proposed with 3 replicates, decreasing the experimental heaviness.

In the framework of standardisation, some more experiment shall be done for testing the use of organic fertilisers with this method. An international ring test will be organised in 2011 to validate the method.

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MO 215

Automated evaluation of ecotoxicologic test systems by image analysis

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Analysis of ecotoxicologic test systems frequently requires the counting of many hundreds of objects on several treatments and replicates. Manual counting of such systems is time consuming, is of limited reproducibility and is difficult to verify and to document. These deficits can be resolved by automated image analysis and particle counting. The ecotoxicology tests which can profit from automated image analysis-based counting include but are not restricted to Collembolan reproduction (OECD 232), Lemna growth inhibition (OECD 221) and honeybee brood studies.

As a first step to establish automated counting of Collembola reproduction studies, optimized computer-assisted digital image acquisition to deliver high resolution images with maximal contrast and minimal reflections was established.

Second, customized macros of the public domain image analysis program ImageJ were created. These macros allow the image analysis and particle counting of any number of images in a folder (batch processing), provide full analysis report of individual images (e.g. non-destructive labelling of individual Collembola, thumbnail collection of all counts, full report of all parameter settings), enable human control and revision if necessary and enable the measurement of additional morphometric parameter (e.g. size or shape).

Third, validation of the automated image analysis-based counting was conducted by comparing it to the manual counting of the same digital images.

This comparison has shown that the automated image analysis delivers comparable results (within the range of variation), but is by far superior with regard to speed, reproducibility, and documentation. Further on sub-lethal endpoints such as size and form of the counted offspring can be taken into consideration.

MO 216

The potential use of 5% peat content OECD soil for earthworm reproduction tests

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The aim of the work presented is to investigate whether a valid Eisenia fetida (earthworm) reproduction test (OECD guideline 222) can be performed using an OECD test substrate of 5% peat content, as used in other OECD guidelines for soil organisms in the terrestrial environment. The result of a successful test with this peat content would negate the need to divide the NOEC of a test substance by a factor of 2 when calculating the PNEC (Predicted No Effect Concentration) of a compound. To accompany this, a more suitable reference substance should also be ascertained, ideally with a log Kow of >2, i.e. with a higher affinity for organic matter so that it will bind to the peat in substrate thus allowing direct comparison between 5% and 10% peat content.

MO 217

Mustard powder and allyl-isothiocyanate (AITC): possible substitutes for formalin as expellants of deep-burrowing earthworms?

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Hand-sorting earthworms from a defined soil volume with subsequent extraction of the deep-burrowing earthworms from the soil below the excavated hole is a widely used and well described method for the determination of size and structure of earthworm populations. The extraction is done using a formalin solution, a method recommended in the current guidance document for field trials. However, the use of formalin has its draw-backs, as it is not only harmful to humans but also to soil organisms and plants. Mustard powder and oil of mustard (AITC, Allyl-Isothiocyanate) hold more favourable toxicological and ecotoxicological properties. They have already been tested as earthworm expellants in field experiments with mixed results, but only for direct application onto the soil surface. The aim of this study was compare the efficiency of aqueous solutions of yellow mustard powder (6 g L^{-1}), brown mustard powder (6 g L^{-1}) and AITC (0.1 g L^{-1}) in extracting earthworms from the soil below hand-sorting holes to that of a formalin solution (2 g L^{-1} formaldehyde). The comparison was carried out at five sites with different soil characteristics and cropping history (3 arable with grass-clover, 2 meadows). The sites are located in southern Germany and the sampling took place in autumn 2010. At each site, four replicates (square holes of 0.25 m^2, 0.2 m deep) per expellant were sampled.

Yellow mustard powder extracted the largest number of earthworms at one site and formalin at the other four sites, but there was no statistically significant difference in the numbers and biomass of extracted earthworms between formalin, AITC and yellow mustard at four of the five sites. Brown mustard was significantly less effective than formalin and AITC at the same four sites and significantly less effective than yellow mustard at three sites. The results of the study indicate that AITC and yellow mustard powder could be suitable replacements for formalin for the extraction of deep-burrowing earthworms from below hand-sorting holes. However, it seems that soil characteristics and/or environmental conditions during extraction influence the efficacy. Further tests under varying soil and environmental conditions should be carried out to evaluate the influence of these conditions on earthworm extraction.

MO 218

Alterations in the endocytotic ability of Eisenia fetida coelomocytes to assess the toxicology profiles of standard soils with different organic matter content and sublethal cadmium concentrations

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Metals are ubiquitous elements in ecosystems that may cause a great variety of deleterious effects at different levels of biological organization. *Eisenia fetida* earthworms are known as important sentinel species for soil pollution assessment, and their immune system is considered a key compartment to study integrated responses to pollutants since represents a major defense line against them. Coelomocytes, free flowing immune cells of the coelomic cavity, have been given special attention for ecotoxicological effects studies due to their sensibility to toxicants exposure. The aims of the present work are, first to determine the optimal number of individuals to use in a routine protocol for toxicity testing of soil, based on testing of neutral red uptake in coelomocytes extracted from the earthworm *Eisenia fetida* exposed in vivo to Cd. Second, to apply this protocol to determine sublethal response profiles for this species, subjected to exposure in soils artificially contaminated with different concentrations of Cd and different organic matter content (%OM). The experimental design consisted in two phases (based on 3 d exposure experiments). The first phase, which determined the optimal number of individuals, was conducted in OECD artificial soil contaminated with three different concentrations of Cd. The second phase was designed using the optimal number of individuals identified in the first phase, and using OECD soil and modified soil OECD with different %OM (added peat). The optimal sample size was determined to be one pool of 5 specimens. A significant decrease in the endocytotic activity of coelomocytes was detected at decreasing concentrations of OM and increasing concentrations of Cd. Indeed, Cd bioaccumulation also decreased at increasing %OM in soil.

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MO 219

Mesocosm soil ecological risk assessment tool for GMO 2nd tier studies

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Ecological Risk Assessment (ERA) of GMO is basically identical to ERA of chemical substances, when it comes to assessing specific effects of the GMO plant material on the soil ecosystem. The tiered approach always include the option of studying more complex but still realistic ecosystem level effects in 2nd tier caged experimental systems, cf. the new GMO ERA guidance: EFSA Journal 2010; 8(11):1879. We propose to perform a trophic structure analysis, TSA, and include the trophic structure as an ecological endpoint to gain more direct insight into the change in interactions between species, i.e. the food-web structure, instead of relying only on the indirect evidence from population abundances. The approach was applied for effect assessment in the agro-ecosystem where we combined factors of elevated CO2, viz. global climate change, and GMO plant effects. A multi-species (Collembola, Acari and Enchytraeidae) mesocosm factorial experiment was set up in a greenhouse at ambient CO2 and 450 ppm CO2 with a GM barley variety and conventional varieties. The GM barley differed concerning the composition of amino acids in the grain (antisense C-hordein line). The fungicide carbendazim acted as a positive control. After 5 and 11 weeks, data on populations, plants and soil organic matter decomposition were evaluated. Natural abundances of stable isotopes, 13C and 15N, of animals, soil, plants and added organic matter (crushed maize leaves) were used to describe the soil food web structure.

HM01 - Characterisation and remediation of contaminated soils and sediments

MO 223

As transport characterization in the vadose zone of the soil: a combined study between field and laboratory experiments

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Heavy metals are major soil pollutants since a lot of former industrial soils are polluted by these contaminants. In the context of risk assessment of contaminated sites, they are of particular concerns because of their toxicity toward human beings. Nevertheless, the vadose zone of the soil is not taken into consideration in this kind of studies though this is where the pollution enters the soil. That is why mechanisms responsible for trace element release in the unsaturated zone of the soil have to be understood. In addition, tools and methods to measure and put forward interactions between contaminants and the solid matrix have to be provided. Thus the aim of this study was to characterize As transport in soils in the field and to put forward mechanisms responsible for its migration in the unsaturated zone of the soil by setting up an original laboratory experiment.

The study site was an in-service wood preserving facility site, located in the north east of France. A regulatory monitoring of the groundwater quality was initiated by a prefectural order in 1998 and 4 water wells are monitored. Water samples are taken twice a year. Soils were contaminated with Cr, Cu and As and we were mainly interested in As release since it was the only trace element measured in groundwater at a concentration above the EU guidelines for drinking water quality. Soils were sandy clay loam. On site, the mean depth of the water table was 6 m from the ground surface; the water table level variation was about 1 m over a year. Soils were sampled and analyze for physico-chemical characteristics and total As content and were submitted to a sequential extraction. In addition, two columns (30 cm high and 12 cm in diameter) were filled with 2 sub-samples of the As contaminated soil (202 and 253 mg/kg) and As release was studied as it could occur on site. Two main phenomena were simulated during these experiments: rain water infiltration and an increase in the water table level.

Field studies in combination with a statistical analysis showed that As release was highly correlated to the water table level. After the results of the laboratory experiments, it was shown that As concentrations at the outlet of the two columns were constant over time. Given that and the results of the sequential extraction carried out on this soil, As release could mainly occur from the soluble and exchangeable fractions of As. Arsenic transport modeling is in progress in order to confirm this hypothesis.

MO 224

Comparative study of chemical species of mercury in contaminated soils from Spanish-colony mining activities in four the Mexican States (Guanajuato, Hidalgo, Queretaro and Zacatecas)

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Amalgamation was heavily used in mining since 1557 in Spanish Colonies in the American Continent. In Mexico and other parts of Latin-America, this process generated mercury containing tailings which were left aside in the mine backyards and with the pass of the years dispersed by rain and wind through vast areas close to different settlements in the Mexican States of Guanajuato, Hidalgo, Queretaro and Zacatecas. Those areas currently represent a potential risk to health and environment since crop farming activities are being developed. The aim of this study was to develop of an integrated, efficient and cost-effective methodology for identifying the mercury species according to their solubility (water-soluble, weak acids, organic acids, and aqua-regia) suitable to characteristic mining contaminated soils of Mexico in order to identify hotspots where remediation or containment activities should be developed. Sites with mining background were selected in the states of Guanajuato (Guanajuato), Hidalgo (Pachuca and Zimapan), Queretaro (Pinal de Amoles and San Joaquin) and Zacatecas (Osiris and La Zacatecana). From the preliminary results of the study, it is estimated that the potential risk is low for the selected sites since mercury is found in stable species (amalgam and sulfide). However, there are some companies that are reprocessing the old tailings to obtain the residual silver left in those materials in Zacatecas and there are mercury extraction activities in Queretaro; this represents a potential risk since these activities might modify the stability of the chemical species of mercury, increasing its solubility. In the case of Hidalgo high concentrations of mercury were found in the nearby of a residential area in the city of Pachuca and ingestion of particles by population is a potential risk.

MO 225

Characterisation of an oil contaminated soil using comprehensive gas chromatography time of flight mass spectrometry (GCxGC-ToF-MS)

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Characterisation of the chemical composition of an oil contaminated soil represents a vital step in the development of an effective remediation strategy. Information on the chemical composition of such complex mixtures is used, as part of a risk assessment, to assess the toxicity and subsequently identify the most suitable technology for treatment. Therefore, the quality of the risk assessment and the probability for a successful soil remediation will improve with the level of chemical characterisation. Currently, analyses of these complex samples is conducted using standard protocols based on gas chromatography with flame ionisation detection (GC/FID) or in a few cases gas chromatography with mass spectrometry (GC/MS). A common feature in the GC traces of oil contaminated soil samples, especially when weathered, is the presence of an unresolved complex mixture (UCM), the concentration of which typically far exceeds the concentrations of the limited number of individual priority pollutants which conventional methods can characterise.

In the present study we report the application of comprehensive gas chromatography-time of flight-mass spectrometry (GCxGC-ToF-MS) for the analysis of soil samples taken from a site contaminated by aviation fuel oil. GCxGC-ToF-MS examination of the oil contaminated soil extract provided superior chromatographic separation allowing the characterisation of a wide variety of petroleum-derived hydrocarbons including toxicologically important components such as branched alkylbenzenes (alkyl substituent: C2 to C13) which have previously been reported to affect the health of marine molluscs and crustacea. The chromatographic profile of the contaminated soil sample matched closely to a typical aviation fuel sample analysed under identical experimental conditions, thus allowing the soil extract contaminant source to be distinguished from other common oil contaminants e.g. diesel. The use of GCxGC in the present study provides evidence of improved chromatographic separation and when coupled to ToF-MS yields more reliable peak assignments from mass spectra than previously possible, thereby allowing vastly increased numbers of individual chemical components to be characterised.

MO 226

Sunflower roots in combination with indigenous soil micro-organisms for treating hydrocarbon-contaminated soil

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Phytoremediation of organic contaminants mainly depends on plant-microbe interactions in the rhizosphere, where, plant roots stimulate microbial populations for enhanced the biodegradation in polluted soil. We conducted a green-house experiment to measure dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere of sunflower roots on a creosote-polluted soil (containing 21.75 mg·Kg-1 of 6 PAHs), and an *in vitro* production of root exudates with 50 seeds of sunflower cv. in sterile conditions in an inorganic salts solution used in mineralization experiments (MM; pH=5,7) to study its effect on the biodegradation of phenanthrene and pyrene (present in creosote). In this way, we may separate the chemical impact of the root exudates from any root surface phenomena indicating that sunflower root exudates have the potential to increase the degradation of xenobiotics by the growth of soil microorganisms. In addition, we performed a characterization of these exudates of sunflower to know their total organic carbon (TOC) and composition. It is important to know the organic matter that these exudates provide to the rhizosphere because, their contribution produces an increase in the size of the heterotrophic microbial community and the concentration of PAH-degrading populations, and it is also important to characterize their composition because little is known about the composition of sunflower exudates and their influence on the elimination of PAHs.

MO 227

PCDD/F impact of a secondary aluminum smelter (ALS) on the surrounding environment

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The aim of the present study was to evaluate the impact of a secondary aluminum smelter (ALS) placed in Piedmont (a region of North West of Italy) and its impact on the surrounding environment. With this purpose we collected ALS emission samples (chimney emissions, fly ash, particulate and wastes), agricultural soils and vegetable and rice samples. PCDD/Fs were measured and expressed as TEQ concentrations according to the international (I-TEF), and World Health Organization toxicity equivalent factors (WHO-TEQ) schemes were calculated. Results obtained indicate a decreasing trend dioxin concentration as the distance from the plant increase, the highest levels were found in soil collected at a distance lower than 500 meter from the ALS, even with values that exceed the reference mean range values for agricultural and natural soils in Piedmont and limit value of 10 pg WHO-TEQ g^{-1} adopted in Italy in soils for green and residential uses. Furthermore PCDD/F concentration decrease from soil though vegetable to rice samples indicating that the contamination is mainly due to atmospheric deposition rather than bioaccumulation in rice plants. To evaluate if the plant investigated might be responsible for PCDD/F contamination in soil collected, Principal Component Analysis (PCA) was performed. Thus source emission and soil samples were included were included. PCA results divide emission samples into two clusters and many of the soil samples collected closest to the plant fall near or inside one of these two clusters.

MO 228

Behaviour and distribution of mercury in soil samples collected from Valdeazogues river (Ciudad Real, Spain)

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The main objective of this study is to determine the behaviour of mercury in the soil of Valdeazogues river (Almadén, Ciudad Real, Spain) by using a six-step sequential extraction procedure developed by CIEMAT (Sánchez et al., 2005). Furthermore, the relationship between the percentage of organic matter in soil and the percentage of mercury associated with the exchangeable and oxidizable fractions is checked.

The results show that total mercury concentrations in soil range from 117 ± 24 to 351 ± 69 mg kg-1. However, the easily available mercury concentration (soluble + exchangeable) is a smaller percentage of total mercury measured in the samples (< 0.16 %). Furthermore, the soluble mer-

cury, in all cases, is less than 0,02 %, so, the leaching process and transport of mercury to surface water and groundwater are very slow. With regard to the distribution of mercury between the different fractions of soil, the metal is associated with more resistant soil fractions, these are: crystalline Fe-Mn oxyhydroxides, organic matter absorbed and the final residue.

MO 229

The artificial sweetener saccharin in soils: sources from agriculture and households, degradation, and leaching to groundwater

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Recent studies have documented the widespread occurrence of artificial sweeteners in the aquatic environment. The sulfoamide saccharin, for example, was detected in most surface waters investigated in Switzerland. Concentrations in lakes correlated with its wastewater burden. Saccharin may also end up in soils through various pathways. Apart from its use as sugar substitute in beverages and food, the sweetener is registered as additive in piglet feed, as it is supposed to facilitate the adaptation to dry feed after the suckling period. Saccharin fed to piglets was largely excreted and, consequently, found in liquid manure at concentrations of 0.3-12 mg/L, where it was stable during two months of storage. Saccharin may thus reach soils in considerable quantities of up to 1000 g per hectare and year with manure application. Furthermore, saccharin is formed in soils by metabolization of certain sulfonylurea herbicides (estimated formation, 1-25 g/ha). Irrigation with wastewater-polluted surface water, fertilization with sewage sludge, and leaks in sewers are further possible sources of saccharin to soils, but these entry pathways were assessed to be of minor importance for saccharin. In batch incubation experiments with six soils, saccharin was degraded with half-lives of 3-12 d. Despite this rapid degradation, saccharin may eventually end up in groundwater, due to high inputs with pig manure and low sorption to soils. Computer simulations predict mean concentrations in soil leachate at 1 m depth of up to 0.3 µg/L. Indeed, saccharin was detected in groundwater at concentrations up to 0.26 µg/L. Under certain conditions, presence of saccharin in groundwater may thus indicate application of pig manure on farmland and leaching to groundwater.

MO 230

Uranium accumulation in the plants of the old mine of Sevilha (Central Portugal) - implications for phytoremediation

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The old uranium (U) mine of Sevilha (Tábua, Central Portugal) is one of several small mines exploited by Portuguese Uranium Company. We decided to evaluate the U accumulation potential of the endemic species. Samples of soil, water and terrestrial and aquatic plants were colleted in the vicinities of the study area. The plant survey did not follow a regular pattern to take into account the dispersion of the species and to sample all the units of the plant community. It was intended to obtain a global view of the response of the vegetation to the accumulation of U. This implied sample collection in the biogeochemical background and within the anomaly. As a result 82 different species we identified and sampled. These species belong to 32 families, five of which are aquatic. All samples were processed at the Chemistry Laboratory of the Earth Sciences Department of University of Coimbra. For determination of mass concentration of U in samples of natural water, soils and plants, it was used the "Fluorat-02-2M"analyzer.
Current U soil contamination on the Sevilha mine ranges from 8 to 560 ppm. In the most abundant families of terrestrial plants, the results show that the Compositae and Ericaceae families have the highest concentrations. For the Composiatae, an average of 4.91 ppm and a maximum of 13.12 ppm was found in Helchrysum stoechas and an average of 4.07 ppm and a maximum of 10.52 ppm was recorded in Hypochaeris radicata. In Erica umbellata an average of 1.70 ppm and a maximum of 7.50 ppm maximum were obtained. Even though the concentrations obtained in this later species are not high it is particularly interesting because it has a high bio-productivity. The water contamination in the vicinities of Sevilha mine results from the natural lixiviation of the wastes used to fill the open pit. Although the U concentrations in waters did not surpass 13 ppb (EPA limit: 30 ppb). Four of the analysed aquatic species proved to be U accumulators. The measured average concentrations were: Riccia fluitans (29.19 ppm), Lemna minor (15.47 ppm), Callitriche stagnallis (9.97 ppm) and Lythrum portula (15.52 ppm). For these species, the observed maximum values were 50.59 ppm, 52.98 ppm, 55.53 ppm and 32.93 ppm, respectively.

MO 232

Organochlorines impacts on plant rhizosphere microflora communities

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The aim of our research is to investigate the organochlorines eco-remediation by plant rhizosphere. Such a research requires a multidisciplinary approach integrating microbiology, chemistry and plant physiology studies. Using standard organochlorines (OCs) [lindane, monochloro-, dichloro-, trichloro-benzenes (MCB, DCB, TCB)], rhizosphere microflora associated to Zea mays plants was studied. Samples were collected on control plants and plants exposed to a concentration of 15µg OCs/g dw during 45 days. Using molecular profiling we found that OCs impacted the rizospheric bacterial communities. Bacterial strains from control and treated plants were isolated and cultivated. These strains were characterized by sequencing the 16S RNA gene. A large diversity of bacteria was found in treated and untreated conditions. Strains from some of these genera (Sphingomonas, Pseudomonas, Xanthomonas, Bacillus) are already known to be involved in the detoxification pathways of OCs [1,2,3], while Sphyngobacteria seemed to be sensitive to the presence of OCs mixtures. Current work concerns test on the sensitivity of the isolated bacterial strains to OCs and their capability to mineralize these pollutants.

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MO 233

Soil management using life cycle impact assessment: environmental state of soil and potential remediation with USEtox model - a decision support

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Heavy metal and soil contamination are industrially connected. Soil contamination bears itself upon an ecological, dynamic perspective where some weakly known processes are still regarded as a source of uncertainty and variability. Risk assessment and life cycle thinking are welcome tools under this uncertainty. UNEP-SETAC Life Cycle Initiative joint efforts harmonized previous work on toxicological life cycle impact assessment into one single tool - USEtoxTM (Hauschild et al). It is available in a stepwise excel tool to estimate comparative toxic units (CTU) from a unit chemical release. Nevertheless, the factors predicted for inorganic substances are still considered as interim factors and effects in terrestrial organisms are not considered. Despite this, the model can be used to assess the environmental state of a determined region due a specific contamination. Thus, we need to quantify the connection between the emission and the resulting health or ecological effects. Two groups of data are necessary to determine the soil inventory: data regarding the affected soil and data concerning a non-affected soil. Thus, we represent both primary (soil contamination) and secondary (soil remediation) impacts over the life cycle of the site. Using average chemical extraction rates from different technological solutions we can use both impacts (primary and secondary impacts) and assess the potential benefits of each solution (over the life cycle) within different land use. Over the study we compare data from the contaminated site (airborne emission, metal concentration on vegetation, epidemiological data) with predicted results and discuss their variability. Moreover, outcomes from contamination/remediation depend on land use. We analyzed the resulting variability by changing simple soil parameters on the model. Finally, the final technological hierarchy is given by multi-criteria analysis also accounting for social and economic aspects. Developments on risk assessment for human health (Carlon et al.) and biodiversity conservation (Critto et al, E. Boriani et al) are used and applied to a case study where metals as Cadmium, Lead, Zinc are measured in different land uses and inhabitants may be under risk. The large contaminated area, the different pollutants found, the range of stakeholders as well as the expenditure and remediation costs pose a rehabilitation challenge.
Keywords: USEtoxTM, soil remediation

MO 234

Endosulfan and cypermethrin-induced oxidative stress in plants and their influence on phytoremediation process

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Phytoremediation involves the use of vegetation for in situ treatment of contaminated soils, sediments, and water. It is applicable at sites containing organic pollutants where plants can uptake and metabolize them and/or enhance the rhizospheric activity. However, some pollutants are known to enhance Reactive Oxygen Species (ROS) formation and it can affect the efficiency of phytoremediation due to toxic effects. ROS produce oxidative damage to proteins, DNA and lipids. Under optimal growth conditions, ROS are produced at a low level in organelles such as chloroplasts, mitochondria and peroxisomes. However, pesticide uptake can dramatically elevate their rate of production. The aim of this work was to study the oxidative stress in edible plants exposed to soils spiked with a mixture of endosulfan and cypermethrin as a measure of plant tolerance for their use in soil phytoremediation. Lipid peroxidation (malondyaldehide content, MDA) and total antioxidant capacity against peroxyl radicals (ACAP) was measure in leaves and roots of soybean, sunflower, colza and alfalfa plants grown in soil spiked with technical endosulfan (Master® 35%, 5 ppm) and cypermethrin (Goti Glex, cis 40-50, 25%, 0.5 ppm) after 15 and 60 days of exposure. Control plants were grown in the same soil without pesticides. Results showed an increase in the ACAP of roots and leaves at 60 days for most species while MDA levels increase only in leaves. These results can indicate that pesticide uptake and translocation may trigger oxidative damage in leaves. However in older plants, growth leads to higher photosynthesis rate and ROS production that enhance the oxidative damage caused by pesticides. Plants grown in contaminated soils showed lower biomass and growth rate than control plants (clean soils) suggesting pesticide toxicity, and the lower MDA levels would be related to changes in lipid types (ie. lower proportion of unsaturated lipids). The knowledge about the induction of oxidative stress by pesticides is necessary for planning phytoremediation strategies and allows selecting tolerant species according to particular sceneries.

MO 235

Effects of apatite mine tailings amendment on toxicity and leaching of shooting derived lead in forest soil

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In Finland, there are roughly 2000-2500 shooting ranges, of which approximately one-third are situating on ground water areas. Dissolution of pellet-derived lead (Pb) into the soil water poses a risk to ecosystems locating in the vicinity of the shooting ranges and, if leached to the groundwater, to human health. Apatite ore beneficiation at the Siilinjärvi carbonatite complex produces mine tailings of versatile chemical and mineralogical properties that may allow the material to act as a sorbent for Pb *in situ*. The aim of the study was to investigate the tailings-induced changes in the mobility and bioavailability of Pb in contaminated shooting range soil and the effects of tailings amendment on soil organisms responsible for decomposition process in the soil. Five test soils of increasing Pb content were collected from a pine forest locating right behind a shotgun shooting range. Soil from an adjacent uncontaminated site served as a control. In the laboratory, half of each soil was amended with the tailings material. The effects of amendment on (i) the toxicity of the soils and (ii) the leaching of Pb, measured using (iia) closed soil extraction and (iib) open lysimeter system, were studied one day after amendment (short-term effects) and after one year of incubation of the soils (long-term effects). (i) Toxicity of the soils was tested using the Collembola reproduction test and the acute toxicity test on earthworms. (iia) In standardized leaching procedure, soils were extracted using 0.001 M $CaCl_2$ solution, and the Pb concentration in the leachates as well as their toxicity to *Vibrio fischeri*, *Lemna minor* and *Daphnia magna* were measured. (iib) In lysimeter assay, water was poured on top of the soil in each lysimeter pot, and Pb concentration of the leachate water collected in the bottom of the pot was analysed. Amending the soil with the tailings reduced instantly the leaching of Pb in a standardized leaching procedure, but increased it in the lysimeter system. The tailings decreased the toxicity and increased soil pH immediately after application. Increased pH is one of the mechanisms through which the tailings amendment can decrease the availability of lead. Further, it is likely that the increased soil pH itself affects the survival and fitness of the organisms. The results of the long-term effects of tailings amendment - yet to be analyzed - will be crucial to reveal the suitability of the tailings to act as a sorbent for lead in contaminated soil.

MO 236

Enhancement of Cd removal efficiency using phosphate-solubilizing bacteria (PSB) in phytoremediation

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Phytoextraction has demonstrated its beneficial use in the removal of heavy metals from polluted soil. Recently, many researches have focused on plant growth promoting rhizobacteria (PGPR) application to enhance metal removal efficiency during phytoremediation. Phosphate solubilizing bacteria (PSB), one type of the PGPR, can solubilize phosphate in soil and provide nutrients for plant growth and increase the bioavailable of metals in soil. This study is conducted to evaluate the effects of the PSB inoculation on removal of Cd from soil in phytoremediation. Pot tests were carried out using artificially contaminated soil with Cd (72.8±2.01 mg/kg). Brassica juncea and Abutilon theophrasti were used and Bacillus megaterium (DSM No. 3228) was used as PSB. The potential of B. megaterium for solubilizing phosphate from tricalcium phosphate (TCP) or soil was tested. While the concentration of phosphorus in the controls was 10.2 and 0.6 mg/L, the concentration of phosphorus in the sample with TCP or soil were 45.5 and 8.2 mg/L, respectively. These indicate that the bacteria have the potential for solubilizing phosphate in soil. Each pot was inoculated with B. megaterium after 4 weeks of growing and re-inoculated at the 6th and 10th weeks. The plants were harvested at the 4th, 6th and 10th weeks. With B. juncea, the Cd accumulation in the roots, stems and leaves was 0.35, 0.39, and 0.09 mg/g of dried plant mass after 4 weeks, respectively. With A. theophrasti, 0.37 and 0.07 mg/g of dried plant was accumulated. When B. megaterium was inoculated, Cd accumulation increased significantly compared to the controls. Interestingly B. juncea mainly accumulated Cd in their roots, whereas A. theophrasti accumulated Cd in leaves. Translocation factor (TF), which is defined as the ratio of metal concentration in shoots to that in roots can be used to evaluate the capacity of plants accumulating heavy metals. With the application of B. megaterium, TF value for A. theophrasti showed a marked increase from 0.19 to 6.68. This indicates that the amount of Cd extracted from soil by roots and translocated into the above-ground regions such as stems, leaves, and flowers was greater for A. theophrasti. The change of metal speciation in soil and the mechanism of heavy metal transport into plants will be also investigated with the PSB application. The findings from this study will enhance the efficiency of phytoremediation using the PSB application of heavy metal-contaminated soil.

MO 237

Root elongation test with Lactuca sativa in assessment of dredged sediment ecotoxicity

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Large amount of sediments are dredged each year for the maintenance of water bodies and waterways. The disposal of dredged sediments is an important environmental problem. Reusing of this material as a soil improver and application on agriculture land is one of the possible options. Application must be regulated based on sediment contamination or toxicity, because of high levels of contaminants frequently present in sediments. Novel directive for the dredged sediments application on agricultural land was ratified in the Czech Republic in summer 2009. Root elongation test according to ISO 11269-1 is one of the four toxicity tests suggested in the directive. The objective of this study was to evaluate the suitability of the root elongation test with *Lactuca sativ*a as a tool for hazard assessment of the dredged sediments. We evaluated 36 sediments samples both from bottom of rivers and ponds and from stockpiles of dredges materials. Contents of heavy metals, PAHs, PCBs and organochlorinated pesticides were analysed in the samples and compared with the limits values suggested in the new directive. Growth of *Lactuca sativa* roots sensitively responded to high content of contaminants (PAHs, heavy metals) in several sediment samples. In some cases, the toxicity was caused by sediment physico-chemical properties.

MO 238

Carbon amendments as a sediment remediation method: ecotoxicological effects in Lumbriculus variegatus

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Light density black carbonaceous particles such as soot, charcoal and activated carbon (AC) have been established to be efficient sorbents for a wide variety of hydrophobic organic compounds (HOCs). Interest has been directed in to use the AC in sediment remediation as a carbon amendment to bind and stabilize contaminants. Despite the promising results from both laboratory and field experiments, some studies indicates that AC addition may not ensure good ecological quality of the sediment but can in fact alter the behavior of organisms. AC addition has been observed to influence among others growth rate, food consumption, lipid content and sediment preference of several organisms. The results of ecological effects of AC addition vary however from no effect to a significant effect even among studies employed with the same organism. The aim of our research was to clarify biological responses in *Lumbriculus variegatus* to AC addition. Within this study coal based AC was used in three different grain sizes and in six concentrations each (percentage / sediment ww). Natural uncontaminated sediment was used in terms to address the attention to the effects of AC additions. A relatively low dose of AC was found to be sufficient to alter food consumption and growth rate of *L. variegatus*. The effective dosage on AC was dependent on a particle size, as the most pronounced effect consequent of smallest sized powdered AC addition with dosage lower than 0,5%. The results of this study indicate that *L. varieatus* is able to detect and avoid AC added sediment by reducing sediment consumption. Interesting question constitutes, whether reduction in HOC bioaccumulation, observed in several studies could in some scale be a consequence of altered behavior of studied organisms. AC addition based dose-response effect was not observed in reproduction in terms of this study. Ecotoxicological effects of AC amendments requires more investigation and finding the mechanism for the effects is especially important. The challenge is to find AC material and dosage that does not have an effect on organisms but despite the fact will be effective enough to bind HOCs and reduce bioaccumulation.

MO 239

Use of toxicity assays for evaluating the effectiveness of groundwater remediation with Fenton's reagent

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A chemical dump site adjacent to the Danish North Sea holds a variety of constituents from pharmaceutical production including sulfonamides, barbiturates, aniline, pyridine, phenols, benzene, toluene, chlorinated solvents, lithium, copper, lead, mercury, etc. An on-going pilot scale project evaluates in situ chemical oxidation (ISCO) using modified Fenton's reagent (H2O2 + chelated Fe2+) as a groundwater remedy. Three injections were performed over a period to test treatment efficacy. Performance monitoring samples were collected from two depths both prior to and during treatment, and analyzed for toxicity and contaminant chemistry.
Organisms exposed to a mixture will react to all contaminants present and, consequently, the toxic effect will represent a sum effect. In contrast, chemical analyses yield information on individual or possibly groups of contaminants but not necessarily all the contaminants. Thus, using a combination of chemical analyses and toxicity assays yields a more robust understanding of the contaminated site and the risk it poses to the environment. Ground water samples were tested via toxicity assay using algae, crustaceans, luminescent bacteria, nitrifying bacteria and yeast (estrogen screening test). Results from the baseline study showed that the two most sensitive species were the marine diatom Skeletonema costatum and the marine copepod Acartia tonsa. It was found necessary to dilute untreated groundwater samples up to 3400 times to reduce the short-term toxicity to the LC10 level. Samples from the upper layer were 2-4 times more toxic than samples from the lower layer. Applying a safety factor of 10 on these results and combined with a dilution model for the recipient indicated that the ecosystem in an area of the North Sea of approximately 1x7 km is affected by groundwater flow from the contaminated site. Chemical analyses showed that PCE and toluene concentrations up to 137 and 60 mg/L, respectively, in the upper layer Total hydrocarbons were up to 94 mg/L. Sulfonamides and barbiturates were found at 600 µg/L and 200-400 µg/L, respectively. After the second treatment with Fenton's reagent the toxicity had increased and now needed 7100 times dilution to reduce toxicity to the LC10 probably due to mobilization of metals. It is concluded that toxicity assay is a useful tool for evaluating samples from contaminated sites and that toxicity assays and chemical analyses supplement and support each other.

MO 240

Microbiotests as screening tools to evaluate potential ecotoxicity of freshwater sediments

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Different methods have been developed to assess the toxic potential of freshwater sediments. Different test batteries composed of bioassays of different organisms (bacteria, algae, seaweeds, micro-crustaceans, and plants), belonging to different trophic levels have been recommended to discriminate among samples and decrease uncertainty in sediment quality assessment (Griest et al., 1995; Nimmo et al., 1995, Cote et al., 1998a,b). Chial and Persoone (2002) mentioned the importance of carrying out tests not only with porewaters, but also with whole sediments. Several exposure phases, such as pore waters, elutriates, organic extracts and whole sediments have also been tested (Bombardier and Bermingham, 1999). Here, we focused our research on whole sediments testing with different solid and liquid phase microbiotests : Microtox® solid and pseudo liquid phase assays, algal solid phase assay, Luminotox solid phase assay and ostracod assay.
To synthesize ecotoxicity information, sediment ecotoxicity index scores were calculated to categorize sediments in order of their potential ecotoxicity. The index formula with its corresponding units has been formulated such that resulting values generally vary from 0 to +∞. In this study, the index scores varied from 17 to 2105. Samples were categorized as marginal hazard potential, moderated hazard potential and high hazard potential when the toxicity index score was less of 50, 50-500 and more of 500 respectively. Finally, results of ecotoxicity index scores were compared with physico-chemical parameters of sediments
Results showed that sediments MIR, CAT and CMA2 presented a high hazard potential, toxic effects were observed in all microbiotests tested. In contrast Maxe and BB2 samples were classified as marginal hazard potential and they did not present ecotoxic effects in all microbiotests. Concentrations of metals in sediments were correlated with the ecotoxicity index scores (Pearson's product moment correlation coefficient and associated p-value). Results showed a correlation with concentration of metals especially with Cr (0.850*), Co (0.860*), Pb (0.925*) and Ni (0.735*). Our results demonstrate that the biological responses of the battery of microbiotests has a strong correlation with some contaminants present in sediments; these mean that this battery can give information about the potential ecotoxicity of freshwater sediments.

MO 241

Addition of reactive media for the geochemical stabilization of mercury in contaminated sediments

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The presence of elevated concentrations of Hg in sediments can lead to formation of methylmercury (MeHg) and subsequent uptake of MeHg in the food-chain. This study evaluated the effectiveness of a variety of reactive media on the stabilization of Hg in water-saturated sediments collected from two contrasting watersheds. The sediments were co-blended with attapulgite clay (ATP), organically modified clay (OMC), sulfur (S), and zero valent iron (ZVI). Batch-style and saturated column experiments were conducted to evaluate the effectiveness of the reactive media for stabilizing Hg. In the unamended sediments, large differences in Hg release were observed both within and between the study sites (release from 100 ng L^{-1} to > 100 µg L^{-1} Hg). In the amended sediments, the release of Hg was reduced by 50 to >99% relative to unamended sediment. The greatest stabilization was observed for sediment augmented with a combination of OMC+ATP and a combination of S+ZVI. Under low-flow saturated conditions, aqueous concentrations of Hg were maintained in the low ng L^{-1} range for more than 6 months. After a hiatus in flow, concentrations of Hg increased in the unamended sediment and sediment amended with ATP. Increases in Hg concentration were not observed for sediment amended with OMC+ATP and with S+ZVI. Sectioning of the columns and analysis for MeHg indicated minor increases in solid-phase MeHg concentrations in the treatment containing OMC+ATP. Increases in MeHg were not observed for the other treatments. Solid-phase analyses showed large accumulations of FeS in the S+ZVI amendment. Geochemical speciation calculations indicated supersaturated conditions with respect to Hg sulfide phases for all treatments. Elevated populations of Fe(III) reducing bacteria were observed in the unamended sediments and the sediments amended with ATP and ATP+OMC. Suppressed populations of Fe(III) reducing bacteria and slightly increased populations of SO_4 reducing bacteria were observed in the S+ZVI column. These results suggest that large decreases in aqueous Hg concentrations can be achieved through the addition of

reactive media, and concentrations in the low ng L^{-1} range can be maintained under varying flow conditions.

MO 242

Characterization of DDT and Mercury contaminated sediment in Toce River and Lake Maggiore (Pallanza Bay)

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To evaluate the need for remedial action in Lake Maggiore (North Italy) and in its tributary (Toce River), sediment characterization activities were performed in 2009-2010. Historically, a DDT manufacturing facility was located on the Toce River and discharged industrial wastewaters containing DDx and mercury to the river. DDT production started in the 1940s and ceased in 1996; radiodating analysis showed that the highest DDx concentrations in sediments are related to the 1970s.

To investigate temporal and spatial distribution of DDx and mercury in sediment and their influence on the whole lake ecosystem, with the objectives of evaluating potential ecological risk and assessing the need for sediment remediation, the following activities were performed:

- chemical concentrations were investigated at various depths in 125 sediment cores. To collect high quality sediment chemistry data, a lightweight sediment-water interface gravity corer was used
- a Sediment Profile Imaging (SPI) camera survey was performed throughout Pallanza Bay and mouth of Toce River to investigate benthos-sediment relationships by documenting gradients in sediment grain size, transport patterns, geochemical processes, and benthic community dynamics
- radiodating analyses were undertaken to better understand sediment accumulation rates and to examine historical trends in chemical loading to the bay
- sediment toxicity to invertebrates was studied with several lines of evidence, including site-specific sediment toxicity studies, site-specific invertebrate community analysis, studies relating DDx tissue concentrations with adverse effects on invertebrates and analysis of benchmark and toxicity testing from major DDx and mercury contaminated sites
- the fish community was investigated by analyzing several species, considering tissue concentrations with adverse effects on fish, comparison of water concentrations with benchmark values and performing fitness evaluation
- fish and invertebrate analyses supported estimation of wildlife exposure to evaluate potential ecological risk.

Bathymetric and morphologic surveys were performed and hydrodynamic parameters were measured to implement a sediment transport model, to understand system variability under different hydrological scenarios.

This poster provides a summary of data collection methods used and the results obtained, as well as conclusions made regarding potential ecological risk and the need for remediation in the lake.

MO 243

Evaluation of a conceptual site model for sediment processes and geochemical conditions in a large industrial port facility, Augusta Bay, Sicily, Italy

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Studies of sediment quality in the Augusta Bay industrial harbor identified elevated concentrations of mercury and other metals and organic chemicals in bay sediments. Previous studies primarily focused on the nature and extent of total mercury in sediments, after which, a large-scale dredging remedy was proposed. An understanding of physical and chemical processes and ecological conditions in the bay had not been demonstrated to the extent necessary for conducting a competent environmental impact analysis and engineering evaluation of the proposed remedy. While it had been demonstrated that contamination did exist, there was no demonstration of bioavailability of any of the contaminants, no indication of the source of ecotoxicity, and no evidence of impact to the bay ecology. It was unclear which substances in sediment posed ecological or human health risks, if any, and therefore impossible to recommend an appropriate, safe and effective sediment remedy.

To address this deficiency, further study in 2008 focused on understanding hydrodynamic processes and the geochemical and ecological conditions in the harbor, and the factors that most influence those conditions. First, a conceptual site model was developed to guide the work and establish a foundation to understand how mercury and other chemicals in the sediment enter the aquatic environment, how they are transported within the bay and deposited in sediment, and where routes of exposure to aquatic life and humans might occur. Hydrodynamic monitoring, sediment transport analysis, and bathymetric and sediment profile imaging surveys were performed in representative regions of the industrial harbor. Sediment, surface water and biota sampling, and ecotoxicity testing and ecological risk assessment were conducted at four stations representing different contaminant distributions, ship traffic activities, and hydrodynamic conditions.

Based on this work, it is apparent that mercury in Augusta Bay does not pose a significant threat to the environment and sediment-associated mercury is tightly bound to the sediment, poses no to low toxicity to benthic organisms, and is largely not bioavailable to aquatic fauna. Furthermore, commercial shipping activities in the harbor do not significantly alter mercury conditions in the bay or create new or greater ecological risks. The proposed large-scale dredging remedy for Augusta Bay is unwarranted, and if implemented, would destroy a complex functioning ecosystem.

MO 244

On the use of ELISA commercial test kits for PCB screening in sediments: practical implications

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Polychlorinated biphenyls (PCBs) are a family of persistent organic pollutants (POPs) of anthropogenic origin constituted by 209 congeners. PCBs bioaccumulate and have been identified as toxic compounds producing a wide spectrum of adverse health effects in biota and humans. Sediments constitute reservoirs of PCB which may be eventually buried together with particles but also remobilized by the effects of sediment resuspension and mixing by benthic organisms (i.e. bioturbation). Sediment is therefore a key compartment in the study of the quality status of water bodies. PCBs have been proposed for the inclusion into the European Water Framework Directive (WFD) priority list, currently under revision. Moreover, the management of dredged sediments requires useful screening and monitoring tools in order to assess contamination levels prior action decision (e.g. immersion, terrestrial treatment, storage, or valorisation). Various screening methods have been employed for PCB determination in different environmental matrixes in the last decades, immunoassays being one of the most employed.

A performance comparison study between a classical reference method for PCB analysis in sediments and a commercial ELISA test kit is presented. A number of sediments covering a wide pollution range (from clean to polluted areas) have been analysed by both a reference and ELISA methods. The reference method was based on a 24h Soxhlet extraction of sediments followed by gas chromatography-mass spectrometry (GC/MS) analysis. A selected immunoassay commercial test kit was employed for the ELISA determination. Briefly, the sediments were extracted by manual shaking during 1 min and ELISA response was measured by spectrophotometric detection. Preliminary results showed a good correlation between the two methods when identifying the pollution gradient but not in terms of absolute concentrations. ELISA provided a total PCB concentration as Aroclor equivalents whreas GC/MS analysis provided the PCB total concentration as 6 indicator PCB (CB-28, 52, 101, 138, 153, 180) in addition to single congener values. Discussion on the most suitable ELISA applications (e.g. monitoring contaminated sites versus EQS compliance checking) and on the adequacy of ELISA for PCB determination in sediments under the current environmental pollution scenario and the new European regulation framework is also presented.

MO 245

Metal concentration profiles in sediment cores of Guanabara Bay: geochronological evolution

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The present research has been pursued in the frame of TAGUBAR Project (Tangential Guanabara Bay Aeration and Recovery, 2002/2007) - Cooperazione Italiana allo Sviluppo - Ministry for Foreign Affairs of Italy. The main target of the TAGUBAR Project was to transfer technologies for the remediation of the Bay of Guanabara (Rio de Janeiro, Brazil).

Guanabara Bay is one of the largest coastal Bays in Brazil, it measures 31 Km from south to north and has a surface area of about 400 km2 of estuarine waters. It is an eutrophic, pulluted system, impacted by largely untreated domestic runoff from the eight million inhabitants of its basin. Because the Bay is situated in a highly populated and urbanized area, it is subjected to chemical contamination from a variety of sources and pathways. Particularly evident in this area is the heavy metal pollution of industrial and sewage. In effect the immediate area surrounding the estuary is highly industrialized and includes petroleum refineries and chemical manufacturers, so the Bay is subject to organic and inorganic chemicals contamination.

This study, conducted in 2005, focused on the distribution analysis of the main heavy metals, of some metalloids and some alkaline earth metals in order to define the possible geochronological evolution in sediments of the Bay. Special enphasys is been done to the speciation of metals in the sediments.

The study of the general distribution of the contamination was conducted to implement strategies for environmental recovery by MODUS System, that is a special mobile equipment for aeration, pollutant stripping and oxidation to be used in shallow or deep water. In Tagubar Project has been discovered that polluted sediments, aerated with mechanical dredging and mixing, can release a very high amount of pollutants, expecially toxic methals.

The results of the geochronological study on the distribution of the elements tested, in some cases showed a clear change in concentration with depth but in general there has been a degree of uniformity in the distribution, probably caused by bioturbation.

MO 246

Regression model of arsenic distribution in water/sediment system of Sevojno

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This paper reports the results on total arsenic concentration analyzed in water and sediments collected in 2009 spring/summer seasons. Water and sediment samples were analyzed for total arsenic content by hydride generation using ICP device (ICAP Thermo 6500 Duo). Study has been focused on the Djetinja river basin of the area of Western Serbia, near Sevojno. Traditionally the Djetinja river and Dragića spring (Sevojno, Serbia) receive pollution from two different sources, in the upper section of the Dragića stream from a primary metal manufacturing sector, Copper Mill Sevojno and, in its lower section, in the Djetinja river, from untreated urban wastes and intensive agricultural activities. The concentrations of arsenic in water and sediment samples taken in spring were higher than in summer. Measured concentrations of arsenic in water of Dragića streams and Djetinja river downstream does not exceed the maximum allowable concentration according to the Regulations on permitted amounts of hazardous and harmful substances in soil and irrigation water in Serbia.

Adsorption of arsenic was studied using equilibrium distribution coefficient as a function of the concentration of arsenic in water and sediment. Regression analysis provided a quantitative agreement of two variables. Pre-defined (based on theoretical knowledge) to be an independent variable - concentration of arsenic in waste water, and the dependent variable - the concentration of arsenic in the sediment. Distribution of arsenic between the water and sediment showed agreement in use of Freundlich's adsorption isotherm with obtained polynomial dependence:

Spring: $y = - 0.0145x2 + 0.0327x - 0.0093$

Summer: $y = 10\text{-}3\ (-0.8259\ x2 + 0.6878\ x + 0.1421)$

Keywords: Arsen, water, sediment, adsorption
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MO 247

Cd2+ and Zn2+ sorption on hydroxyapatite in the presence of EDTA in aqueous solution in equilibrium state

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Contaminated soil and water presents an unacceptable risk to human and ecological health and must be remediated. The removal of heavy metals from natural waters and soils needs an special attention because their variety of toxic effects. Among different treatments the adsorption technology is attractive due to its efficiency, economy and simple operation. Hydroxyapatite (HAP-$Ca_{10}(PO_4)_6(OH)_2$) is approved and recommended as a effective metal ions binding material.

The binding process on HAP is controlled by number of factors including pH, contact time, type of metal ions in a solution, HAP physico-chemical properties, ect. The fixation of metal ion on HAP surface may take place through one or more simultaneous mechanisms of: ion exchange, surface complexation, dissolution of HAP to form new metal phosphates, and substitution of Ca^{2+} ions in HAP by other metal ions during recrystallization. In soils and natural waters metal ions complexation by organic ligands and competing sorbates must be considered as well. Altough the sorption of heavy metals on HAP has been extensively studied, the dominating mechanism of complexation in the presence of chelating agents is under discussion.
The removal of Cd^{2+} and Zn^{2+} from aqueous solutions by hyrdoxyapatite was investigated with and without EDTA being present. Batch experiments were carried out using synthetic hydroxyapatite with Ca/P 1.60 and a specific surface area of 40.2 m^2/g in the pH range 4 to 9 (25 $^{\partial-\partial}$C; 0.1 M KNO_3). The initial concentrations of Cd^{2+}, Zn^{2+} and of EDTA were 0.002 M. The solid-solution ratio was 2 g/L.
The amount of Cd^{2+} and Zn^{2+} removed from the solution increased with increasing pH, reaching ≈ 90 % at pH 9. The presence of EDTA reduced the HAP removal capacity to 13 % due to the formation of $[CdEDTA]^{2\partial'}$ and $[ZnEDTA]^{2\partial'}$ in solution. In a binary solution (Cd+Zn) the competition of metals reduced individual sorption capacity 10-15 % compared with the single component solutions but the total adsorption maximum was approximately constant (0.8 ± 0.1 mM/g). In a Cd-Zn-EDTA-solution the Cd^{2+} sorption was reduced but the sorbed amount of Zn^{2+} did not change remarkably. In any case the sorbed amount of Zn^{2+} was higher compared with Cd^{2+} and was less affected by solution composition. The solubility of HAP increases in the presence of EDTA at pH values above 6, mainly due to the formation of $[CaEDTA]^{2-}$. The surface composition of solid phases was analysed by X-Ray Photoelectron Spectroscopy (XPS).

MO 248
Effect of farm management practices on concentrations of halogenated pesticides in New Zealand streams
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Halogenated pesticides, especially organochlorine pesticides (OCPs), have raised global concern due to their long persistence, low biodegradability, wide distribution in the environment, and chronic adverse effects on wildlife and humans. Because of their high lipophilicity, the bioaccumulation of small fractions of halogenated pesticides can pose potential hazards in the long term. In New Zealand, pesticides have brought considerable financial benefit and no other method of weed and pest management has been as cost-effective. However, financial benefits of using pesticides are often outweighed by negative environmental and health effects. Most OCPs are banned in New Zealand; nevertheless, their persistence means that residues continue to be detected in sediments and other environmental matrices. A number of studies have been published that compare the environmental impacts of different farm management systems. However, to the best of our knowledge, no studies have compared the concentrations of pesticides in stream sediments from conventional, integrated, and organic farms. Hence, the present study aims to determine if different farm management programs affect pesticides concentrations in streams that pass through farms.
The sediment samples being used in this study were collected from farms at five locations on the South Island, New Zealand. The samples were mixed with diatomaceous earth (DE) and then extracted with n-hexane-acetone (1:1 v/v) using accelerated solvent extraction. The extraction cells were loaded with additional DE and Florisil to remove excess moisture and macromolecules, respectively. The final extracts were analyzed for dieldrin, endrin, endrin aldehyde, α-HCH, γ-HCH, trans-chlordane, cis-chlordane, trans-nanochlor, cis-nanochlor, endosulfan I, endosulfan II, endosulfan sulfate, and chlorpyrifos using GC-ECNI/MS. The data was analyzed using SPSS software. The results indicated that the concentrations of dieldrin, endrin+endrin-aldehyde, γ-HCH, and sum of endosulfans were higher in the streams passing through conventional farms compared to integrated and organic farms. Interestingly, the concentration of α-HCH was higher in streams passing through organic farms compared to integrated farms and no significant relationships were found for chlorpyrifos or the sum of chlordanes.

MO 249
Characterization of heavy metal contamination of sediments from Douro River basin, Porto, Portugal
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Douro River is the second most important river in Portugal, travelling a total of ca. 900 km until the Atlantic Ocean, approximately 200 km being in the Portuguese territory. Its estuary is located between the cities of Porto (north side) and Vila Nova de Gaia (south side), in Northern Portugal. This area, besides being highly industrialized is also densely populated, and Douro River estuary receives large amounts of untreated sewages as well as continuous discharges of a total of eight wastewater treatment plants. Therefore, the occurrence of significant metal contamination in this area, particularly of sediments, could be expected. Sediments act as important reservoir of many hazardous chemicals, including heavy metals, which make them a secondary source of pollution. This is particularly important when sediments are eroded and transported further downstream (due to flood and resuspension processes). Along the course of the river to the sea, transportation, dilution and redistribution of sediment-associated metals occurs, and this may have an important impact on the chemical and ecological quality status of the estuaries. In order to assess the metal contamination of Douro River basin sediments, samples were collected during 11 campaigns from March 2009 to July 2010 on 6 different sampling sites along the estuary, at both margins. The 66 samples collected were preliminarily evaluated regarding humidity and granulometry. The fine fraction (< 63 μm), as well as a certified reference material (CRM) of estuarine sediment, were then submitted to a microwave-assisted acid digestion (ca. 0.5 g of dry sediment were digested in high-pressure Teflon vessels with 6 ml of suprapure concentrated nitric acid) and the metal content in the obtained solutions were determined by ICP-MS. Preliminary results show Ni, Cu and Pb levels around 50 μg/g, suggesting that the Douro River basin is significantly contaminated by these heavy metals.
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MO 250
Evaluation of DDx degradation in Sediments of Lago Maggiore (Pallanza Bay): a case study of historical contamination
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As part of a series of sediment characterization investigations performed in 2009-2010 in the Lago Maggiore (North Italy), radiodating analyses (210Pb and 137Cs) were undertaken in 9 sediment cores to better understand the sediment accumulation rates in the Pallanza Bay (Lago Maggiore) and to examine historical trends in chemical loading of DDx to sediment. Historically, a DDT manufacturing facility was located on the Fiume Toce (that inflows into Pallanza Bay) and discharged industrial wastewaters containing DDx to the river.
In details, radiodating analyses (137Cs, 210Pb) were performed on sediment cores from 9 stations, chosen to span a range of likely sediment accumulation rates. For each station, the sediment accumulation rate was determined based on the activity of 137Cs and 210Pb in 2-4 cm depth increments of each core. Cores recovered for assessment of sediment accumulation rates were also analyzed for DDT isomers and metabolites.
In parallel, sediment chemistry was investigated collecting and analyzing DDT isomers and metabolites (but not investigating isotopes) at various depth in about 80 sediment cores collected throughout the bay.
For both radiodating and chemical analysis, coring relied on a lightweight sediment-water interface gravity corer, to collect undisturbed cores and high quality data.
The assessment of linked geochronology (and their extrapolation to the whole bay) and chemical data improves the comprehension of degradation and DDT fate inside the bay, together with the understanding of sedimentation behavior and historical trends in chemical loading to the bay. Results show that concentrations of DDx have overall declined significantly relative to both the highest measured concentrations of each analyte at depth, as well as relative to concentrations measured contemporaneous with the 1986 peak in 137Cs activity. For DDT, the most significantly elevated DDT concentrations occurred in the mid-1960s; DDT degradation to DDD or, in minor part, DDE, appears correlated to sedimentation rates in the different location of the bay. This poster presents methods and results with major focus on the ratio DDT/DDx in sediments and considerations about degradation and DDx environmental fate.

MO 251
Application of NEBA and sustainability analysis to evaluate sediment management options for ports and harbors
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Sustainable sediment management is a comprehensive approach for addressing the long-term management and conservation of clean and polluted sediments. The goal is to maintain current and future services, including human use and ecosystem-based, while balancing the broader environmental, economic, and societal concerns. Typically, sediments are managed on a project-by-project basis without the benefit of a comprehensive, sustainable strategy to reduce costs and improve environmental benefits. Net environmental benefit analysis (NEBA) is one tool increasingly used to forecast different sediment management and remediation decisions. NEBA, incorporating a set of specific quantified ecosystem service metrics, is a framework that provides a scientific basis for balancing the investment costs and labor with the environmental and societal benefits imagined during decision-making. A NEBA identifies the "breakpoints"where costs become disproportionate to the benefits gained. By doing so, sustainable sediment management activities can be identified that minimize impacts on ecological and human use services and maximize value to the public. This presentation presents two case studies demonstrating the use of NEBA and different sustainability evaluation tools to examine the environmental, economic, and social benefits and costs of different sediment management alternatives for addressing PAH and metal-contaminated sediments in a coastal river that has been subject to decades of industrial pollution and storm water discharges. The presentation illustrates the concepts of air, carbon, ecological, energy, and water footprinting and the tools used to identify the tradeoffs between different management options and select the best practical option. Applied appropriately, and with careful consideration of uncertainties, tools such as NEBA and sustainability assessment frameworks are useful to balancing the disparate environmental risks, project costs, economic benefits, and ecological impacts typically involved in the evaluation of port and harbor projects.

LC01 - Developments in Life Cycle Impact Assessment (LCIA)

MO 254
Are chemical scores better assessed using USEtoxTM or the classical less sophisticated CDV from the EU Ecolabel?
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Science & Environnement, GENEVA, Switzerland
USEtoxTM, a multimedia fate modelling, proposes new characterisation factors (CF) for human toxic impact and freshwater ecotoxic impacts to be used in product LCA. Calculated on more than 3000 cghemicals, these CFs constitute a new ranking of chemicals based on their hazard proprieties. USEtoxTM is now also consired by the French consumer product carbone footprint information scheme (under development) to be the basis for the calculation of two additional criteria: human and eco toxicity. Nowadays, Life Cycle Assessment and the EU Ecolabel are the two tools used to identify on the market environmentally friendly products, but do they provide the same answer? Both tools also provide a ranking of chemicals based on their hazard properties, but are the top 10-20 most important toxics identified by these two tools the same? Is there a risk that consumer products get the wrong label?
The comparison exercise between the EU Ecolabelling and the USEtoxTM ranking score have demonstrated that both models identify the same five chemicals of highest concern for the environment and that there is an overall 'ok' agreement between the two models ranking scores. The difference between the two models can probably be explained by differences in the degradation or ecotoxicity data. By using similar fate and effect data in both systems, it is likely that the correlation will become stronger. From a product labelling perspective though, one could conclude that using a simple model as the CDV approach is probably good enough and certainly less subject to variability in the results. The CDV requiring only two parameters while USEtoxTM more than 15, without mentioning the complexity in calculating score.
In any case, if both system are used for product comparison and/or labelling, a list of agreed and peer-review CF must be published to be used by all practionners to avoid that ranking of chemicals or product changes due to a change in the data use for the assessment.

MO 255
Critical review of USEtoxTM and comparison of chemical ranking scores with the EU Eco-

label and the EUSES model

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In the last years, the concern for the ecological impact of chemicals in the environment has largely increased and with it the development of tools to assess the risk/hazard of chemicals. Nowadays, Life Cycle Assessment (LCA) appears as a new tool to address this issue, creating a debate with the Risk Assessment (RA) community. In may 2010, after several years of intense work and many workshops held with LCA and RA experts, the consensus model USEtoxTM was officially launched and promoted as a tool for policy makers and regulators.

There might be however a risk of confusion among decision makers and regulators about what USEtoxTM can really do. This confusion comes from the fact that USEtoxTM uses the same wording as RA, suggesting USEtoxTM looks at the risk assessment of chemicals, whereas it rather looks at the hazard associated with chemicals. Can however the same conclusions be drawn from LCA and RA model predictions? Nowadays, Life Cycle Assessment, the EU Ecolabel and the RA based EUSES model are the three tools used to identify environmentally friendly products on the market. But do they provide the same answers? Those tools also provide a ranking of chemicals based on their hazard properties, but are the same top 10-20 most important toxics identified by these three tools? Is there a risk that consumer products get the wrong label?

To attempt to answer those questions, first a critical review of the USEtoxTM model has been carried out. Moroever, the ecotoxicological impact risk of 69 chemicals have been assessed using those 3 different methods: USEtoxTM, the EU Ecolabel and EUSES. These three have very different ranking methods covering complex life cycle assessment approach, simple critical dilution volume approach and classical risk assessment approach, the two last ones being methods already extensively validated.

Preliminary results of chemical ranking using the USEtoxTM and the EU Ecolabel indicate that, despite showing similar trends, the two models agree on the ranking of the five first chemicals only. The large uncertainty associated with complex modelling and the quality of the initial ecotoxicological data, seems to be partly responsible for the observed discrepancies. The large difference in risk/impact assessment observed for a few chemicals have been further investigated to better understand what causes two models with the same objective to have very different prediction, as this could lead to very different decision my policy makers.

MO 256

Normalization references for USEtoxTM-based toxic impact categories: North American and European economic systems

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As an optional step of the life cycle impact assessment (LCIA) phase in the ISO standards, normalization aims to express the magnitude of the impacts by comparing the characterized results against a common reference situation - the normalization references. In this study, we used inventories of two economic regions, North America and Europe, to calculate normalization references for the three currently-modelled USEtox™-based impact categories, i.e. freshwater ecotoxicity, human toxicity, divided into cancer effects and non-cancer effects. Base years for the references are 2004 for Europe and 2006 for North America. The normalization references have been calculated using recommended factors as well as with interim factors, as needed. It is found that, in spite of different inventory assumptions, the normalization references fall within the same order of magnitude for both North America and Europe. By analysing the most contributing substances, metals turn out to dominate the impacts in both regions. This may be explained by the interim status of the characterization factors (CFs) for metals, which might be overestimated in the current model. Part of the explanation may also lie in the incomplete coverage of organics in both the inventory and the CF databases. With respect to the intended global character of the USEtox™ model, different approaches to determine normalization references of other economic systems (e.g. Asia or world) are discussed in relation to these findings. Overall, we thus recommend the use of the provided set of normalization references for USEtox™, but we also advocate 1) to perform an update as soon as a more comprehensive inventory can be obtained and as soon as characterization factors for metals are revised; 2) to consider extension to other economic systems in order to allow normalization in USEtox™ to be used on a global scale.

MO 257

Spatial differentiation of chemicals in Life Cycle Impact Assessment for ecotoxicity and human toxicity

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The ability to consider influence of regionalization is recognized as one of the challenge in order to increase relevance of human toxicity and ecotoxicity in life cycle assessment.

In recent years, several spatially distributed fate and transport models of chemicals, i.e. models allowing spatially explicit representations of contaminants from a given spatial distribution of sources were developed.

A key issue to be addressed in the development of the life cycle impact assessment methods and corresponding characterisation factors is the level of spatial detail required in the life cycle impact assessment and the related uncertainties assessment. A cross comparison among several models were performed to determine conditions which warrant spatial resolution for characterisation factors concerning ecotoxicity and human toxicity.

A set of organic chemicals representing large range of substances with different physical chemical properties is used in the evaluation under different emissions scenarios, such as multiple source vs. point source emissions.

Three models (Mappe, Impact world, Usetox) are used to assess fate of chemicals, from the local to the global scale. Global default values of characterisation factors derived from Usetox are compared with the results of Impact world and Mappe to highlight the relevant drivers of impacts and the main constraints in calculating fully spatial resolved characterisation factors at fine resolution.

The results will help practitioners to identify situations for which spatial differentiation in the life cycle impact assessment of toxic chemicals should be considered relevant as well as how to create consistency between inventory and impact assessment regarding regionalization.

MO 258

Interspecies correlation estimates for ecotoxic effects in Life Cycle Impact Assessment

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Ecotoxicity is often excluded as impact category in Life Cycle Assessments (LCAs), because information is lacking on the fate and effects of many chemicals. This study uses Interspecies Correlation Estimation (ICE) statistical models to enhance the effect dataset to be applied in Life Cycle Impact Assessment (LCIAs). Next to laboratory tests and QSAR-estimations, ICE models have been developed as an additional approach to estimate the toxicity of chemicals to both aquatic organisms and terrestrial wildlife. The acute toxicity value of one species (i.e. the surrogate species) is used to produce correlation toxicity values for multiple species. In this study, we used ICE to estimate hazardous environmental concentrations for 50% of aquatic species, and hazardous doses for 50% of wildlife species. The data were subsequently applied to derive Effect Factors (EFs) for LCIA. As the introduction of estimated data brings additional uncertainty in the input data, the uncertainty in EFs based on ICE needs to be weighted against the uncertainty in EFs based on a low number of experimental data only. The poster shows the EFs based on ICE model estimates, together with their uncertainty ranges. It also compares EFs based on ICE and EFs based on small experimental datasets.

MO 259

Case study to evaluate USEtox and Critical Dilution Volume (CDV) as methods for assessing the ecotoxicity impact of laundry products

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There is increasing interest in the assessment and comparison of the environmental impacts of products. Schemes such as Grenelle de l'Environnement, currently under development in France, aim to assess and communicate the impacts of consumer products for a few selected impact categories, including freshwater ecotoxicity. This poster presents the results of a case study to compare USEtox and Critical Dilution Volume (Eco-labelling) for assessing the ecotoxicity impacts of laundry products. The scope of the study focused on the end-of-life stage, i.e. when the products are discharged after use into the wastewater system, whereas the 'cradle to factory gate' impacts have been excluded. The poster presents the results of the case studies and discusses the application and limitations of each method within the wider context of assessing product impacts on water quality. A second aspect of the study was to broaden and refine the characterisation factors (ChF) of detergent ingredients published by the USEtox team and to evaluate differences in calculation approaches within the USEtox tool. This shows a need for a review/evaluation process of USEtox ChFs.

MO 260

USEtoxTM for assessing impacts of cosmetics products for the French regulation of environmental footprint of mass market products

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New environmental regulation for footprint calculation of mass market products distributed in France is currently implemented following the indications of the Grenelle II law. Companies will be requested to provide a quantified assessment of environmental performances of products covering several impact categories such as resource depletion, greenhouse gas (GHG) emissions, water or air pollution. Whilst impact categories are easily quantified using existing methods, new ones such as aquatic ecotox are more complex to address. Nevertheless, this impact category is crucial for environmental footprinting of products directly emitted in water (shampoo, detergents). For those products, the ADEME has identified USETox as the most suitable model for assessing aquatic ecotoxicity in LCA. Applying USETox is promising in terms of results interpretation but requires the development of a specific expertise and the collection of new environmental data for product characterisation. In that frame, Cycleco proposed to the ADEME to provide support for producers and manufacturers of cosmetics or detergent products and ingredients. The developments are done by Cycleco. The overall purpose of the project is to (1) Support companies for assessing the aquatic ecotoxicology footprint of their products; (2) Develop a database providing USETox characterisation factors for cosmetic ingredients and detergents available via the ADEME LCA database for environmental footprinting. Cycleco has already provided to the French ADEME and to the French ministry of Environment a guideline for implementing USETox to be used in the French Environmental Footprinting. Such a large scale experiment for USETox in a considerable step forward, and for that Cycleco was required to produce data enabling large scale use of the model for the cosmetic sector. Thus Cycleco has developed more than 500 new factors in order to cover most of the ingredient used for cosmetics products. In a further development a complete database for cosmetic ingredients will be produce, making USETox easy to use for all cosmetics producers. Beyond the production of the 500 new characterisation factors, the footprinting of several rinsable product (mainly shampoo) have been done on the basis of these new data using LCA, and the main results are presented in the poster highlighting the importance and complexity of assessing ecotoxicity of cosmetics products for the calculation of environmental footprint.

MO 261

Evaluation of toxicity in cotton production and toxicity impact assessment methods

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Toxicity from cotton production is a major concern for cotton producers as retailers and consumers begin to incorporate environmental sustainability in their decision making process. We selected five methods that provided index values for each pesticide, without requiring any other parameter input. Four of these methods (CML, Impact 2002 +, ReCiPe, and TRACI) were part of a more complete life cycle assessment methodology and accessible in SimaPro software. The fifth method, EIQ, available in Microsoft Excel, was a method used specifically for assessing toxicity from pesticides. EIQ is a US-based model. Its assumptions and calculations are somewhat more straightforward and explicit. In addition, its impact categories (consumer, farmworker, fish, bees, etc) are more explicit. EIQ also has the most comprehensive list of index values for pesticides used on cotton. However, EIQ's methodology appears to have less capability of distinguishing between magnitude of toxicity of pesticides. It is unclear and beyond the scope of this study to understand how well these methods would perform when comparing cotton production with other agricultural products, or comparing cotton production with other textile production. Ultimately, each of the three methods has its strengths, and is a valuable tool in estimating toxicity from cotton, and all agricultural production. While the methods do correlate, they are not equal. Therefore method selection should depend on the needs of the analysis, such as which pesticides are under study, the type of toxicity being studied, and whether the study is specifically for toxicity from pesticides, or something more broad, including multiple environmental impacts.

MO 262

Life Cycle Impact calculation at global scale and at site-specific scale - damages on coastal waters and soils in the Milazzo Peninsula (North-Eastern Sicily, Italy) -

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The Milazzo Peninsula, located in the North-East of Sicily (Italy) is a highly polluted area. Since the last decades, many studies show an increase of the number of cancers and lung infections in this area. The coastal environment is also stressed and an important line of coast is forbidden to swimming due to pollution. Two main anthropogenic activities seem to contribute to the environmental damages: a thermo-electric plant and a refinery.

The main objective of this study is to calculate the damages effectively caused by those two activities, in order to analyze their real contribution to the damages and to understand if there can be other causes.

The Life Cycle Impact Assessment is used in order to better estimate the respective damages on two environmental compartments: the waters (marine and fresh) and the soils.

Seasonal sampling campaigns are realized to collect soils and waters. Initially, heavy metals, trace elements, Polycyclic Aromatic Hydrocarbons and hydrocarbons are analyzed in order to determine the effect and fate factors of the pollutants at a local scale. The analytical methods are ICP"ðMS for the heavy metals and the trace elements, and chromatography for the hydrocarbons, linear and aromatic. In a second time, we calculate the terrestrial and aquatic ecotoxicity generated by these pollutants. Two methods are used. The first one is a "global"method using the software SIMAPRO, european databases and two calculation methods (Recipe and Impact 2002+). The second one is a site-specific approach with a specific characterization of the toxic emissions.

The aims of this poster are: 1) to analyze and interpret the pollutants concentrations that have been found in the different compartments; 2) to assess the impacts and damages upon the chosen compartments using the "global-scaled"method; 3) to assess the impacts and damages upon the chosen compartments by the site-specific approach; 4) to compare the results of both approaches.

MO 263

Regionalized global atmospheric source-to-receptor modeling for LCIA acidification

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This paper aims to provide regional worldwide source-to-receptor fate factors for acidifying emissions (NOx, SOx and NHx) within life cycle impact assessment. For this purpose, the three dimensional global scale tropospheric GEOS Chem model was selected. Model simulations were run for the 2005 year of reference. A novel methodological approach was then developed to derive emission-to-receptor matrices with a global 2x2.5 (latitude x longitude) horizontal grid. This new approach overcomes current modeling limitations requiring time-intensive calculations at the expense of a reduced spatial coverage and/or lower spatial resolution. The results of this approach were evaluated against the results from another atmospheric model, EMEP. To do so, the 2x2.5 grids were aggregated at a European country level resolution and compared with source-to-receptor values from the unified EMEP model. Comparison showed good agreement for the NOx and an acceptable agreement for the SOx. For the NHx, close range deposition was systematically lower than EMEP model predictions. Consequently, a correction factor was applied to match EMEP reported local deposition. Comparison also revealed that the source-to-receptor matrices generated with this approach slightly underestimate the overall deposition originally simulated by the GEOS Chem model by about 10 to 17%. This means that when GEOS Chem predicts that 96% of an emission will deposit, this percentage drops to 80% when using the source-to-receptor matrices. Continental fate factors were calculated by aggregating the respective 2x2.5 air grid results using area weighting. Results showed that at a continental level, 40 to 70% of an emission will deposit within the same continent and 10 to 30% will deposit into seas and oceans.

MO 264

Review on methodology for LCIA of marine eutrophication

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As part of the ongoing EU FP7 project LC-Impact (www.lc-impact.eu) new life cycle impact assessment (LCIA) methods are going to be developed and tested on industry cases. Among the life cycle assessment (LCA) impact categories in focus are aquatic eutrophication. As related to especially the marine environment very few and restricted attempts have yet been done on trying to include eutrophication in LCA. The aim of LC-Impact is to develop both a global and a spatial (and temporal) differentiated model, as both central fate processes, sensitivities of receiving environments (e.g. differences in limiting nutrient and variations in this over the year) and the resulting damage can show important spatial variations. Both midpoint and endpoint (damage) modelling are included and the aim is to base the damage modelling on dose-response curves expressing the correlation between the (increase in) nutrient concentration and the potentially affected fraction of species in the marine ecosystem. This poster will present a review of the very limited existing attempts on how to include marine eutrophication in LCA and discuss alternative methodologies on how to model the environmental mechanism of this impact category.

MO 265

Globally applicable, spatially explicit assessment of non-toxic air pollutants

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1. Introduction

This paper presents intake factors (iF) and characterization factors (CF) regarding human health effects for the pollutants NH3, NMVOC, NOx, SO2, primary particulate matter (PPM), and also accounting for the reactants ozone and secondary inorganic aerosols (SIA). Research on LCIA for transboundary pollutants have been reviewed and it is suggesting that spatially differentiated fate modelling may be crucial. The present work aims to fill the gap of consistent global modelling by developing an approach to derive globally applicable and spatially explicit values, and by providing a comparison between models with a different degree of spatial resolution.

2. Materials and Methods

Within the ongoing EU-funded project LC-IMPACT (2010-2012) the "Development and application of environmental Life Cycle Impact assessment Methods for imProved sustAinability Characterisation of Technologies"is pursued. Within the area of "Non-toxic pollutant impacts"the objective is to develop globally applicable, but spatially explicit, life impact assessment methods and characterization factors.

Within the TM5-FASST modelling framework, the world is divided into 54 regions. Each region serves as a source region and each grid cell (resolution 1°x1°) of the whole world serves as receptor region. Population data and concentration response functions are applied in order to calculate the iF and relevant diseases. A new methodology for evaluating emissions due to transport activities has been applied taking into account the urban increment for emissions in urban areas. Finally, the application of monetary valuation is demonstrated in order to derive specific damage cost estimates per unit of emission by including and excluding equity weighting.

3. Results

A detailed comparison will be made between iFs derived by the TM5-FASST model and by the EcoSenseWeb integrated assessment tool in order to assess the inherent uncertainties and implicit variability in the estimates. For primary particulate emissions specific conditions (i.e. height of release, adjacent population density and distribution) are taken into account, and therefore, iFs and CFs are calculated for several archetypes such as, urban, non-urban and remote sources with different height of release. Finally, iFs and CFs suggested in different LCIA methods have been compared with the findings of this work and further results from LC-IMPACT, as well.

MO 266

Development of damage assessment method for biodiversity caused by global warming

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According to Millennium Ecosystem Assessment (2005), climate change is recognized as one of the main factors of biodiversity damage. Though several damage assessment methods used in LCIA have been developed to quantify the damage of biodiversity caused by land use and chemical exposure, there are still few methods for climate change. Therefore the aim of this study is to develop a damage assessment method of biodiversity caused by climate change using EINES (Expected Increase in Number of Extinct Species) as an indicator of extinct risk.

The procedure of the method is as follows. First the future temperature change and the amount of rainfall change caused by CO2 emission were predicted using MAGICC (Model for the Assessment of Greenhouse-gas Induced Climate Change) model. Secondly, the change of future distributions of species by future climate change was projected based on bioclimatic modeling (also called niche-based model). Then we linked the results of bioclimatic models (shifts and reductions in species' ranges) to extinction risk based on the IUCN Red List Criteria (IUCN 2001). Thirdly, EINES was calculated with an inverse of the time (years until extinction) which was estimated from extinction risk of IUCN Red List Criteria. Finally, the extinct risk of Japanese vascular plant was estimated based on the method.

MO 267

Accounting for the potential value of temporary carbon storage: the issue of choosing relevant time horizons

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Currently, climate change is an impact category of great importance in LCA, but the issue of possible climate change mitigation potential from temporary carbon storage is not considered consistently. Several proposals for accounting for the climate change mitigation value associated with temporary carbon storage have been made; however most of them are based on an interpretation of the 100-year time horizon, adopted from the Kyoto Protocol. The consequences of this interpretation are discussed here, and it is illustrated how such a relatively short time horizon is hiding the long-term impacts of anthropogenic released CO_2 in the atmosphere, thus risking to 'green wash' the use of fossil fuels by employing temporary carbon storage as compensation for their release. Based on an overview of the complete carbon cycle on Earth, another approach is suggested here: For temporary carbon storage to provide real long-term climatic benefits, storage should at least be ensured for a thousand-year timescale, in order to facilitate simultaneously removal of carbon from the near-surface layers of the carbon cycle down to deeper layers, from where it is not as easily released again. However, as there is also a key issue of not crossing potential nearby climatic tipping points, there may also be a climatic benefit from more short-term storage solutions, buying society time to become more carbon neutral before the carbon is released. Such short-term storage value should however be accounted separate from the long-term climatic benefits, illustrating that they address two different parts of the climate change problem.

MO 268

Environmental performance of electric taxis compared to diesel taxis in Copenhagen: a case study including the impact of traffic noise upon human health

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Noise may well be one of the most pervasive issues affecting human well-being and yet it is largely ignored in everyday LCA practice. The marginality of noise within LCA can be explained by the particular characteristics of noise emissions as a pollutant, and by several methodological shortcomings (a problematic selection of health effects and dose-effect relations, the nonlinearity of impacts) that still hinder the inclusion of transport noise as an established impact category within LCA.

In this work, the environmental performance of a hypothetical taxi fleet is assessed for the city of Copenhagen. Two scenarios are compared: the first one considering diesel vehicles, the second one considering full electric vehicles (EV). Both scenarios take into account best available technologies. The impact categories covered include global warming, non-renewable cumulative energy demand and impact upon human health. Moreover, this work presents the first real-world application of the assessment method for the inclusion of the impact of road traffic upon human health within LCA proposed by Franco et al. Said methodology builds upon the incremental approach to assessments proposed by Müller-Wenk, whose calculation assumptions and procedures are modified in order to improve the accuracy of assessments. The IMAGINE traffic noise emission model is used to model the emissions of EV and Diesel taxis, and the resulting sound power emission levels are computed. The use of said traffic noise emission model leads to faster, more versatile assessments of health impairment due to traffic noise.

Earlier quantifications of health damages attributable to traffic were made in terms of DALY units. In this research study, self-reported annoyance -derived from the dose-response functions developed by Miedema et al.- is proposed instead as the primary indicator for health impairment due to traffic noise. Hence, the midpoint indicator is still comparable with other endpoint categories. Moreover, geo-referred information reported by EU member states can be easily included in the exposure assessment. This straightforward use of indicators termed in plain,

natural language (e.g. '[additional] number of persons annoyed by traffic noise') has the benefit of presenting results in a manner that is intelligible to both policy-makers and the general public, while being comparably effective in communicating the impacts of noise.
Keywords: noise, electric vehicles, LCA, number of persons annoyed

MO 269

Endpoint characterisation factors (CF) in the unit of [DALY per Bq] and damage costs [Euro per Bq] for selected radionuclides releases during normal operation of nuclear fuel cycles

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Characterization Factors

A simplified approach for characterization factors (CF) for assessment of potential human health impacts related to radioactivity for a selected number of radionuclides has been developed. Updated data on relation between Bq and manSv, according to UNSCEAR reports from 1993 and 2000 [1], has been used. This approach is site generic because for the UNSCEAR reports typical population densities for certain processes have been assumed. The dispersion and exposure modelling, i.e. the relation between emission [Bq] and dose [manSv] is included in these dose factors. The approach has been implemented in the EcoSenseWeb tool [2] in order to evaluated impacts due to nuclear fuelled electricity generation. However, the CF can also be applied to other sources with similar environmental conditions.

Effect Factors

The factors relating collective dose [manSv] to human health impact, are based on the ICRP60 [3] recommendations, i.e.:
0.05 cases per manSv for fatal cancers
0.12 cases per manSv for non-fatal cancers and
0.01 cases per manSv for hereditary defects.
Moreover, updated factors from ICRP have been compared.

Damage Factors

These endpoints are weighted in the following way
1.62 DALY per non-fatal cancers
16.2 DALY per fatal cancers and
37.5 DALY per hereditary defects.
In addition, latest WHO 2002 statistics are applied, ensuring a consistency with the treatment of cancers used for the human toxicity category, with average of 11.5 DALY per case of cancer against 17 DALY per case, as reported by [4].
The results have been compared with other LCIA methods, e.g. based on [4] or [5], on the basis of DALY per unit of activity emission.

Damage Costs

For monetary valuation the DALY have been multiplied by the value of a life year lost (VOLY-acute) of 60,000 $Euro_{2000}$ per year (based on l[6]).
Finally, discounting with declining discount rate is applied to the most important substances, i.e. Rn-222, H-3 and C-14.

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MO 270

Impacts of agricultural land use changes on ecosystem services

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Land use is considered to be an intensive human activity that aims at exclusive use of land for certain purposes, such as agriculture, and adapting the properties of land areas in view of these purposes. All basic land use activities result in either damage to or benefits for ecosystem quality. The ecosystem quality can be expressed in certain ecosystem services, such as carbon stock, erosion regulation, and nitrogen cycling. Over the years the idea has been evolving that life cycle oriented methods should encompass spatial analysis of ecosystem services, since these form the basis of planetary activities and human well-being. In this research we focus on the changes in ecosystem services due to the expansion of land area used for agriculture in need to increase production of rapeseed, corn, and other crops. The objective of this research is to provide quantitative estimates for the changes in ecosystem services, that can be applied for life cycle assessment (LCA) purposes. To calculate this, the Environmental Policy Integrated Climate model (EPIC) will be used, which applies the global land use database Global Land Cover (GLC) 2000. EPIC is a global spatial and temporal agri-environmental model, which simulates bio-physical impacts on homogenous response units characterised by altitude, slope and soil class. The major processes included in EPIC are weather simulation, hydrology, erosion, nutrient and carbon cycling, pesticide fate, crop growth and competition, soil temperature and moisture, tillage, cost accounting, and plant environment control. The output of the model can be crop yields, hydrology (PET, runoff, percolation), sediment transport, N-leaching, green house gases, and soil carbon sequestration. Model output can be aggregated to e.g. province, country, ecozone, or biome level. The poster will show results of changes in CO2 emissions, erosion potential, and biomass stock due to an increase in crop production in various world areas. These results can serve as input in life cycle inventories or serve as midpoint indicators in life cycle impact assessment.

MO 271

LCA biodiversity indicators using cocoa (Theobroma cacao) as a model crop

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Biodiversity conservation is one of the hot topics of today's research and policy developers. Cocoa production is reaching an all-time high and as its production increases, cocoa forests offer unprecedented opportunities for biodiversity conservation through production as well as a continued threat to destroy the very biodiversity that it could help conserve. There is a definite need to develop a metric for measuring biodiversity in cocoa plantations; literally dozens of articles have been written on the value of biodiversity, the conservation benefits, the pest control, the longevity and sustainability it adds to cocoa production, and the possible economic benefits of cocoa. Corporations, farmers, and governments looking to the future want to reap these benefits and select a crop system for cocoa that will help eliminate the boom and bust cycle that it has suffered and will be sustainable over centuries. For these reasons and more, a metric is needed to assess the biological diversity found in cocoa plantations under different management systems.
One method for assessing biodiversity posited has been to survey target species that are deemed "ecologically significant", keystone species who may reflect a greater total biodiversity or greater number of ecosystem services proffered. Determining which species or taxa should be targeted presents significant difficulty and requires extensive knowledge of the particular ecosystems, and cocoa is now a global crop. Traditional indices of biodiversity may also help, but they tend to be hamstrung by specific requirements of the ecosystem to which they are applied. Species counts by taxa, by species, along with relative abundances may profer a sense of species richness and species diversity. Unfortunately, such overarching ideals are not feasible due to the dearth of primary data from the disparate regions and production systems.
Further considerations also include the necessity of measuring endemic biodiversity to total biodiversity. If the cocoa farms are considered biologically diverse, but the majority of the species are exotics, one of the original purposes of preserving biodiversity in the region has become moot. As with all factors in LCA, for a method of cocoa production to be touted as a "best practice"or as sustainable, a metric for biodiversity must be at least understood before establishment. That metric is what this research attempts to develop through the address of the aforementioned issues.

MO 272

Water use impacts in life cycle impact assessment

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Current LCA methodology is lacking in the area of evaluating the environmental impacts of water use. Typically freshwater consumption is presented in the form of a volumetric quantity. Without further information regarding type of water use, location of water withdrawal, indication of consumptive versus degradative use, and in-stream or off-stream use with respect of the quantity of water reported tells little about the impact. Furthermore, it is important to assess the status of the local environment which the volume of freshwater is withdrawn.
There is currently no agreed standard which to assess water scarcity. However, there are many proposed indicators used to assess water resources vulnerability. The majority of water stress indices are a function of freshwater demand and supply. In order to accurately assess freshwater stress or scarcity, an evaluation must be done at the basin level. Idealistically, water consumption by each sector (agricultural, domestic, and industrial) would be easily measured and available at the basin level for the entire globe. However, such data does not yet exist.
Other risk indices incorporate per capita water demand, environmental water requirements, and physical and economic water scarcity. Each index provides a different approach to assessing water resources vulnerability and are all informative evaluating corporate risk. By incorporating multiple indicators into the LCIA for water use, a comprehensive evaluation of local freshwater vulnerability can be made. Only after the status of local freshwater resources is determined, can the impacts of water use be determined.
Developing a method to quantify the variables surrounding the status of local freshwater resources is critical in order to evaluate the impact of freshwater use.

MO 273

Toward consistent emergy calculations with LCI databases: implementing the emergy algebra

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Life Cycle Inventory (LCI) databases like Ecoinvent are used for evaluating resource depletion and ecosystem quality within Life Cycle Assesment (LCA). LCA ignores, however, part of ecosystem services (material and energy flows) that are damaged by human activities and should therefore be included in the assessment. Emergy concept could fill this gap. Emergy is defined as the quantity of energy (solar energy, in MJse) directly and indirectly necessary to support a given system and its level of organization, providing a measure of the global Nature's efforts required to make a product or service available. The main drawback hampering the applicability of emergy calculations is the lack of detail and transparency of its allocation procedure. The use of LCI database for emergy calculation is therefore highly relevant to solve this issue. Unfortunately, Emergy requires a set of algebric allocation rules that are completely different to those applied in LCI. Two major problems are to be faced: 1) all the multi-output processes (and related natural resources) shall be included without LCA-like allocation but even though dealing with a square A matrix; 2) specific rules for co-products, splits and feedbacks make allocation at each node of the network depending on the surrounding nodes and links. This study provides a consistent conceptual and technical framework to definitively solve these problems, which is illustrated through an example of complex system based on ecoinvent unit processes. Datasets of the co-products of a multi-output process are kept separated but are assigned the full amount of inventory data of the multi-output process. The additional co-product generated by each dataset is then calculated. Conventional LCI calculations are performed to obtain the matrix s x A including all the mass and energy flows of the network. A novel algorithm for emergy algebra has been developed in Matlab to this purpose and applied to the s x A matrix. The emergy results obtained have been successfully re-calculated using Emsim, software for emergy calculation, by drawing the network as a graph. The framework is therefore validated on a complex case study. The approach is being applied to the whole Ecoinvent database. The framework will allow consistently introduce emergy in LCI and LCA, sound calculation of specific emergy of products (impossible task so far), generating a huge impact in the practice of sustainability assessment.

MO 274

The analysis of environmental impacts

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Environmental Impact Assessment (EIA) is an issue of major concern for every industry, for every nation and for every product that is in the market. The EIA constitutes one of the very important steps of Life Cycle Assessment (LCA). EIA constitutes one of the four steps in performing LCA. The definition goes further and includes the evaluation and implementation of opportunities to affect environmental improvements. It is very important to understand the nature and the limits of the impacts in order to be able to proceed with a plan to improve them. The environmental impacts occur because during the process there is a material and energy transformation that leads to the increase of the entropy. This will lead to various emissions and wastes. However, the environmental impacts could be divided into two categories: The impacts that are impeded in the process, that are unavoidable, and their magnitude depends on the efficiency of the process;

the impacts that are peripheral, the impacts affected by factors located in other than the main processes. The impacts that are included in the second category, the peripheral impacts, could be easily minimized with the implementation of certain actions. The impeded impacts require a very thorough knowledge of the production process in order to be able to set up a program of improvements.
This work will analyse these two environmental impact categories and show examples of production processes.

MO 275
Using multi-criteria decision making models in the framework of LCIA analysis
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Life Cycle Impact Assessment (LCIA) focuses on a charge of consumption and emissions produced during LCI to specific categories of impact attributable to known environmental effects. Moreover, also aims to quantify, with appropriate characterization methods, the magnitude of the overall contribution to the process or the product has against the effects concerned. The structure of a LCIA consists of some compulsory elements (mandatory elements) which convert the results of inventory in appropriate indicators that can be used directly or as a basis for optional subsequent evaluations LCIA.
In accordance with approved international standards, the study has to complete the selection of environmental effects to be considered, the results of the allocation being chosen LCI to environmental effects (classification) and the calculation of different indicators category, such as Nutrification Potential, Ozone Detection Potential, Global Warming Potentials, etc. While, the comparison between environmental indicators calculated with reference values (normalization) and the determination of the importance of individual environmental effects (weighing) are optional. In a lot of approaches, such as Eco-indicator method and Panel approach, the values of different environmental impact indicators are aggregated by weighted sum, obtaining a value that allows us to rank or classify the alternatives.
This implies, among others, the elicitation of trade-off weights and to accept the complete compensation among evaluations, for example good values on some indicators can be perfectly balanced by bad evaluations with respect to other indicators.
In this paper, we emphasize the possibility to aggregate multi-criteria evaluations using different approaches and operators, actually more suitable for environmental problems. More precisely, values of different indicators can be aggregate in a more flexible way, using operators not necessarily perfectly compensatory, allowing partial compensation and/or not transitive preferences, including also the possibility to model interactions among criteria. In the case of sorting, i.e. ordinal classification, for example, some reference profiles or only exemplary classification of a sample of well known objects can be introduced as preference model of decision maker. The output of these models can be outranking relations or decision rules, whose appropriate exploitation allows to built up the recommendation (choice, ranking or sorting) required.

MO 276
Life Cycle Impact Assessment: research needs and challenges from science to policy making
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In the Communication on Integrated Product Policy (IPP), the European Commission committed to produce a handbook on best practice in Life Cycle Assessment (LCA). In this context, JRC led a "science to policy"process which resulted in the ILCD International Reference Life Cycle Data System (ILCD) Handbook. ILCD Handbook is a series of detailed technical documents, providing guidance for good practice in Life Cycle Assessment in business and government. For Life Cycle Impact Assessment (LCIA), the Handbook provide guidelines to methods and assessments to analyse the emissions into air, water and soil, as well as the natural resources consumed in terms of their contributions to different impacts on human health, natural environment, and availability of resources.
Those guidelines come from a comprehensive process of selection of methods based on a set of scientific and stakeholder acceptance criteria and involving extensive hearings of domain experts, advisory groups and the public. In this "from science to policy"process a number of research needs, critical issues and challenges for Life Cycle Impact Assessment emerged.
Robustness of models and reliability of characterisation factors must be the basis for further development in Life Cycle Impact Assessment (LCIA). To foster the robustness and acceptability of existing and new methods, some of the points under discussion will be presented

LC02 - Developments in Life Cycle Sustainability Assessment (LCSA)

MO 282
Development of a Sustainability Business Assessment (SBA) tool in British American Tobacco (BAT) to support sustainable decision making
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This poster summarizes some of the tools and business enablers being developed in BAT to support sustainable decision making. The focus of the tools is on the provision of information to better understand the consequential supply chain impacts of product and process design.
Life Cycle Analysis (LCA) includes the 'seed to stub' model, and has been used to develop the Sustainability Business Assessment (SBA) tool across Group Research & Development (GR&D). The SBA has been incorporated into project management in the design community, and provides a disciplined approach towards the assessment of the environmental, social and economic implications related to a new product, process, material or innovation to the business. These studies compare existing impacts to those created by the introduction of a new material or innovation, and provide an opportunity to mitigate consequential impacts along the supply chain.
The environmental part of the SBA is delivered through LCA studies. For example, water and energy use, and emissions to air and water. The social part of the SBA includes the provision of health and safety procedures, and use of robust scientific methods.The economic part of the SBA includes cost of machinery, processes and capability and continuity of supply. A case study illustrating the use of the SBA will be presented.
The LCA database also enables the creation of Interactive-reports which allow supply chain communities to run business scenarios and compare the environmental impacts (for example: CO2 impacts related to transport decisions).

MO 283
Use of aluminium vs. iron metals in the car industry. Comparative evaluation made by using LCA and external costs methodologies.
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Aim of the study is the evaluation of the environmental advantages and potential reduction of external costs that can be achieved through substitution of the traditional iron based materials (pig iron and steel) with aluminium in the production of road transport vehicles. Data about circulating car and lorry fleets, mileage, vehicles composition, etc., refer to year 1997.
Quantification of environmental impacts has been carried out by using LCI data provided with the SimaPro software.
Results show that primary aluminium production determines external costs per unit mass higher than those due to iron and steel production (3.900 lire97/kg, 460 lire97/kg, 760 lire97/kg respectively). However, secondary (80%) aluminium production determines much lower costs (700 lire97/kg), very similar to those than iron and steel. These data indicate the importance of aluminium recovery and recycling.
Fiat Research Centre states that for a 1000 kg car a weight reduction of 10% determines a consumption reduction of 6% , similarly to what behold Alcan Europe and other in the document "Aluminium for the future generations".
Two different scenarios have been simulated that include a short term modest weight reduction (10%, scenario A) and a mid term higher weight reduction (20%, scenario B): for both scenarios has been assumed the option of using primary (A.1, B.1) and secondary (A.2, B.2) aluminium in substitution of iron and steel.
The study indicates that a vehicle weight reduction obtained through a progressive substitution of iron with aluminium in some components determines a reduction of the external costs evaluated over the whole vehicle life.

MO 284
Development of an evaluation method of sustainability for events from the view point of environmental, economic and social aspects
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Towards realizing the sustainable society, it is becoming an urgent task to estimate the various kinds of events; sports, conference, music festival, etc. Based on tripod aspects of environmental, economic and social issues, an evaluation method of sustainability for events is developed.
First, all kinds of the data of event (construction of temporally structure, goods, food and drinks, services, transportation, etc) are collected. Then resource consumption of fossil fuel, minerals, water and economical ripple effect are calculated using the database developed by Tokyo City University based on IO method. CO2 emission is calculated using 3EID and process method.
For environmental aspects, Life Cycle Inventory Analysis is performed with resource consumption of fossil, mineral, and water, and GHG emission. Then Life Cycle Impact Assessment is performed with LIME2.
For economical aspects, economic ripple effect analysis is performed considering employment using IO method.
For social aspects, considering added value, water consumption, CO2 emission by a event, influence of human health, education, food production, water for living, social assets, then social impact assessment is performed by calculating the influence for the HDI(Human Development index).
Then sustainability assessment for events is performed by combining and arranging the tripod evaluation above for environment, economic and social aspects.
A sustainability evaluation for a sports event is shown as an exemplification of this method.

MO 285
Social Life-Cycle Assessment of waste management options. Some preliminary results.
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This paper presents the preliminary results of a Social Life-Cycle Assessment (S-LCA, as defined by the UNEP-SETAC Guidelines) of the available waste management options in Spain and Portugal, which is performed within the framework of the LIFE+ project FENIX - "Giving packaging a new life"(2010-2013).
The integration of social aspects in environmental analyses is increasingly being recognized as very important (Benoît and Mazijn, 2009); however, S-LCA is still in its infancy and needs to be consolidated and tested in a wide range of real case-studies. Since most social impacts of technologies and management options depend very much on the local conditions (e.g. economy, social and institutional structure, culture), the choice of the indicators has to be made according to the characteristics of the problem to be tackled.
In our case, a first list of 13 impact categories were defined, based on a thorough literature review. Then, questionnaires were filled out by three groups of social actors, namely: (i) 10 Spanish Regional Governments, (ii) 20 Spanish and 9 Portuguese Management Units (MUs), and (iii) both the Spanish and the Portuguese green dot holders. The 13 impact categories were thus given individual scores by the representatives of the consulted social actors, leading to a final ranking, which was obtained selecting the first five categories in the ranking of each group of social actor. Finally, an indicator was chosen for each impact category, in order to assess the impact in the respective social dimension of the available waste management options.
The first preliminary conclusions are as follows: 1) when analysing the social impact of technologies or management options, it may be difficult to adopt a life cycle approach, because impacts taking place in different stages of the supply chain are difficult to evaluate and to aggregate in one indicator; 2) in some cases, data availability represents a serious hindrance; 3) it is most important to adopt a large range of point of views, involving different categories of social actors.

MO 286
Energy sustainability in medium and small communities
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The Covenant of Mayors (CoM) is a European initiative by which towns and regions voluntary commit to reduce their CO2 emissions. The formal commitment is to be achieved through the implementation of a Baseline Emission Inventory (BEI) and a consequential Sustainable Energy Action Plan (SEAP).
The Comunità Montana Valle Sabbia (CMVS) is a group of municipalities located in the northern area of Brescia district (Italy), between the Garda lake and the Alps, that have joined the

CoM. In the area of CMVS the industrial sector is well developed, so there is little improvement margin with regard to CO2 reduction, as demonstrated by BEI analysis; on the contrary, this study has highlighted that consistent benefits could come from the reorganization of residential and tertiary sectors in some municipalities and of the residential sector and public transports in others.
Based on results obtained during the BEI step, the SEAP has identified a group of actions to reduce energy consumption and GHG emissions in the CMVS: improvement of buildings envelope to reduce thermal transmittance; replacement of old and low-efficiency central heating mainly in municipal buildings; substitution of present municipal public lighting with low-energy consumption systems; integrated photovoltaic systems on buildings to reduce grid electricity demand.
Besides these deeds, all concerning the sphere of public tertiary and private residential sectors, which anyway could not be sufficient to comply to the EU directive 20/20/20, it has been suggested a PV power plant of 6 MW, able to serve many Municipalities, that would help reduce actual emissions in the atmosphere.
In accordance with the CoM guidelines the estimates were carried out following the Life Cycle Assessment method, able to provide a complete evaluation about both current situation and actual benefits as a result of the proposed actions, without which the result is underestimated.

PE01 - Data-driven, knowledge-based, and QSAR modelling in ecotoxicological assessment

MO 290
Results evaluation in spatial decision models
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The use of spatial decisions support systems for environmental management allows considering expert knowledge though the integration of multiple criteria in a GIS basis. As environmental systems are highly complex, these models present an inherent uncertainty. Due to this fact, results evaluation must be performed to check if the model outputs correspond to stakeholders' expectations. These methods allow evaluating how the information given in model's input affects its outputs.
The objective of this work was to perform results evaluation in GIS. For that, the exploratory method was applied to a case study of land classification. The first step was developing scenarios based on the initial model. For each scenario, the weight of one criterion was set to zero and the weights of the remaining criteria were redistributed accordingly. Then, the results for each scenario were compared to the initial map, identifying the changes in each pixel.
The results allow a better understanding of the developed model. Besides that, the use of GIS allows presenting the results in a graphic way, facilitating its evaluation. This method has proven to be useful for model calibration.

MO 291
Determination of polycyclic aromatic hydrocarbon emission sources from Cartagena Bay, Colombia
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The Cartagena bay is an estuarine in the Caribbean Sea which received fresh waters from Magdalena river through the Dique channel. Several commercial activities are developed along of this Bay such as petrochemical industries, harbours, and tourist attractions. However, all these activities produce an environmental implication that it has not been measured yet. Therefore, 16 priority polycyclic aromatic hydrocarbons (PAHs) analyzed by gas chromatography coupled with mass spectrometry (GC/MS) in sediments from Cartagena bay were used to determinate the possible emission sources through approach of multivariate analysis to know what can be produce the major impact. PAHs are largely produced by mean of the combustion or pyrolysis of organic matter. High levels of PAHs in the environmental can be related with the risk to develop cancer in human. Results showed that the profile of PAHs of the Cartagena bay implicate that the pollution is caused by oil spill, combustion of gasoline and diesel.

MO 292
Modeling toxicity endpoints using artificial neural network ensembles (II)
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Today and for the foreseeable future, it is important to utilize the best possible analysis of knowledge (both data and technique) to save and protect the environment. The use of in silico models are at the forefront of the battle surrounding the development and waste management of chemicals. A common theme in Green chemistry is the application of useful models to reduce the need to perform costly, time consuming, and often inhumane in-vivo experiments, such as lethal dose/concentration and Draize tests for skin or eye irritation. Unfortunately, making a useful toxicity model is often more difficult than it sounds mainly due to the involvement of multiple biological mechanisms and conflicting experimental measurements.
In this work, artificial neural network ensembles (ANNEs) were used to generate robust models for a number of toxicity endpoints including acute rat toxicity (LD50), fathead minnow LC50, Tetrahymena pyriformis IGC50, Daphnia magna LC50, reproductive/developmental toxicity, and bioconcentration factor. These results are compared with other QSAR methodologies used in the Environmental Protection Agency's Toxicity Estimation Software Tool (T.E.S.T.) v3.3 which include multiple linear regression, hierarchical clustering, nearest neighbor, and other similarity-based methods. In almost all comparisons made using the training and external test sets specified by the EPA, ANNEs proved to be more robust and showed superior performance in terms of RMS error for regression models and false rate for classification models. Accuracy within the applicability domain of each model was further improved by using Kohonen mapping (independent of the endpoint values) to select new training and external test sets.
Finally, all models were optimized by applying focused curation techniques beyond averaging the available experimental data. One curation technique not often publically addressed is the examination of predicted outliers in both training and test sets identified by preliminary models. Another technique is to verify that the structure of each chemical exists in one or more reliable data sources. If a structure cannot be identified the chemical is flagged for further curation. Examples and the relative finalized models are discussed, as the techniques can be useful when dealing with large data sets where rigorous literature mining is not feasible.

MO 293
Predicting species sensitivity distributions of chemicals with limited ecotoxicity data
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Efforts to reduce the risk of a certain compound to aquatic life may increase the total risks arising from alternative compounds. In order to reduce the total risks arising from the use of chemical compounds, we need to quantify the risks of the target and alternative compounds with common metrics. Species-Sensitivity Distribution (SSD) is a powerful tool that can provide a common metric for quantifying ecological risk of compounds. Derivation of SSD is commonly hampered by lack of ecotoxicity data. In this study, we propose an approach to fill data gap on ecotoxicity data of compounds to allow us to compare and evaluate risks of the target chemical and its alternatives with SSD-based common metrics. We have compiled available acute aquatic data and calculated SSDs for several dozen of chemicals. The calculated SSDs were categorized by different chemical classes. Based on the categorized data, we have developed a linear model to generate SSDs of chemicals with limited ecotoxicity data. Hazardous concentrations (HCs) from the estimated SSDs using the linear model were well predicted within a factor of 2 for Class 1 (non-polar narcosis) chemicals. The proposed approach has potential for predicting SSDs for chemicals with limited ecotoxicity data. A SSD prediction using bayesian approach and the domain of applicability and the management of uncertainty are discussed during presentation.

MO 294
Physicochemical characterization of mixed polybrominated/polychlorinated derivatives of Persistent Organic Pollutants with QSPR
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Mixed polybrominated/polychlorinated Persistent Organic Pollutants (Br/Cl-POPs) are formed in various natural and anthropogenic processes. Since empirical studies confirm that its physicochemical properties and biological activity are comparable to the properties of their polychlorinated and polybrominated counterparts. it is evident that Br/Cl-POPs may also have negative effects on the wildlife and human health. Regarding that environmental concentrations of Br/Cl-POPs in all environmental compartments are increasing, detailed studies aimed at performing a comprehensive risk assessment for this class of mixed POPs is of special interest.
The main problem in the risk assessment for mixed Br/Cl-POPs is the huge number of congeners, theoretically possible substitution combinations. On one hand, this leads to difficulties with identification of the particular congeners present in the environmental samples. On the other one, physicochemical characterization of such a large number of compounds could not be performed with empirical methods in a reasonable time period.
The problem can be solved by employing such computational techniques as Quantitative Structure-Property Relationships (QSPR) modelling combined with combinatorial chemistry. We generated all possible polybrominated/polychlorinated congeners for six groups of organic compounds (benzenes, PXBz; biphenyls, PXBs; dibenzo-p-dioxins, PXDDs; dibenzofurans, PXDFs; diphenylethers, PXDEs; naphthalenes, PXNs) with the ConGENER software package. This resulted in 23,739 mixed congeners in total. Then, molecular geometries of the congeners were optimized and quantum-mechanical descriptors were calculated at the level of semi-empirical PM6 method. Based on these descriptors and the existing or newly developed QSPR models, we predicted logarithmic values of five physicochemical properties, including: Henry`s law constant (log KH), subcooled liquid vapor pressure (log PL), solubility in water (log SW), and three partition coefficients (log KOW, log KOA and log KAW). Finally, we have estimated the environmental behavior of the studied compounds by comparing the values of the predicted properties with the corresponding values for well-studied polychlorinated and polybrominated POPs.

MO 295
Global vs. local QSPR models for persistent organic pollutants
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Experimentally determined data on the key physicochemical parameters for halogenated congeners of Persistent Organic Pollutants (POPs) are available for a limited number of compounds. In the absence of experimental data, a range of computational methods can be applied to fulfil those data gaps. One of the widely used techniques is Quantitative Structure-Property Relationships (QSPR) approach. There are two ways to develop the QSPR models: using a more complex global model or fitting a simple local model that covers a specific class of chemically related compounds. The main purpose of the study was to investigate, if local models have significantly better explanatory and predictive ability than global models with wider applicability domains.
We have compared in pairs predictive performance of global and local QSPR models to predict the water solubility, vapour pressure and octanol-water partition coefficient of the Cl/Br-substituted congeners of benzenes, biphenyls, diphenyl ethers, dibenzofurans, dibenzo-p-dioxins and naphthalenes. The comparison was based on the measurement of the goodness-of-fit, robustness and prediction ability recommended by the Organization for Economic Cooperation and Development. In addition, we employed t-Student's pairwise test to analyze statistical significance of the models' residuals.
The comparison between the predictive performances of local and global models, indicated that the local models fit better to the experimental values and were more precisely than the global ones. However, any statistically significant differences between the measured and calculated values for both types of models (local and global) were not found. Based on the obtained results, we concluded that since global models fulfil all quality criteria recommended by OECD, they should be applied in practice in state of more time consuming procedure of modelling the particular groups of POPs one-by-one. This is extremely important from the economic point of view, especially when the number of novel chemicals identified in the environment is growing exponentially.

MO 296
Development of QSAR for soil toxicity of benzene-based compounds to Folsomia candida and validation of soil pore water concentration
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The rapid development of industrial chemicals and the persistence of existing compounds in soils make it necessary to establish less time and resource consuming methods to determine possible toxic effects on soil organisms. Since the enormous improvements in computable software, quantitative structure activity relationships (QSARs) may provide an effective tool to overcome this challenge in bigger complexity. Toxicity to the springtail Folsomia candida was determined for nine chlorobenzenes, aniline, five chloroanilines and 5 chlorophenols, applying ISO guideline

11267. Toxicity in LUFA 2.2 and OECD artificial soils was compared, using reproduction as the endpoint. EC10 and EC50 were the chosen test parameters, with the focus on the EC50. In an attempt to develop a QSAR, toxicities were related to the Kow of the test compounds. Chlorobenzenes and chlorophenols showed a decrease in the effective concentration with increasing log Kow in nominal and modeled pore water concentration, respectively. In contrast the chloroaniline test series showed a decrease in toxicity in nominal concentration and an increase in toxicity in calculated pore water concentration with increasing log Kow. Based on the two calculated effective concentrations (EC10/EC50) a second experiment was designed to validate the pore water hypothesis, which described that only the freely available concentration in the pore water is causing toxic effects. For eight chlorobenzenes and 4 chloroanilines solid phase micro-extraction (nd- SPME) with polyacrylat coated fibers were used, to determine pore water concentrations at three time points over a four week standard toxicity test. The fibers were equilibrated over 4 weeks in soil - water suspension with 2 grams soil and 6 ml water. Subsequently, the fibers were extracted in cyclohexane and analyzed by GC-ECD. Only LUFA2.2 natural soil was used in the model confirmation experiment. The relative high volatility of the compounds leads already to high losses during the spiking and test preparation steps and a depletion of the total soil concentration before the actual test. The results indicate the losses of compounds during all steps of preparation and losses in the bioavailable fraction during test performance. However, the slopes of the regression models remained comparable. We therefore conclude that the pore water hypothesis is confirmed, but strongly advice to include pore water analysis for model validation in future experimental designs.

MO 297

Modeling ecotoxicity risks at the river basin scale: integration of the species sensitivity distribution (SSD) concept

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Traditional and simplistic approaches evaluate ecological risks of chemicals by calculating hazard indexes based on environmental concentrations and LOEC/NOEC values. Another methodology is using a concept of species sensitivity distributions (SSD), which has been traditionally used for predictive purposes, i.e. estimations of environmental quality criteria for e.g. pesticides (calculation of HC5 values). In the present study, we develop a model based on SSDs for retrospective assessment of ecotoxicological risks at the river basin scale. Existing ecotoxicological data were compiled for a number of priority chemicals (industrial chemicals, pesticides and their byproducts), that has been selected with respect to their relevancy in the model basins of the Zenne river (Netherland) and Odense river (Denmark). The ecotoxicity data were thoroughly assessed for their quality and resulting datasets served for calculation of SSD parameters of individual chemicals (mean and S.D. of the log-normal SSD). Integration of the SSD parameters with measured and/or modeled concentrations in the river basin is used to calculate PAF or msPAF values (potentially affected fraction or multisubstance PAF) for different temporal scales, which allow direct comparison of ecotoxicological risks at various localities within the investigated river basins. The outcomes of the model and its limitations will be discussed [Supported by the EU FP7 project AQUAREHAB and by the project CETOCOEN (no. CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund].

MO 298

Acute toxicity to Daphnia magna test: comparison of predicted vs Actual EC50 results

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With the implementation of REACH, the use of QSAR's to predict various ecotoxicological properties of chemicals is widely recognised. The results obtained from Acute Toxicity to Daphnia magna Tests are of importance for the risk assessment and labelling of chemicals, and hence this endpoint has been selected for the purpose of this comparison study.
The suitability of the program was assessed by comparing predicted values against experimental results obtained from regulatory testing of new and existing chemicals.

MO 299

Evaluation of test methods for measuring toxicity to sediment organisms

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There are several internationally agreed tests available for investigating the potential long-term effects of substances, metabolites (or transformation products) and non-extractable residues on sediment-dwelling organisms. These standardised test methods focus on benthic invertebrates (e.g. *Chironomus, Lumbriculus*). However, as identified by CEFIC-LRI in their ECO17 call, there is a lack of methods for assessing potential effects on microorganisms, plants and animals from different taxonomic groups and for 'read across' from freshwater to marine sediment tests. The European Commission guidance for REACH identifies that specific effects of chemicals on plants and micro organisms are not covered by the currently available and regularly accepted test methods. Here, we provide a data- and model driven approach to improve the risk assessment for contaminants in sediments using (a) transparent protection goals, (b) underpinned model concepts how to link exposure and effects, (c) a single species test battery that covers appropriate focal species representative for different exposure pathways and sediment communities, (d) data from higher-tier experimental population and/or community studies, and (e) integrated state of the art exposure and population effect models, so that a tiered risk assessment scheme is achieved.

MO 300

Toxic effects of nine polycyclic aromatic compounds on Enchytraeus crypticus in artificial soil in relation to their properties

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The aim of this study was to compare the toxic effects of selected two- and three-ringed PAHs and their N-heterocyclic analogues with one or two nitrogen atoms on the survival and reproduction of Enchytraeus crypticus in the artificial soil. If the toxicity was expressed as soil pore-water concentration (µmol/L), the toxicity showed clear pattern and dependency on structure and properties of the compounds. This was primarily related to the different lipophilicity of the compounds after that the toxicity significantly increased with increasing Kow value. This relationship indicates nonpolar narcosis as the general toxicity mechanism of the tested compounds. In addition, significant correlation of the ionization potential (IP) and toxicity of PACs has been identified by multidimensional QSAR models. The suggested importance of IP can help to explain deviations from proposed nonpolar narcosis model. Unraveled relationships between PACs structure and properties and their toxicity could help to the prediction of their effects in soil.

MO 301

Covert toxicity of compounds by prediction

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Covert toxicity of compounds by prediction

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There are two interesting groups of organic compounds with very useful effects and which are said to be nontoxic. One group involves nootropics (or smart drugs), the other one perfluorinated aliphatic acids. Nootropics favourably affect a damage of central nerve system - cognitive ability and improve the activity of brain. They are used for memory or attention disorder. Their dosage is high, they are not metabolised and are totally excreted from the body. Their effect is explained by an increased supply of the brain by oxygen and nutrition and by eliminating free radicals. They should have no adverse or site effects. No severe consequences can be found by a literature survey, although some are sometimes mentioned. Possibility of nausea, a feeling a pressure in one´ s head, vomiting or allergic reactions are mentioned in a worst case. The global historical industry-wide emissions of total perfluorinated carboxylic acids were estimated to be thousands tonnes. It was estimated that the majority (about 80%) of perfluorinated carboxylic acids have been released to the environment from fluoropolymer manufacture and use. Their properties like negligible vapour pressure, solubility in water or bad degradability suggested that they would accumulate in surface waters. The estimated mass in various environmental compartments confirmed that surface waters, especially oceans, contain the majority of perfluorinated carboxylic acids implying a long environmental residence time. It was concluded that, in addition to atmospheric transport/degradation of precursors, ocean water transport could significantly contribute to their long-range transport even to Arctic region. The Tubifex assay predicts a chronic effect of perfluorinated carboxylic acids to aqueous organisms living in aqueous or muddy media even in low concentrations. Predictive expert system HAZARDEXPERT as a part of PALLAS (CompuDrug International Inc.) predicts actually no toxic effects of nootropics, but indicates a low, but not zero, probability of teratogenic effects. It evokes a question what consequences this fact may have for youngsters taking nootropics the whole life.

MO 302

Atom centred fragments (ACF) to estimate and decrease QSAR modelling uncertainties

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Proper application of a QSAR models requires to consider the applicability and limitations of the model with respect to each individual case. In this context, the applicability domain of a model needs to be regarded.
However, even within the applicability domain the reliability of a single prediction is not obvious. The presented approach provides a mean to estimate the model uncertainty. With a validation set of compounds and their experimental values, model errors of similar compounds can be taken into account to estimate the prediction uncertainty for a test substance. The similar compounds are obtained from atom-centred fragments (ACF). The ACF method decomposes molecules into structural fragments consisting of a central atom and bonding neighbours. Similarity then is calculated by comparing the occurrences of the ACFs. Furthermore, the model errors for the similar compounds can be used to refine the model prediction.
The performance of the presented technique is shown with several examples for environmental partitioning and ecotoxicological endpoints. Several applications are implemented in the software system ChemProp.
Financial support by the European Commission through the project OSIRIS (Contract No. 037017) is gratefully acknowledged.

MO 303

Chemical domain of QSAR models - extension to generic expressions

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The chemical domain of estimation models can be characterised by an analysis of atom-centred fragments (ACF) from the training set. ACFs decompose molecules into individual atoms and their neighbourhood. The chemical domain of a test compound then is determined by examining the occurrences of the ACFs of this compound in the training set.
Basically, this approach is not restricted to estimation models, but can be applied to any compound set. The only requirement is the availability of all chemical structures. However, in real world applications this often is not the case. Data sets may include generic expressions as compound classes, mixtures of isomers, etc. An approach to overcome this limitation is presented for three important types of generalisation. These are variable alkyl substitutions, multiple occurrences of hetero atom fragments, and ring substitution patterns.
The study was funded by ERASM, Brussels. In addition, financial supported by the EU via the integrated project OSIRIS (contract No. 037017) is acknowledged.

MO 304

Screening for new dioxin-like compounds with the classification SAR model

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Dioxin-like compounds (DLs) is a subclass of persistent organic pollutants (POPs), showing

structural and toxicological similarities to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Currently about 100 of toxic Dls have been identified. But taking into account that other policyclic hydrocarbons can exist as congeners and be substituted by Cl, Br or both, the total number of potentially toxic congeners is very large. Due to limited time and costs of laboratory tests, it is necessary to create screening methods that allow selecting those compounds, for which empirical research should be of the highest priority.
Thus, the aim of this study was to develop a novel classification method, capable to select those congeners (from 182 058 of theoretically possible brominated, chlorinated and mixed derivatives of 12 congeneric groups includes: acenaphthenes, anthracenes, biphenyls, biphenylenes, dibenzofurans, dibenzo-p-dioxins, dihydroanthracenes, fluorenes, naphthalenes, phenanthrenes, xanthenes, 9-fluorenones), which exhibit the same level of toxicity as 2,3,7,8-TCDD.
We generated chemical structures among the groups of compounds containing chlorine, and/or bromine. The structures were generated using ConGENER software. Next, we optimized geometries of the structures and calculated for them a set of 26 topological and quantum-mechanical descriptors (using semi-empirical PM6 method and DRAGON software respectively). Simultaneously, we collected all available experimental data on dioxin-like toxicity (toxic equivalence factors, TEFs, and relative effect potencies, REPs). Also empirically confirmed information about particular congeners which are not toxic were aded. We divided the collected data, into two classes: toxic (TEFs and/or REPs > 0.0001) and non-toxic. Compounds from both classes, were then utilized for training and external validation of the classification SAR model. The SAR model was developed with two chemometric methods: linear discriminant analysis (LDA) and k-nearest neighbor (kNN) non-linear classifier. The most optimal set of descriptors was selected with a genetic algorithm (GA). The best model has been developed for a set of 182 058 compounds, to found new dioxin-like compounds.

MO 305
Exploring the QSARs for OH Tropospheric Degradation of VOCs using freely available online descriptors
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Tropospheric photochemical air pollution has impacts on scales ranging from local to global. The reactions of organic pollutants in the atmosphere with OH radicals, NO_3 radicals, and ozone are of great concern from an ecological standpoint for risk assessment measurement such as degradation pathways, atmospheric lifetimes and fate of these compounds. It was already established that reactions with hydroxyl radical (-OH) is the most important pathway of day time removal of organic pollutant in atmosphere because of the reactive nature of OH radical to react practically almost with every volatile organic compounds (VOCs) in the troposphere. Due to limited availability of experimental gas phase rate constant data for chemicals, alternative theoretical approaches like QSAR/QSPRs are often practiced to predict the high risk of organic chemicals and to reduce the time consuming, expensive and difficult experimental procedures.
In this study we developed QSAR models for hydroxyl radical tropospheric degradation rate of 460 VOCs, using HOMO, LUMO from Hyperchem minimization in addition to separately freely available online molecular descriptors (from the CADASTER online platform- www.cadaster.eu) or descriptors calculated from an updated version of DRAGON software. The Genetic Algorithm as Variable Subset Selection (GA-VSS) was used to select the relevant molecular descriptors in the modeling step (Ordinary Least Squares (OLS) regression). Three splitting criteria [K-ANN, k-means cluster and random on response] were applied for verifying the external predictivity of developed QSAR models, with special emphasis on model applicability domain which was verified by the leverage approach. The statistical qualities of the models developed from the pool of online descriptors were comparable with those obtained from the DRAGON descriptors and, most importantly, the GA selected, in addition to HOMO, descriptors with comparable mechanistic meaning, from completely different pool of input descriptors.
So it can be suggested to use online freely available descriptors to increase the reproducibility of the models for the safety of environment and for REACH.

MO 306
On the agreement of external validation parameters for linear regression QSAR models
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The evaluation of linear regression QSAR models performances, both in fitting and external prediction, is of pivotal importance. While leave-one-out (LOO) Q^2 internal validation technique (cross-validation) is well established, different external validation parameters have been proposed in the last decade: Q^2-F1 (Shi), Q^2-F2 (Schuurmann), Q^2-F3 (Todeschini), r^2m (Roy) and the Tropsha-Golbraikh method. These parameters usually are in accordance, making one confident of a model predictivity, but doubts arise when they give contradictory results. In these cases the QSAR model developer should understand which one of the aforementioned parameters is "the best". However this is not an easy task, mainly because no one of these parameters could be considered "the best"in every situation. We are thus looking for a simpler method to evaluate the external predictivity of the models, independently on the set composition. In our opinion, the simplest method consists in the quantification of the similarity among the experimental data of external test set versus the corresponding values calculated by the model. In this study our new method has been used as a reference and we have evaluated the number of contradictory and agreeing results on validation parameters by means of 210.000 simulated datasets. A wide range of possible scenarios has been generated and, concerning the more realistic ones, 95% of agreement has been found among our method and all the aforementioned validation parameters together. Our proposed method is the most precautionary among those analyzed. We have verified that disagreements among results is related to two possible situations: a) the external data points are well predicted (good matching), while at least one of the validation parameters rejects the model (rare), b) the matching is not good and one or more validation parameters accept the model (less rare). The second alternative is more dangerous for QSAR models, thus a deeper analysis of the results is suggested. Our method, verified also on real models, has been proposed as a tool to be used in addition to the aforementioned external validation parameters to find out this kind of critical models with doubtful predictivity.

MO 307
Environmental chemoinformatics for REACH
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The implementation of the EU legislation concerning the registration, evaluation, authorization and restriction of chemicals (REACH) requires demonstration of the safe manufacture of chemicals and their safe use throughout the supply chain. REACH encourages development of new in vitro test methods and replacement of animal tests wherever possible by alternative methods. These goals are not achievable without well-trained personnel with a broad expertise and knowledge in both experimental and computational areas of environmental sciences. The requirements for such scientists, however, are not limited to the REACH implementation itself. Large companies and SMEs could be interested to employ such specialists to perform risk assessment and prioritization of molecules in the development stage. Therefore, the primary objective of the Environmental Chemoinformatics Marie Curie Initial Training Network (http://www.eco-itn.eu MC ITN ECO) is to contribute to the education of a new generation of scientists, environmental chemoinformaticians, who will receive advanced training in both environmental and computational methods. To achieve this goal the ECO is educating its fellows using expertise and knowledge of its partners in various complementary computational and experimental areas of environmental sciences. The additional training is offered by means of Winter and Summer Schools and includes both theoretical and practical courses. The internships to the laboratories of associated partners allows fellows to learn new methods and to broaden their knowledge in the field. Currently, ECO employs 10 long-term fellows working towards their PhD in laboratories of partners. The ECO also offers a flexible system of more than 30 Short Term Fellowships to Early Stage Researchers who are invited to apply and can stay in laboratories of partners from 3 to 12 months (see http://www.eco-itn.eu/positions). The experience with recruitment of fellows as well as direction of outgoing studies in environmental chemoinformatics within the project will be presented.

MO 308
ANTARES project: an evaluation of non testing methods for REACH
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Animal testing in Europe is expected to increase for the activities related to the REACH legislation. Currently in Europe a lot of animals are used per year for laboratory experiments.
To correctly evaluate the impact on the environment and human health, industry will pay a high cost (billions of euros), millions of animals will be killed, and it is questionable if the number of laboratories is sufficient to cope in a reasonable time with the need to produce the toxicity data. To solve all these problems, the REACH legislation promotes the use of non-testing methods (NTM).
The term NTM refers to all the approaches used to predict the effects of chemical compounds without the use of the real chemical compound, but on the basis of the chemical structure only and comprises a series of different tools whose commonality lies in the identification of a relation between chemical structure and exhibited activity or toxicity.
There are different types of NTM to assess activity and/or toxicity of chemicals, among them Quantitative structure-activity relationship (QSAR) methods are the most used. QSAR is the process by which chemical structure is quantitatively correlated with a well defined process, such as biological activity or chemical reactivity.
In this context a LIFE project, named ANTARES, has been funded to evaluate existing NTM and their feasibility for REACH purposes.
As the project has the main target to analyze the use of NTM in accordance to REACH, and to identify suitable method, the first step has been to identify relevant criteria for comparing QSAR methods.
A list of them has been established. They have been divided into main (more important) and additional criteria. For each of them a particular score has been decided based on the importance of the criterion.
Using the overall score we will first identify available QSAR models which could be used for REACH. Then, if there are more than one method for the same endpoint, we will rank them, using the proposal scoring system.
The availability of checked and validated non-testing methods will provide a huge, immediate benefit, producing new important knowledge, abating the costs and time needed to get the information on chemicals, and saving animals. The introduction of these methods will reduce the use of animal tests not only for their use by chemical industry, but in the other sectors too.
Financial support from ANTARES project (LIFE 08 ENV/IT/000435) is gratefully acknowledged.

MO 309
MISTEX: A web-interfaced database to standardize and facilitate data collection, storage and cross requests in ecotoxicology
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Today, global change and accelerated xenobiotics production play active roles in the rising contamination risk of ecosystems and subsequently of human health. The ecosystem answer to such environmental perturbations (physical, chemical and/or biological) is studied in all compartments of the biosphere (air, soil and water) and at all levels of biological organization. Depending on the level of biological organization, answer may strongly differ, with major changes in terms of biochemistry and physiology, of life history traits (survival, growth, reproduction), of demography, or of structure and functioning, at the cell, individual, population or ecosystem levels, respectively. Concomitantly, exponentially increasing amounts of data are collected, which may concern any kind of species, toxic compounds, life traits and environmental conditions. The need for a unifying database is then become crucial in order to standardize the way theses data are collected and stored, and to facilitate cross requests in view of meta-analyses.
The relational database MISTEX (Modelling, Inference and Simulation for Toxicological Evaluation of Xenobiotics) was imagined to meet such requirements, grouping together raw data from any kind of experiment in a consistent way and keeping intact the context in which they have been acquired. At present, MISTEX stores measurements as raw data (number of survivors, length[3DOTS]) associated with all meta-data that allow their unique identification (geolocation, species, compound[3DOTS]). MISTEX works through a Web interface, which provides among others a data capture sheet to collect them in a standard way and directly send them to

the database. From MISTEX, simple or cross requests can be done, for example to gather all data on a particular species or a particular trait whatever the studied compound. This will later allow one to combine its own data with other information resources, to design further complementary experiments, to elicit prior probability distributions in view of Bayesian inference, or to validate predictions with dynamical mechanistic models.
Its flexibility, its genericity, its user-friendly nature and its evolvability make MISTEX apart from other existing databases. Works are currently in progress to couple MISTEX with R packages - some of them already exist (lattice, drc), some others are to be thought up - in order to directly link shared data with graphical viewing, statistical analysing and mechanistic dynamical modelling tools.

PE02 - PBPK modelling in ecological risk assessment

MO 312

Using bird and mammal body burden modelling in pesticide risk assessments: a regulatory perspective

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The current EU initial assessment of the acute risk to birds and mammals exposed to pesticides in plant protection products involves derivation of a risk quotient based on acute toxicity data (LD50) and theoretical estimation of exposure in daily dietary intake. The acute risk assessment can be refined by taking account of physiological processes (e.g. absorption, elimination) and ecological factors (e.g. feeding rate). Body burden models, such as EFSA (2005)[1], attempt to address such issues, calculating the time taken to achieve a lethal dose and comparing this to the time taken to induce cessation of feeding. Such models are potentially useful in acute risk assessments given that the options for refinement are limited. However, there are many uncertainties in using the models from a regulatory perspective. Selection of an appropriately robust model is the first challenge as model validation is limited by a lack of relevant field data. The validation process is also problematic due to imperfect information on the exact conditions (e.g. feeding rate) that lead to mortality and (likely) lack of availability of input parameters for the species involved. The applicability of the models for different types of chemicals and modes of action is also uncertain. Perhaps the most challenging stage though is the selection of parameters with which to populate the models. These must be derived from studies that are not designed to produce such data. It also often involves extrapolation of data between birds and mammals, with limited information regarding relevance. There are also complications when attempting to increase the ecological relevance of the models e.g. incorporating mixed diets. The final stage of predicting acceptable risks is consequently problematic, with many interpretations being possible depending upon whether best-case or worst-case parameters are used. The aim of this poster is to present some of the uncertainties associated with this approach with the aid of case studies. We highlight areas where future research could be focused to enable these models to be used with more confidence within risk assessments and ensure protection goals are being met.

[1]EFSA (2005) Opinion of the scientific panel on plant health, plant protection products and their residues on a request from EFSA related to the evaluation of pirimicarb (Question No. EFSA-Q-2004-160). *The EFSA Journal*, **240**, 1-21.

MO 313

Avian blood sampling for toxicokinetic modelling using dried blood spots (dbs)

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Physiologically-based pharmacokinetic (PBPK) models have been used by the pharmaceutical industry for many years in drug development, typically using mice and rats as test subjects. Improvements in analytical instrumentation and dried-blood spot (DBS) sampling techniques have reduced the volume of blood needed per sample. Typically 60 -100 µl need to be collected for a triplicate DSB sample (~15 µl per spot). For northern bobwhite (Colinus virginianus) that volume is considerably below the generally recommended limit for blood sample size of 1% of the bird's body weight. The DBS method of blood collection has also simplified the handling and processing of the samples prior to analysis. Therefore, study designs could be modified to take advantage of these improvements. Smaller blood volumes allow for decreases in the time interval between sampling events for an individual animal or, in the case where only a single sample was taken from an animal, multiple samples could now be collected. This would result in a reduction in the number of animals used.
However, anatomical and physiological differences between birds and mammals needed to be considered when developing techniques for collecting the blood samples for ecotoxicological studies with bird species. Collection methods considered were toe clipping, and venipuncture using a lancet, and using a needle and syringe. Sites for venipuncture included the jugular veins and the wing veins (cutaneous ulnar vein, also known as the brachial).
Toenail clipping for avian blood sampling is generally considered a painful technique and a sample could potentially be contaminated if the test substance is presented through the diet. Toenail cuts tended to bleed slowly and clotting was a problem. Lancing a wing vein also had similar problems with slow bleeding, clotting and the potential for contamination. Using a needle and syringe for blood collection and rotating the collection sites among the jugular and wing veins, left and right sides for both, was the preferred technique for collecting the necessary blood volume. This poster presents more a more detailed description of the procedure.

MO 314

Toxicokinetic of perfluorooctane sulfonate (PFOS) following oral exposure under realistic dose. Implication for bioaccumulation assessment. Preliminary study with rabits.

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The bioaccumulation of chemicals in biota and the potential for biomagnification through the food chain represent essential elements for a proper environmental risk assessment. Toxicokinetic approaches have been increasingly employed to estimate final concentration of chemicals on top predators and hence to asses the potential biomagnification. This kind of studies comes from the simplistic lipophylic-equilibrium theory allowing to investigate other chemicals with different patterns of accumulation. That is the case of perfluorinated compounds (PFCs) which are preferentially bound to proteins and retained in the blood and liver of animals. However, pharmacokinetic studies with PFC in mammals are limited and mostly carried out at relatively high doses. Consequently, the first objective of this study was to characterize the toxicokinetic of a PFC; concretely perfluorooctane sulfonate (PFOS) in rabbits (Oryctolagus cuniculus) as representative mammal. Also, the oral exposure to PFOS was conducted under realistic chronic dose (0.1 µg/kg at day). Rabbits were fed with PFOS-contaminated food by gastric intubation during three months for assessing their uptake. Subsequently the contaminated food was eliminated and the assay continued during other three months for covering the depuration period. All through the experiment 2mL of blood were sampled weekly and PFOS was analysed. From the experiment, the toxicokinetic of PFOS have been estimated obtaining elimination blood rate of 76.25 ± day -1. The toxicokinetic parameters were included into a previously developed biomagnification model. This model was designed to estimate daily chemical concentrations covering the possible processes taking place within this period of time (uptake, distribution and elimination of chemical). The calibration study with PFOS toxicokinetic parameters demonstrated the capacity of biomagnification model to produce proper estimations of expected concentrations. Therefore, this model could be considered as a useful tool for chemical risk assessments.

MO 315

Toxicokinetic of perfluorooctane sulfonate (PFOS) following oral exposure under realistic dose. Implication for bioaccumulation assessment. Preliminary Study with chickens.

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The bioaccumulation of chemicals in biota and the potential for biomagnification through the food chain represent essential elements for a proper environmental risk assessment. Toxicokinetic approaches have been increasingly employed to estimate final concentration of chemicals on top predators and hence to asses the potential biomagnification. This kind of studies comes from the simplistic lipophylic-equilibrium theory allowing to investigate other chemicals with different patterns of accumulation. That is the case of perfluorinated compounds (PFCs) which are preferentially bound to proteins and retained in the blood and liver of animals. However, pharmacokinetic studies with PFC in birds are limited and mostly carried out at relatively high doses. Consequently, the first objective of this study was to characterize the toxicokinetic of a PFC; concretely perfluorooctane sulfonate (PFOS) in chickens (Gallus gallus) as representative bird. Also, the oral exposure to PFOS was conducted under realistic chronic dose (0.1 µg/kg at day). Rabbits were fed with PFOS-contaminated food by gastric intubation during three months for assessing their uptake. Subsequently the contaminated food was eliminated and the assay continued during other three months for covering the depuration period. All through the experiment 2mL of blood were sampled weekly and PFOS was analysed. From the experiment, the toxicokinetic of PFOS have been estimated obtaining elimination blood rate of 137.99 ± day -1. The toxicokinetic parameters were included into a previously developed biomagnification model. This model was designed to estimate daily chemical concentrations covering the possible processes taking place within this period of time (uptake, distribution and elimination of chemical). The calibration study with PFOS toxicokinetic parameters demonstrated the capacity of biomagnification model to produce proper estimations of expected concentrations. Therefore, this model could be considered as a useful tool for chemical risk assessments.

MO 316

Toxicodynamic experiment for different time-variable exposure regimes of the insecticide chlorpyrifos on aquatic arthropods

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Non-target organisms may be exposed to fluctuating concentrations or sequential pulses of pesticide contaminants. Currently, evaluation of the potential adverse effects of pulsed pesticide exposure on non-target aquatic organisms is considered as a major challenge. The Threshold Damage Model (TDM) is a process -based model to predict the acute effects of pulsed pesticide exposure on the survival of aquatic invertebrates and consists of a toxicokinetic part in which uptake and elimination are described and of a toxicodynamic part including processes such as damage and recovery. In the latter step, internal thresholds are compared with hazard values and then translated into survival. The model assumes stochastic death. Here we present data from a Toxicodynamic experiment (TD) in the laboratory with the model substance chlorpyrifos. The survival experiment is designed to (1) get information about faunal responses to time variable exposures and how species may differ in sensitivity (2) parameterize the toxicodynamics part of the TDM for different species on basis of the two endpoints mortality and immobilization. Toxicodynamic experiment quantified survival of Cloeon dipterum and Chaoborus obscuripes to varying patterns of chlorpyrifos exposure over 34 and 31 days, respectively. The toxicodynamic parameter values for the killing rate constant (kk), recovery rate constant (kr), the threshold and background mortality were estimated by fitting the TDM with the experimental observed survival data per species. This was done with the least-squares method based on Levenberg-Marquardt algorithm implemented in the program Open Model v1.2 . *C. obscuripes* showed a delayed effect in immobility and mortality without any recovery where as *C. dipterum* responded directly to the exposure and some recovery occurred within 2 to 6 days. In general, the effect of the pulses became less when more time was given for elimination and potential recovery, as was intended by the experimental design. The TDM was able to predict the experimental data well (R^2: 0.94 and 0.91 for *C. obscuripes* and *C. dipterum,* respectively*)* however, not all parameters were robust e.g. the threshold parameter.

MO 317

Toxicokinetic-toxicodynamic (TKTD) modelling of survival of Gammarus pulex following exposure to multiple pulses of the fungicide propiconazole

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In aquatic environments, toxicant concentrations fluctuate over time. The risks they pose to organisms are currently evaluated by comparing time-weighed averages (TWA) of observed concentrations to short term toxicity data. However, in multiple pulse exposures both elimination of compounds and organism recovery between pulses play a role in the effects of subsequent contaminant pulses. The current study aims to predict survival following multiple pulse exposures using toxicokinetic-toxicodynamic (TKTD) models with differing assumptions of survival: an individual tolerance distribution (IT) and stochastic death (SD).

A short term toxicity test, toxicokinetic (TK) experiments and a long term pulse toxicity test were conducted with the aquatic invertebrate *Gammarus pulex* exposed to the fungicide propiconazole. The pulse toxicity test included treatments with two pulses and different recovery intervals in between and one constant exposure treatment with the corresponding TWA concentration calculated from the pulse treatments. TKTD models assuming IT and SD were fitted to the survival data and the goodness-of-fits were compared. In addition, the goodness-of-fits when simulated internal concentrations based on TK test were included or excluded were compared. Also, the models were fitted to short term toxicity data and it was tested how well they predict the effects in multiple pulse exposure.
The results show that propiconazole is eliminated fast from *G. pulex*. In addition, two possible metabolites were observed; however, the concentrations remained low which indicates that they were either formed very slowly or eliminated very fast. The long term toxicity test showed that survival at the end of the experiment was higher in treatment with TWA concentration when compared to pulse treatments. Based on the TKTD model, toxicodynamic processes were slower than the elimination of propiconazole, i.e. recovery from damage was the dominant recovery process. Altogether, the survival was better characterised by the model assuming individual tolerance in the *G. pulex* population. The IT model with and without simulated internal concentrations estimated similar overall recovery. This indicates that the TK test was not necessary when predicting the survival.

MO 318
A TK/TD model for sublethal effects on Myriophyllum spicatum
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Ecological models, particularly TK/TD models, are capable to refine risk assessments for plant protection products. In this work a previously published growth model for Myriophyllum spicatum was extended to a TK/TD model by integrating respective toxicokinetic and toxicodynamic parts. Uptake and distribution kinetics are modeled by physicochemical properties of the substance tested and species-specific parameters. These species-specific parameters are derived from published literature. The toxicodynamic sub model describes the reduction of growth rates based on internal concentrations and EC_{50} values of the investigated chemicals.
First comparisons between experimental laboratory data for growth inhibition under different exposure scenarios (static and short term) and calculated growth curves demonstrate that the model can simulate reversible growth reduction in Myriophyllum spicatum properly. An extrapolation to effects under field conditions is possible by considering realistic climate and environmental conditions. The ability to use arbitrary exposure patterns as an input for the toxicokinetic sub model enables a realistic risk assessment for plant protection products taking into account specific predicted environmental concentrations in surface water.

MO 319
Mechanistic modelling for risk assessment of cadmium and silver toxicities on abalone populations
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The purpose of this study was to link toxicokinetics/toxicodynamics and subcellular partitioning for assessing the susceptibility and growth inhibition risks of abalone Haliotis diversicolor supertexta exposed to waterborne and foodborne cadmium (Cd) and silver (Ag). We reanalyzed the published data on growth inhibition and subcellular partitioning associated with the present mechanistic model to explore the correlations among elimination (k_e), detoxification (k_d), and recovery (k_r) rate constants and to assess the growth inhibition risk. We found a positive correlation among k_e, k_d, and k_r in abalone exposed to Ag. We also employed a life-stage based probabilistic assessment model to estimate the growth inhibition risk of abalone to environmentally relevant Cd (5 - 995 μg L^{-1}) and Ag (0.05 - 9.95 μg L^{-1}) concentrations in Taiwan, respectively. The results showed that abalone had a minimum 20% probability of growth inhibition risk exposed to Cd, whereas Ag exposure was not likely to pose the risk. When abalone population exposed to the same Cd and Ag concentrations, the Cd- and Ag-exposed maximum biomasses were estimated to be 0.0039 and 0.0038, 61.61 and 43.87, and 98.88 and 62.97 g for larva, juvenile, and adult, respectively. Our study offers a useful tool to detect the potential growth biomass of abalone populations subjected to Cd and Ag stresses and also provides mechanistic implications for a long-term ecotoxicological risk assessment in the realistic situations.

MO 320
Predicting internal concentrations of chemicals in fish based on PBPK and one-compartment models
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European Union regulations require comprehensive testing and risk assessment before a chemical might be approved for use. In ecological risk assessment of chemicals in water, fish play a very important role, being the only vertebrate representative in freshwater systems. For this reason it would be very useful to simulate the relationship between external and internal dose in fish, and to define internal dose of a chemical using toxicokinetic modelling. Thus far, quantification of chemical toxicity is generally based on measurements of external exposure; however, in order to understand and interpret toxicological effects well, using internal concentrations of chemicals is more suitable. The goal of this study was to predict internal concentrations in fish based on the hypothesis that physiologically based pharmacokinetic models simulate internal concentrations of chemicals in better agreement with measured data (in fish tissues and whole body) than one-compartment approaches. To achieve that aim, two one-compartment models and one physiologically based multi-compartment model (PBPK) were used in order to simulate internal concentrations of chemicals in fish and fish tissues. Only respiratory uptake routes were considered for both model types and were described by mass-balance differential equations. Toxicokinetic models were compared with measured data of chemical internal concentrations in rainbow trout and fathead minnow. These two fish species were chosen because of their wide application in toxicity testing and the availability of their physiological parameters.
For internal concentrations of chemicals both in fish tissues and in whole body, the PBPK model outperformed one-compartment models; however for rainbow trout, agreement between each of the model and measured data was higher than for fathead minnow.

MO 321
Health risk assessment of a PFOS and PFOA mixture by using a whole body pharmacokinetic model
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Despite their increasing use in human health risk assessment, the application of physiologically-based pharmacokinetic (PBPK) models for the characterization of chemical mixtures is still incipient. Notwithstanding, humans are exposed to multiple substances rather than to individual chemicals. The purpose of the present study was to develop a single-compartmental PBPK model with the capacity to integrate chemical pollutants with different physical-chemical and toxicological properties. The model was applied in a specific case-study: the cumulative exposure of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) through dietary intake and water consumption, the most predominant routes of exposure. While PFOS was listed under the Stockholm Convention in May 2009, PFOA is a serious candidate to enter that list. In recent years, the concentrations of these as well as other perfluorinated compounds have been analyzed in environmental (water), biological (blood) and food samples collected in Catalonia (NE of Spain), as part of a large monitoring investigation to trace the predominant sources of exposure. The PBPK model here developed was validated by comparing theoretical values with experimental data on PFOS and PFOA levels in drinking water, foodstuffs and blood.

MO 322
General unified theory of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology
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Toxicokinetic-toxicodynamic models (TKTD models) simulate the time-course of processes linking exposure concentrations to toxic effects on organisms. Unfortunately, even for an apparently simple endpoint like survival, a large number of very different TKTD approaches exist. Examples are the critical body residue (CBR) approach, the critical target occupation (CTO) approach, the damage assessment model (DAM), the DEBtox model for survival, and the threshold damage model (TDM). These differ in their underlying hypotheses and assumptions, although these are usually not explicitly stated. To clarify the underlying assumptions of the various modelling approaches, and to show how they relate to each other, we developed a "General Unified Theory for Survival"(GUTS). From GUTS, a large range of existing TKTD models for survival can be derived as special cases, but also mixed models may result. For example, GUTS can take on the extreme philosophies of the CBR models (immediate death and a distributed threshold in the population) and hazard models (death is stochastic at the level of the individual), but also a combination of these two extremes (stochastic death with a distributed threshold). We envision that GUTS will help to increase the application of TKTD models in ecotoxicological research as well as environmental risk assessment of chemicals. It unifies a wide range of apparently unrelated approaches, clarifies their underlying assumptions, and facilitates further improvement in the modelling of survival under chemical (and other) stress.

MO 323
An algal toxicokinetic model for population level ecological risk assessment
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Various herbicides are used for weed prevention in paddy fields. These herbicides flow directly out from paddy fields to rivers through drainage channels, and are detected from April to August in river waters in Japan. Moreover. these herbicides are highly toxic to algae, and therefore there is a higher concern for the aquatic risk caused by paddy herbicides. Population viability is a main endpoint of ecosystem protection, and therefore it is needed to assess timed effect and subsequent population recovery considering these time-varying exposure. The aim of this study is the development of an algal toxicokinetic model to predict algal population dynamics under time-varying herbicide exposure for population level ecological risk assessment.
We investigated the effect of herbicide pretilachlor, which is widely used in paddy fields in Japan, on the growth and mortality of the green algae Pseudokirchneriella subcapitata. According to the standardized test guideline, a 72 h algal growth inhibition test was conducted. Dead cells in subsamples were stained with the green nucleic acid dye SYTOX-Green, and live and dead cells were counted separately using flow cytometry. Moreover, cells in the algal growth inhibition test that were exposed for 72 h were inoculated with herbicide-free fresh medium, and their growth was monitored to investigate population recovery after timed exposure. Algal population dynamics are described based on the logistic model and toxicity are described by growth inhibition and mortality. Model parameters were deterimned from these test results, and model prediction of algal population dynamics was consistent with the measured values.

MO 324
Prediction of the blood/air and fat/air partition coefficients from chemical structure
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For physiologically based pharmacokinetic (PBPK) modelling, partition coefficients between tissues and environmental compartments are required. Two important parameters are the distribution of organic chemicals in the blood/air and fat/air system. Available human and rat data have been collected from various literature sources and evaluated. The number of available data is rather limited, yielding a set of experimental blood/air partition coefficients for 279 compounds, fat/air data for 124 compounds only.
The data sets have been employed to develop new estimation models for these properties at body temperature. The models are superior to existing literature approaches based on Abraham-type linear solvation energy relationships (LSER). The new models estimate the blood/air and fat/air partitioning through two different descriptors. The model performances depend on the descriptor quality. They yield squared correlation coefficients of regression of $r^2 = 0.88$ and 0.90, respectively.
The study was supported by the EU projects 2-FUN (contract No. 036976) and OSIRIS (IP, contract No. 037017).

RA02 - Aquatic and terrestrial mesocosm and field studies - Messages from complex systems to academia, regulators, and industry

MO 327

Lab-to-field assessment factor evaluation for the nickel surface water pnec

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To develop chemical benchmarks that are protective of natural systems, regulators must account for the unknowns involved when extrapolating information from a few test species in simple laboratory environments to the complexity of natural ecosystems. In this paper, we examine the sources of uncertainty associated with the predicted no effects concentration (PNEC) for nickel (Ni) in surface water. In the European Union risk assessment for Ni (ECB 2008), the PNEC for surface water is derived from the HC5 calculated as the 5th percentile of the species sensitivity distribution (SSD) based on chronic EC10/NOEC values for 31 species divided by an assessment factor (AF) of 2. Because the HC5 is derived using the Biotic Ligand Model (BLM), it also accounts for site-specific bioavailability. In a 4-month mesocosm study (Fraunhofer 2010), the BLM-based Ni HC5 value, based on the chemistry of the mesocosm test water, was 3.5 to 5.7-fold lower than the multispecies NOEC. Accordingly, an additional AF would not be required for the HC5 to be protective of the community NOEC. In a field study in the UK (WCA Environment 2009), ambient Ni concentrations relative to population- and community-based benthic macroinvertebrate indices indicated that deterioration in ecological quality is unlikely to occur at the HC5 Ni concentration. Of 632 sites in the Forum of European Geological Surveys (FOREGs) database where Ni concentrations were measured, and where corresponding dissolved organic carbon, pH, and hardness measurements were within the calibration range of the Ni BLM, just 1% had an ambient Ni concentration exceeding the HC5, while 7% had ambient Ni concentrations exceeding the HC5 divided by two. De Vries et al. (2010) concluded that the HC5 level, for any chemical stressor, is a protective threshold for changes in biodiversity, as defined by species richness and heterogeneity, and noted that changes in concentrations at and below the HC5 would not be detectable. Given the resilience of ecosystem function, coupled with the protectiveness of the Ni HC5 when compared to mesocosm- and field-based effects data, we conclude that the uncertainty within the aquatic effects assessment is sufficiently understood, and that the HC5 provides an adequate level of protection for freshwater systems.

MO 328

How mine drainage waters affect lotic systems: results from a Triad-based Environmental Risk Assessment of a disused mining area in Tuscany (Italy)

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Aim of this study was to determine the potential environmental impact posed to a lotic system by effluents from a disused mining area in Tuscany (Italy). To analyze the impact of mine drainage water inputs, a Triad-based Ecological Risk Assessment, encompassing chemistry, ecotoxicology and ecology, was carried out along a small water-body, up and downstream the confluence with the channel collecting mine waters.

Chemical analyses were focused on determining metals and inorganic contaminants concentration in water. Water toxicity was evaluated utilizing different model organisms, i.e. bacteria, algae, protozoa, crustaceans. Sediment toxicity was determined testing interstitial water with protozoa and nematodes, and bulk sediment with seeds and ostracods. Finally, the benthic macroinvertebrate community structure was analyzed as ecological endpoint.

The elements of interest in the mine waters of the study area are iron, manganese and sulphates: although a gradient in chemical concentrations was observable between the up- and downstream sites, levels were below safety thresholds in both sites. Only mild toxic effects were detected on protozoa after exposure to superficial and interstitial water and on seeds exposed to bulk sediments; no alteration was evident in genotoxicity endpoints on the two model organisms, i.e. protozoa and seeds. Interestingly, benthic macroinvertebrate community showed a similar structure in both up- and down-stream sites; surprisingly, a significant reduction of benthic diversity with respect to the stream collecting mine effluents was detected both in the up- and downstream sites. The integration of the Triad data into an environmental risk index (EnvRI) applying an Expert Decision Support System shows a low level of risk, below the safety threshold (0.25), due to the mine effluent inputs (EnvRI= 0.15). Pollutant sources in areas upstream the study sites seem to contribute to the degradation of the lotic system, reducing the biological quality of the stream. In fact, higher values of EnvRI are obtained for both the up- and downstream sites (respectively 0.30 and 0.31) when considering a third unpolluted reference point in the mine area. A risk of biodiversity decline in the study area is evident, even if it is not related to the mine drainage input. In fact, the high biodiversity of the macroinvertebrate community structure (EBI= 10) in the stream collecting mine drainage waters suggests low bioavailability of pollutants for the biota.

MO 329

Estimating safe concentrations of four heavy metals (Cu, Zn, Cd, and Mn) from field effects on species richness of riverine macroinvertebrates

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The ecological impacts of heavy-metal contamination on aquatic ecosystems are a major concern worldwide. Ecological risk assessment is thus necessary for effective risk managements. In this context, the estimation of the safe concentrations (SCs), such as predicted no effect concentration (PNEC) and environmental water quality criteria, at which unacceptable effects are most likely to be avoided, is critical. Although such concentrations are usually determined from laboratory toxicity tests to assess effects on individual-level traits, the real goals of ecological risk management are to protect populations, communities, and ecosystems in the natural environment. Information on metal-induced field effects can therefore provide useful insights into the SCs in the natural systems. Despite previous studies evaluating the effects of heavy metals on aquatic organisms, knowledge useful for estimating the safe concentrations is limited.

We estimated SCs of heavy metals (copper, zinc, cadmium, and manganese) from macroinvertebrate survey data collected from over 400 individual sites in UK, US, and Japan. Benthic macroinvertebrates are widely used to evaluate the anthropogenic impacts on freshwaters, and we used EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness as a metric in this study. The relationship between dissolved concentrations of each metal and EPT richness were determined using quantile regression (the 90th quantile). Additionally, for this analysis, we used a function to identify a threshold concentration below which no effects were observed- taken to be the SC. The SCs (and 95% confidence intervals) of copper, zinc, cadmium, and manganese were estimated at 8.1 (1.9-20.8), 60 (26-405), 0.07 (0.01-0.45), and 3.9 (1.0-21.7) µg/L, respectively. We compared these values for copper, zinc, and cadmium with PNECs from EU risk-assessment reports and environmental water quality standards in the UK and US. Despite being derived from laboratory toxicity tests, most of these laboratory-based SCs overlapped with our estimates. To our knowledge, this is the first study to estimate the SCs from geographically widespread field data on macroinvertebrate surveys.

MO 330

Requirements for Terrestrial Model Ecosystems (TME) in Environmental Risk Assessment - influence of coring sites and management on microarthropod communities

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Terrestrial Model Ecosystems (TME) can serve as an intermediate tool between laboratory and field tests in the Environmental Risk Assessment (ERA) of plant protection products and have been proven to be a suitable test system for a refined risk assessment. These outdoor test systems realistically represent the field situation and allow the examination of effects on complex soil communities at different trophic levels. At RWTH Aachen University in cooperation with Bayer CropScience a structural higher-tier TME-test system in soil ecotoxicology has been developed which is now considered to be ready for routine operations.

The initial situation of a TME study as well as the test conditions in the course of a one year study can be quite variable depending on the selected habitat for coring the test units and the management of the TME. Habitat selection and maintenance of the soil community are crucial issues, determining the representativeness, reproducibility and thus the validity of a test. Important aspects regarding the choice of the TME habitat are 1.which target community (off-crop/in-crop) we want to test, 2. are there minimum requirements concerning the diversity and community structure of the TME site and 3. which management strategies are suitable to sustain a stable test community over a one year period?

To answer these questions we tested the influence of different habitat management strategies on the soil microarthropod community (Collembola, Acari) in TME from two different coring sites. Additionally we tried to examine whether the management of the vegetation (mowing, fallow, mulching) within the test units (TME) has an influence on the population dynamics of the soil microarthropod community. The results indicate that our strategy of TME testing over the last years fulfils essential criteria of a semi-field test system within the ERA.

MO 331

Benthic microcosm; tool for toxicity estimation on ecosystem level

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Sixteen week outdoor benthic microcosm experiment was conducted to evaluate the effects of sewage sludge on benthic invertebrate colonization from May to September 2010 in intertidal mud flat. Re-colonization of macro/meiobenthos and chemical characterization were observed and analyzed using 2 factor ANOVA design (3X3X3); three sewage concentrations, three segment groups within microcosm, and three replicates for each treatment group. Changes of macro/meiobenthis, ignition loss (IL), COD, total sulfur (TS) and water contents (WC) were observed every three week period. Sewage concentration was major effect on changes of IL, COD, TS and WC with time, and the number segments within microcosm showed significant effects on changes of benthic community. This study was focused on the segmentation effects on recruitment of benthos within microcosm and revealed that segmentation can improve the colonization of benthic community in toxicity test at ecosystem level. Accordingly, segmented microcosm (small plastic basket) can be a useful tool for toxicity estimation at ecosystem level.

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MO 332

Comparative sensitivity and recovery of structural and functional endpoints to fungicide chronic exposure in artificial streams

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Ecological risk assessment of chemicals at the community level is usually based on the measurement of various parameters that derived from those used in ecological studies. Although these parameters are well suited to assess the acute/short term responses to toxicants, they may be sometimes less efficient in documenting indirect or long term responses, including recovery of the stressed systems. Furthermore, most of these parameters refer to the structure of the communities whereas there is a growing interest in the assessment of functional consequences of chemical stressors. A 5-month long experiment was performed into 40-m long outdoor flow-through experimental streams (volume : 6 m^3) to compare the responses of structural (taxonomic richness of invertebrate community and abundances of the various invertebrate groups, periphyton composition and biomass) and functional (alder litter breakdown, food web functioning) parameters to pesticide exposure. After a two-month stabilization period, 4 streams were treated with thiram, a dithiocarbamate fungicide (nominal concentrations: 35 and 170 $\mu g\ L^{-1}$; 2 replicates per concentration). Continuous exposure was carried out for three weeks and four untreated streams were kept as controls. Exposure was followed by a two-month long recovery period. In this presentation, the temporal dynamics of water physico-chemical characteristics (including thiram residues), periphyton composition and biomass, benthic invertebrate community structure and litter breakdown in control and treated experimental streams are presented. The sensitivity and the recovery dynamics of the various endpoints are compared and discussed.

MO 333

Ecotoxicology study of new butachlor CS formulation in Korean rice paddy field

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Butachlor CS(capsule suspension) formulation was newly developed to decrease risk of oriental weatherfish which is the major biometer of Korean agricultural ecosystems. Ecotoxicology studies of acute, Semi-field and agricultural field study were performed using oriental weatherfish. Acute toxicity study was performed by test guidelines of Rural Development Administration, Korea. Semi-field study was experimented in rice paddy field which was divided to 3 plots(each plot 2.5 x 4.0 m). Each plot contains 5 cages with 20 oriental weatherfish. Mortality and abnormal behaviour were under the criteria of observation for 23 days after the application of butachlor CS formulation. During the experiment, paddy water and soil were sampled and analyzed. Agricultural field study was performed at the conventional rice cultivation conditions. The paddy field(1500m2) was divided to 3 plots(500m2), sprayed butachlor CS formulation and harrowed

at the same time, and then planted rice. Each test plot contained 1000 oriental weatherfish which were 10 cages with 10 weatherfish, and 900 out of cages. Bird netting was set to protecting from the birds. Mortality and abnormal behaviour were under the criteria of observation for 21 days after the treatment of butachlor CS formulation. During the experiment, paddy water and soil were sampled and analyzed. According to results of the acute toxicity study, butachlor CS formulation's risk was lower than six times compared to already-in-use product. Cumulative mortality of butachlor CS formulation in semi-field study was resulted in significantly lowered to 5% compared to that of 64 ~ 97% of already-in-use product. In agricultural field study, the cumulative mortality of oriental weatherfish was 0% in the case of butachlor CS formulation. In cnclusion, new developed butachlor CS formulation was very low ecological risk to the oriental weatherfish.

MO 334

A multi-trial approach for estimating potential ecotoxicological effects of an industrialized area

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The aim of this study was to evaluate, by means of biomarkers and the analysis of contaminants and trace metals, the toxicological impact caused by the emission from an hazardous waste incinerator plant using the Italian wall lizard (Podarcis sicula) and the leaves of downy oak (Quercus pubescens) as biomonitor species. The study area is one of the most important gold and silver manufacturing area in Europe and is also characterized by the presence of an hazardous waste incinerator and two of the major national roads: the A1 highway and the high speed railway line. The lizard for its physiological and ecological characteristics is a powerful bioindicator; it has a limited home range and it is widely distributed in the urban environment. The downy oak has a wide distribution into the study area and is characterized by the presence of leaf with hairy surface that entrap the particulate matter. In order to obtain a more representative sampling design of the flow of incinerator emission into the atmosphere it has been applied a theoretic diffusion model which it has been validated through the detection of the cadmium concentration as a tracer metal accumulated into the leaves of downy. After this phase, the downy oak was used as a vegetal bioindicator to evaluate the presence of trace elements accumulated on the leaf surfaces by the atmospheric deposition. In a second phase, a set of biomarkers (EROD activity, uro- copro and proto-porphyrins, Comet assay and ENA assay) and the levels of contaminants (PCB and Dioxins) and trace metals (Cd, Hg, Pb, Zn) were evaluated in 47 specimens of the Italian wall lizard collected in sampling station that has been selected according to the diffusion model data. The results showed a good relationship between the theoretical model and the concentration of trace metal into the leaves. The results of the biomarkers and the analysis of contaminants evaluated in the Italian wall lizard, showed an high sensitivity of the bioindicator species. In conclusion the results obtained underline how the use of a multi-trial approach based on biomarker responses and contaminant levels in bioindicator species is a simple and effective method for the evaluation of possible impacts caused by a contamination from any type of industrial plant.

MO 335

Ecological diagnosis of pollution impacts by weight of evidence

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A recent survey of sediment quality in Melbourne and the Yarra Valley found elevated concentrations of a range of pesticides (1). Because contaminants typically occur in combination, and guideline concentrations exist for few of the detected pesticides, predicting the ecological impact of contaminated sediment is a complex issue. We applied a weight of evidence approach to three complementary sediment toxicity bioassays to diagnose both the severity and type of ecological impact. We measured community compositional change in a field microcosm bioassay, deformity rates in wild Chironomus spp, and growth, emergence and survival in laboratory-reared C. tepperi. The field microcosm bioassay provided an ecologically relevant benchmark by estimating the impact of sediment pollution on invertebrate community composition. Pesticides and heavy metals were linked with two distinct types of microcosm community change. In wild Chironomus spp, heavy metals were specifically associated with mentum deformities, while pesticides were associated with deformities of the pectin epipharanges. Sites where impacts were identified by both laboratory C. tepperi bioassay and wild Chironomus spp deformity rates tended to also display community impacts in the field microcosm bioassay. We conclude the C. tepperi test was the most sensitive of the three bioassays, deformities suggested more severe toxicity, and microcosm community change indicated the most severe toxicity. The combined bioassays provide useful evidence regarding the type of contamination responsible for the observed toxicity.

(1) Rose G, Allen D, Allinson G, Allinson M, Bui A, Wightwick A, Zhang P. (2009). Melbourne Water and DPI agrochemicals in Port Phillip catchment streams-project summary report on 2008-09. DPI Tech Rpt

MO 336

Characterizing off-field vegetation for NTA terrestrial mesocosm studies

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In contrast to in-crop environments, the habitat for studies with non target arthropods in natural off-field environments can as a matter of fact not be standardized. Because the composition of the arthropod fauna is closely linked to the local flora there is a clear need to characterize the vegetation of such a study site. This is not only needed to assess the within study variation in habitat characteristics as a basis for blocking, but also to enable a future classification/comparison of off-field studies. Here we present a simplified standard method to assess plant diversity and relative abundance of grassland vegetations. The method involves releves of units consisting of eight small squares, using presence/absence sampling, performed on regular grid imposed over the study site. The data analysis uses Twin Span and correspondence analysis to quantify similarities in vegetation structure within the study site.

RA04 - Environmental risk assessment and management of Plant Protection Products (PPPs) and biocides

MO 339

Is the composition of the small mammal prey guild a factor that influences the risk to predators from anticoagulant rodenticides?

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The small mammal prey guild in Ireland is restricted compared with that in Britain but still includes species most likely to consume anticoagulant rodenticides (AR) baits, the biocides that are the most widely used globally to control rodents. We hypothesise this restricted prey guild will enhance secondary exposure of predators and the associated risk of secondary poisoning. We determined, from the presence of liver residues, the exposure of 115 foxes (Vulpes vulpes) to ARs (predominantly second-generation anticoagulant rodenticides) that were shot or killed by traffic in Northern Ireland (NI). Exposure to the more toxic ARs, flocoumafen and brodifacoum, was more prevalent than in foxes from elsewhere in the UK and reflect greater use of these compounds by farmers in NI. Exposure to ARs generally was greater (% of animals exposed and/or magnitude of residues) in lowland than upland animals, probably due to greater availability and consumption of AR-contaminated small mammals in lowland habitats. Overall, 84% of the foxes tested had detectable liver residues and exposure was greater or similar to that in samples of foxes from Scotland and from England and Wales, despite the British samples being biased towards suspected poisoned animals. High exposure in NI foxes is most likely explained by greater predation of commensal rodents and those non-target species most likely to take AR baits. Our data are consistent with the hypothesis that the reliance of small mammal predators in Ireland on a restricted prey guild enhances their exposure to ARs. We argue that Ireland may be a sentinel for the UK in terms of likelihood of AR-mediated effects on predator populations.

MO 340

Estimation of emission from treated wood: evaluation of experience under the EU Review Program of the Biocidal Products Directive

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Chemicals such as biocides contained in Wood Preservatives, can leach from the wood to the environment being a cause of environmental and health concern. Once in the environment, the biocide residue can be immobilised and degraded in soil, or transferred to the other compartments in the environment.

In 1998, Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products was adopted. The Directive aims to provide a high level of protection for humans, animals and the environment. Wood Preservatives are defined as Product Type 8 and are among the priority substances to be assessed.

For Annex I inclusion, an environmental assessment is required. For Wood Preservatives, this methodology requires the determination of: leaching rates from treated wood, exposure rates, and environmental effects. The main issue of the Review Program for biocidal substances (2000-2004) was that none of the existing draft guidelines were validated and the lack of international harmonisation. The Leaching Workshop organised in 2005, aimed to solve this issue.

The majority of the substances used in Wood Preservatives have now been assessed in the Review Program. In addition product authorization has also started. As a consequence, there is a need to re-evaluate the results on the Leaching Workshop in the light of the experience gained so far in the Review Program. In addition, ongoing recent developments within the OECD and CEN have to be considered

MO 341

Harmonising the exposure assessments for rodenticides: finalising the revision of the Emission Scenario Document for PT14.

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The Technical Guidance Document (TGD) published by the European Commission provides guidance for the overall risk assessment of chemical substances including active substances for biocidal products. Specific guidance on the exposure assessment of these active substances has been provided for in Emission Scenario Documents (ESDs), which were developed per biocidal product type in the EUBEES 2-project.

The first active substances for biocidal products of the first priority list (product types 8 and 14) have been included in Annex I or IA of the Biocidal Product Directive 98/8/EC and experience has been gained with the use of ESDs.

More specifically, during the evaluation of active substances for product type 14 (rodenticides), the need for a revision of the guidance related to the assessment of primary and secondary poisoning became clear and subsequently an update and harmonisation of this assessment was endorsed at the 23rd Meeting of Competent Authorities.

Currently, the revision of the ESD for product type 14 is ongoing, which will include an updated section on the issue of primary and secondary poisoning of non-target animals exposed to rodenticides as well as a harmonisation with other risk assessment schemes (i.e. EFSA). Additionally, the issue of exposure of target- versus non-target animals and possible risk management measures will be addressed.

MO 342

Risk assessment of fungicides and bactericides: are we using the correct data?

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Risk assessment is conventionally performed by comparing measured or predicted environmental concentrations with a concentration that should protect the ecosystem on a long-term perspective. This protective concentration is calculated based on a set of ecotoxicological data and we assume that the diversity of the data from this set represents the diversity of species we can find in the ecosystem. In reality, ecotoxicological data are collected in databases and the species represented are mostly limited to daphnids, some algae and some fish, that do not represent the richness of the ecosystem. This is particularly problematic when trying to evaluate the risk of specific acting compounds like fungicides and bacteriocides that have a mechanism of action developed to affect fungi or bacteria. Indeed, neither fungi nor bacteria are well represented in ecotoxicology databases. In this study, we tested two fungicides, metalaxyl and propiconazol, and one bacteriocide, triclosan, on different bacteria and yeasts. The results were compared with species sensitivity distribution curves constructed with conventional ecotoxicological data obtained from databases. In general, the yeasts and bacteria were not more sensitive than the other species. However, for both metalaxyl and triclosan, yeast and bacteria were among the most sensitive species.

Furthermore, we also tested one substance, triclosan, on a natural microbial community, which was shown to be more sensitive than any single species. Indeed, 3 ug/l was sufficient to change the phylogenetic composition of the community. It seems therefore crucial to include effect data obtained with yeasts, bacteria, fungi and even whole microbial communities when calculating protective concentrations for fungicides and bacteriocides.

MO 343

Are HC5 values derived from toxicity tests with fish, invertebrates and primary producers protective of aquatic fungi?

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Aquatic ecological risk assessment for fungicides in Europe does not currently assess risk to fungi. Rather, regulatory acceptable concentrations based on ecotoxicological data for fish, invertebrates and primary producers are assumed to be protective of all other aquatic organisms. Here we explore the validity of this assumption by investigating the effects of a fungicide applied at the HC5 concentration (derived from single species toxicity tests on fish, invertebrates and primary producers) on the abundance and functioning of aquatic hyphomycetes in semi-field studies.
The study was performed in enclosures placed in an experimental ditch system at Sinderhoeve experimental station, The Netherlands. A single application of tebuconazole (238 µg/l) was applied to four enclosures and another four enclosures were used as controls. The experiment ran for 52 d and aquatic hyphomycete abundance and leaf decomposition rates in enclosures receiving tebuconazole were compared to those of control enclosures. In addition, the consumption by invertebrates (*Gammarus pulex*) of leaf material from treatment and control enclosures was compared in an additional laboratory experiment. Tebuconazole exposure caused some change in the abundance (as conidial production) of the dominant aquatic hyphomycetes species (*Anguillospora longissima* and *Tetracladium setigerum*), but had no effect on leaf decomposition rates. There was, however, a significant reduction in the consumption rate of Gammarus pulex when fed tebuconazole-exposed leaf material.

MO 344

Fungal community structure and functioning in arable and non-arable streams

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Microbes, in particular fungi, play an important role in the ecology of streams by making energy and nutrients locked away in dead organic matter available to aquatic organisms. Agricultural practices may impact stream communities by altering habitats or by reducing water quality through increased nutrient and pesticide concentrations. The risks of pesticide exposure to aquatic animals and plants are well studied, but little is known about the risk posed to fungi; despite many pesticides being fungicides. Here we investigate the impact of arable farming, including pesticide application, on the structure and functioning of aquatic hyphomycetes, a key group of stream fungi. Fungal community structure, biomass, leaf decomposition rates and utilization of leaf litter by shredding invertebrates (i.e. *Gammarus pulex*) was assessed for 5 pairs of arable and non-arable streams in Lincolnshire, United Kingdom. Arable streams supported lower fungal diversity, lower fungal biomass and lower leaf decomposition rates than non-arable streams. However, the consumption of leaf material by shredders was increased when leaf material was conditioned in arable streams.

MO 345

The effects of three pesticides on the growth of Euglypha rotunda (Rhizaria; Euglyphida): is this species a good bioindicator?

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In this study we investigated the effects of pesticides on a common soil testate amoeba species (*Euglypha rotunda*). *E. rotunda* and other free-living euglyphid soil protozoa play important ecological roles in the soil microbial loop as bacterial predator and in the cycling of silica. However, effects of pesticides have never been tested on these organisms. Imbalanced selection of model organisms in ecotoxicology could potentially cause erroneous assessment of true environmental impact of pesticides and pollutants on soil biodiversity and ecosystem function. We therefore evaluated the effect of three pesticides, the herbicide glyphosate (Roundup active ingredient), the fungicide carbendazim (Carbendazim SA 60 FL active ingredient) and the insecticide thiacloprid (Alanto active ingredient) on the growth of *E. rotunda*. We assessed population growth of *E. rotunda* after exposure at following concentrations ranges: 0.1 - 10 mg/L glyphosate, 0.1 - 2 mg/L carbendazim and 10 - 1000 ng/L thiacloprid cultured in NCL 0.1% media during 21 days of incubation. The results showed that both the fungicide and the insecticide decreased the growth of *E. rotunda*, even at low concentrations. Thiacloprid inhibited totally the growth of *E. rotunda* in all the range of concentration tested. Carbendazim caused a total growth-inhibition at a concentration of 1 mg/L and a partial growth-inhibition at a concentration of 0.1 mg/L. By contrast, glyphosate stimulated the growth of *E. rotunda* at concentration of 10 mg/L but no effect was detected at 1 mg/L. These results show than *E. rotunda* reacts differently when exposed to different pesticides and is highly sensitive to some such as thiacloprid. We therefore suggest that it could be a good bioindicator to be tested to evaluate the effects of pesticides.

MO 346

Does the length of the interval between repeated pulses influence the effect of the pesticide permethrin on Hyalella azteca?

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Exposure of non-target aquatic organisms to pesticides is likely to occur in short pulses following periods of drain flow, surface run-off or spray drift. Since standard aquatic toxicity tests for acute effects assessment are primarily based on continuous and maintained exposure periods of 24 to 96 hours, there is a mismatch between laboratory and field exposure patterns. This has potential implications for standard risk assessments and could result in over- or underestimation of risk.
The aim of the present study was to examine the effect of different time intervals between two pulses of the pyrethroid insecticide, permethrin, on the freshwater amphipod Hyalella azteca. Permethrin is used in mosquito control and to control a wide range of insect pests on various crops and is known to be highly toxic to aquatic invertebrates. H. azteca is widely distributed throughout North America and is a common food source for birds, fish and large invertebrates. It is therefore considered to be an ecologically important species. In addition, H. azteca has been extensively used as a test organism and is generally sensitive to contaminants.
H. azteca were exposed to two repeated pulses of one hour each with different intervals between the two pulses. The nominal exposure concentrations were 0, 0.3 or 0.9 µg/L, and the intervals between the pulses were 0, 6, 24, 48 or 144 hours. After each pulse the organisms were transferred to clean water. Survival was recorded immediately after the pulses and again after 10 days from the start of the experiment.
The implications of having different intervals between pulses will be discussed.

MO 347

Invasive freshwater crayfish: a new ecosystem component and its challenge to risk assessment

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Due to their high commercial value exotic freshwater crayfish have been introduced to many freshwater ecosystems. Being very mobile and able to escape designated water bodies over land crayfish soon spread far beyond their initial points of introduction. Today almost every European country harbors at least one species of exotic crayfish.
Being carriers of the fungal infection known as crayfish plague (lethal to indigenous crayfish) and strong ecosystem engineers through mechanical interference or active predation freshwater crayfish heavily impact macrophytes, invertebrate and vertebrate communities in many ways and therefore are often considered pests. However, invasive crayfish have also become an important part of the diet of larger fish, birds, and mammals. In combination with strong crayfish feeding on organic matter this might result in an additional exposure route in the food chain.
Although being hardy creatures, its large size and aggressive territorial behavior make most crayfish challenging test subjects in the laboratory. In this poster we present several tried techniques used in crayfish testing and provide solutions covering the challenges crayfish pose to risk assessment.

MO 348

Population-level effects of spinosad and Bacillus thuringiensis israelensis in Daphnia magna: comparison of laboratory and field microcosm exposure conditions

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Because exposure to toxicants not only results in mortality but also in multiple sublethal effects, the use of life-table data appears particularly suitable to assess global effects on exposed populations. The present study uses a life table response approach to assess population-level effects of two insecticides used against mosquito larvae, spinosad (8 µg/l) and *Bacillus thuringiensis var. israelensis* (*Bti*, 0.5 µl/l), on *Daphnia magna* (Crustacea: Cladocera), under laboratory versus field microcosms conditions. Population growth rates were inferred from life table data and Leslie matrices under a model with resource limitation (ceiling). These were further used to estimate population risks of extinction under each tested condition, using stochastic simulations. In laboratory conditions, analyses performed confirmed the significant negative effect of spinosad on survival, mean time at death, and fecundity as compared to controls and *Bti*-treated groups, population growth rate λ was lower under exposure to spinosad. In field microcosms, 2 days after larvicide application, population growth rate was significantly lower under spinosad exposure conditions than under control and *Bti* exposure conditions. Simulations performed on spinosad-exposed organisms led to population extinction (minimum abundance = 0, extinction risk = 1), and this was extremely rapid (time to quasi-extinction = 4.1 one-week long steps, i.e. one month).

MO 349

Status of guidance documents for environmental risk assessment of plant protection products at EFSA

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Since 2006, EFSA's PPR Panel has been responsible for the update and development of EU Guidance Documents (GDs) for Environmental Risk Assessment (ERA) of Plant Protection Products. Since October 2008, the EFSA Pesticide Steering Committee (consisting of Member States, the Commission and EFSA representatives) has been responsible for setting priorities for needs of new Guidance or updating existing GDs for risk assessment of Plant Protection Products (PPPs). An update on the ongoing work, as well as an outlook of ongoing activities in the PPR Panel, will be given:
Revision of the GD on Persistence in Soil, New GD on Emission from Protected crop system (Green-houses and Covered crops), Revision of the GD on Aquatic Ecotoxicology and the Revision of the GD on Terrestrial Ecotoxicology.
Besides the update and expected time-lines for the ongoing activities, an outlook for upcoming requests for revision or new GDs and Opinions will be presented.

MO 350

Database compilation on pesticide ecotoxicological tier 1 study endpoints using IUCLID 5.2 as data entry software

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In 2009, EFSA's PPR unit has received two mandates for revising the current Guidance Documents (GDs) on Aquatic Ecotoxicology (SANCO/3268/2001) and Terrestrial Ecotoxicology (SANCO/10329/2002) regarding the placing of plant protection products (PPPs) on the market. In this context, the validation of Toxicity Exposure Ratios (TERs) used in these GDs is needed. As a requisite for this, it was decided to create a database on ecotoxicological properties assessed at Tier 1 level for all available active substances and PPPs dossiers submitted under Directive 91/414/EEC, that contain also higher tier ecotoxicology studies. The data entry has been outsourced in 2009 according to EFSA procurement procedures and fully carried out at EFSA premises by ChemService.
The database contains all available tier 1 study endpoints for aquatic organisms, bees, non target arthropods, non target plants and soil organisms, for the dossiers where higher tier ecotoxicology data for any of these taxa are available. This amounts to 227 dossiers corresponding to 104 active ingredients and 123 PPPs. The data has been collated using IUCLID 5.2 software which has a data structure based on the OECD harmonised template for reporting chemical test summaries. This option was selected since the harmonised template is an international data standard which is

used for instance by ECHA for the REACH process and for the submission of biocidal products dossiers according to the EC Directive 98/8. The IUCLID interface ensures that entered data is in a format suitable for querying and statistical analysis, the query plug-in tool assists in data quality checks and the IUCLID system supports the export of data in XML format to facilitate data sharing.
The database will be further populated entering tier 1 study endpoints for all available dossiers, i.e. also those not containing related higher tier studies, following a mandate received by the European Commission (EC) in 2010, to compile such a database covering all available ecotoxicology tier 1 data. These data are intended to be used by the EC and the European Member States in the context of the new legislation Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market, Regulation (EC) No 1185/2009 concerning statistics on pesticides, and Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides.

MO 351
Risk mitigation measures in the framework of the council Directive 91/414 EU
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According to Directive 91/414/EEC pesticides are listed in Annex I ("positive list") if at least one representative use does not pose "unacceptable"effects to humans and to the environment on the basis of a risk assessment process. EFSA (the Pesticide Risk Assessment Peer Review Unit - PRAPeR) is responsible for the peer review of risk assessments of active substances in Europe. The EFSA conclusion lists reference values and endpoints, identifies particular conditions that may need to be considered in relation to the risk, and the critical areas of concern. In the particular conditions of the conclusion, risk mitigation measures proposed in the ecotoxicology section are included.
If a potential high risk would be identified at the higher tier risk assessment for non-target species then risk mitigation measures were necessary to reduce the adverse effects of the active substance for the non-target species.
Different risk mitigation measures are contemplated for birds and mammals, aquatic organisms, bees, non-target arthropods and non-target plants (guidance documents on risk assessment for birds and mammals, SANCO 4145/2000, aquatic ecotoxicology, SANCO 3268/2001 and terrestrial ecotoxicology SANCO 10329/2002) at EU level. .
Guidance document "landscape and mitigation factors in aquatic ecological risk assessment"is currently available to harmonise the mitigation measures for aquatic organisms. However, there are currently no guidance documents to harmonise the mitigation measures required for birds and mammals, bees, non-target arthropods and non-target terrestrial plants.
The aim of this work is to give an overview of the mitigation measurements presented for the active substances suggested in the EFSA conclusion for non-target species. Critical issues and the need of further improvement will be discussed.

MO 352
Hellenic scenarios for bird focal species: the case of the cotton fields
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In Europe, the risk to birds from plant protection products (PPP) needs to be evaluated under the provision of Council Directive 91/414/EEC and associated documents. In many cases, a refined risk assessment is necessary, for which the identification of actual bird species more likely to be exposed to a PPP is often a pre-requisite. While several studies have been conducted in typical crops of central temperate Europe, less information is available for crops specific to Mediterranean countries, like cotton.
In this study, we present information about bird species observed in cotton fields in two regions of Hellas (Kopaida and Larisa) in two field seasons in spring and summer using two bird survey methods ('line transects' and 'point counts').
The most frequent and abundant species found in cotton fields in the two regions were the house sparrow and the barn swallow. The crested lark was often observed in Larisa but much less frequent in Kopaida. Other candidates for focal species were yellow wagtail, european bee-eater and greater short-toed lark. Among the larger species, common kestrel and lesser kestrel (Falconidae), carrion crow, european magpie and eurasian collared dove were identified as the most common species. The selection of bird focal species for the refined risk assessment of plant protection products used in cotton is further discussed.

MO 353
Foraging habitat of hedgerow bird species in arable land and its relevance to in-crop exposure to plant protection products
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In Europe, the risk to birds from plant protection products (PPP) needs to be evaluated under the provision of Council Directive 91/414/EEC and associated documents. In many cases, a refined risk assessment is necessary, for which the identification of actual bird species more likely to be exposed to a PPP is often a pre-requisite. While several farmland species are clearly exposed as they use primarily the in-crop field as foraging habitat, there is little information about potential exposure of those hedgerows species living in field margins adjacent to arable fields.
In this study, we provide information regarding the relevance of the in crop area as a habitat use for the hedgerow bird species in lowland-farming landscapes in order to provide a background against which decisions concerning risk assessment from the pesticide applications might be evaluated. The study was conducted in spring and summer in during 2009 in two predominantly agricultural areas of Hellas (Kopaida and Larisa) that differed in the relative frequency and complexity of hedgerows between the arable fields. Main crops were cotton, cereals and alfalfa. Birds were observed using the 'point count' method conducting a high number of observation sessions (459 sessions), each lasting 10 minutes (total observation time=4590 minutes). The number of species and individuals and time (minutes) spent by each individual in all habitats (mainly cotton but also in alfalfa and cereals) was recorded.
In Kopaida (160 sessions), a total of 35 species (of which 12 hedgerow species) was observed. In Larissa (299 sessions) the total number of species was 33 (9 hedgerow species). In both study areas, irrespective of the type and size of hedgerows, only a minor proportion (2 out 21) hedgerow species visited and potentially fed in the crop. The two hedgerow species visiting the crop (Remiz pendulinus and Acrocephalus schoenobaenus) spent a small proportion of the total observed time. Despite the different agricultural landscapes in Kopaida and Larisa, the behaviour of the hedgerow bird species was similar: feeding events within the crop appear to involve a minor proportion of the foraging time of hedgerow species. The extrapolation of these results to other regions and their implication for the regulatory risk assessment of PPPs in Europe is further discussed.

MO 354
Probabilistic ecological risk assessment of eleven paddy herbicides
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Probabilistic ecological risk assessment of 11 herbicides, commonly used in Japanese paddy fields was conducted. The effect assessment was based on species sensitivity distribution (SSD). The acute EC50 values of standard toxicity tests for aquatic primary producers were collected from available literatures and then fitted into lognormal distributions. Predicted environmental concentration (PEC) was calculated using an environmental model defined by the Ministry of Environment, Japan. The regional variations of PEC were quantified using Monte Carlo analysis. A joint probability curve was derived by comparing SSD and PEC distribution, and the area under the curve was defined as expected potentially affected fraction (EPAF) for quantitative risk index. The highest EPAF was 6.2% for bensulfuron-methyl.

MO 355
Higher-tier surface water risk assessment according to ELINK
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For a herbicide a higher-tier aquatic risk assessment was performed. One pillar was a mesocosm study. A lower-tier exposure calculation only provides a single worst-case drainage/run-off entry peak and does not take into account the duration of the exposure. It was challenged by authorities whether the exposure pattern in the mesocosm study covers the real environmental exposure situation for run-off and drainage entries with respect to peak height, duration, and area under the curve. Based on the recommendations of the ELINK workshop and using representative FOCUS surface water scenarios the suitability of the mesocosm study was demonstrated. For that purpose the "envelope curve concept"was applied comparing exposure concentrations observed in the mesocosm and time-resolved PECsw originating from FOCUS Surface Water Step 3 calculations. By this procedure it was shown that the exposure pattern used in the mesocosm study covers the run-off and drainage exposure pattern at any time with a safety factor of at least 2.

MO 356
New strategies in the risk assessment of plant protection products in soils
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The risk for soil organisms arising from the exposure to plant protection products (PPP) is assessed by relating the effect concentration to the calculated predicted environmental concentration in the soil matrix (PECsoil). Currently, the PECsoil calculations are based on simple assumptions, e.g. on an homogeneous distribution of the substance in the first 5 cm of the soil profile. But, since neither the chemical properties of the applied substances nor the habitat preferences of the soil organisms are taken into account, the appropriateness of this approach in the risk assessment (RA) of PPP has been questioned. The German Federal Environment Agency (UBA) is currently working on the improvement of the risk assessment procedure for soil organisms as part of the national authorisation process of PPP. In this context, a re-evaluation of literature data and of earthworm field studies has been undertaken, with the aim of corroborating the key hypotheses on which the newly developed strategy is based and to assess the implications that will arise from the implementation of the new procedure for the outcome of the risk assessment of plant protection products in soil.The methodology chosen followed these step:s
- Functional characterization of life form types of Central European soil invertebrates (e.g. oligochaetes, microarthropods) at crop sites regarding their exposure to PPPs -with particular regard to different exposure pathways.
- Identification of "focal communities" of soil organisms regarding the exposure to PPP for combinations of crop types and tillage operations as indicators for the soil biocenosis.
- Analysis of the results of earthworm field studies with PPPs which were performed in the context of the registration of PPPs, in particular regarding of the representativity of the respective community on the control plots, the physico-chemical properties of the test substances as well as the sensitivity of the individual life form types.
- Indication of parameters for the derivation of the ecological relevant exposure concentrations of PPPs for soil organisms based on their life form types and exposure scenarios
- Discussion of possible effects of modified exposure calculations on the interpretation of the results of ecotoxicological standard tests with soil invertebrates, including the question whether existing test designs are still appropriate or should in future be modified.

MO 357
Plant protection products authorization in Poland. Actual and future requirements.
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The submitted dossiers for plant protection products should contain study reports and calculation data for active substances and their metabolites according to requirements.
Physical and chemical properties of releasers and metabolites of their hydrolysis are essential for the risk assessment.
Assessment of predicted environmental concentration (PEC) for soil (PECS), ground water (PECGW) and surface water including sediments (PECSW and PECSED) should be based on proposed use of plant protection product (GAP) and European guidelines - FOCUS Working Group Guidance Documents. According for actual requirements, for Poland there are particular requirements for ground and surface water. The calculation for PECSW and PECSED are realized for STEP 1-2, STEP 3 and STEP 4.
For the ground water - all European scenarios are required, for the surface water - D3, D4 and R1 scenarios are obligatory. For some crops there is no scenarios for D3 or D4 or R1. In that case the worst scenarios is used as representative for Poland.
In future - Poland will be treated as Central Region State and according to such requirements all information should be submitted.
If metabolites properties confirm potential hazard for environment, then additional calculations for them are necessary and required. Evaluation of submitted reports and data for each active substance and its metabolites are considered for risk assessment. The final risk assessment and conclusion should include all available study results and information and should be compared with proposed GAP.

MO 358

Lack of an understanding in correlating various legislations in an effort to harmonize a strategic solution to environmental risk assessment and management - The Indian experience

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The Insecticides Act, 1968 and Rules, 1971 is the major legislation which deals with the regulation of PPP's and biocides. However, there are other legislations which are remotely associated with this Act., but with a poor understanding of their implications in making regulation harmonized amongst all enacted legislations in an effort to have a more effective risk management strategy. At present there is a poor understanding of any kind of risk management strategy in the country. The proposed Pesticide Management Bill 2008 too fails to address such concerns, which is still pending approval from the Lok Sabha (Parliament of India). The paper attempts to highlight the lack of a national commitment towards a harmonized approach to evaluating risk management in the country.

MO 359

Hot-spot identification & management for surface waters in context of the German National Action Plan for Sustainable Use of Pesticides: the methodological framework

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New EU legislation on the sustainable use of pesticides requires member states to produce 'national action plans' (NAP) on pesticides. The German NAP is designed to provide incentives for further risk reduction with the overall goal to reduce the risk potential by 25 % until the year 2020 in relation to the risk potentials calculated for a reference period from 1996 to 2005. A core component of the NAP is the management of hot-spots. They are defined as temporally and spatially defined fields of action with increased risk associated with plant protection products. Hot spots can occur due to specific environmental conditions (e.g. high water body density, high soil porosity), due to wide-spread and frequent use of a few plant protection products or due to high-frequency, crop and pests-related use of a specific plant protection product over a single area in connection with other conditions, such as frequent rainfall. Hot Spot detection & management follows a stepwise approach: The target of step one is a ranking of regions according to their aquatic risk potential using the indicator model SYNOPS-GIS and a subsequent in depth analysis at stream segment level for TOP regions. These in depth analysis apply probabilistic and spatial methods for more realistic exposure analysis linking critical source areas to receiving stream segments. The method requires recent regional pesticide use data, large scale soil data (1 : 50 000), weather data and explicit crop locations from the land parcel identification system (LISP) (EC No 796/2004). Results are aggregated to watershed units recognized also by the water framework directive (WFD). This common regional backdrop assures the link between activities of the WFD with the NAP. Monitoring data collected within WFD activities are used in the second step for validation purposes. In the third step existing regional risk mitigation measures (RMM) are reviewed and new management strategies are defined in cooperation with the official local extension services. These activities aims at providing regional "tool boxes"of RMM in order to strengthen the voluntary cooperation of farmers in water protection at local level. As the final step a model based control of success is performed to assess the regional effectiveness of the selected RMM before putting those into practice. This step is a partial run through step 1. First results of the implementation of this methodological framework are collected in North Rhine-Westphalia.

MO 360

Hot-Spot identification & management for surface waters in context of the German national action plan for sustainable use of pesticides: first results

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Within the National Action Plan the indicator model SYNOPS is applied for the analysis and detection of spatially defined regions with increased risk (hot spots) associated with plant protection products. These can occur due to specific environmental conditions (e.g. high water body density, high soil porosity steep slope), due to wide-spread and frequent use of a few plant protection products or due to high-frequency, crop and pests-related use of a specific plant protection product in connection with climate conditions, such as frequent rainfall.

In this study the provisional detection of such hot spots was conducted with SYNOPS-GIS in North Rhine-Westphalia on the basis of surveys on pesticide use and weather data from 2009 and extended GIS datasets such as the "Land Parcel Identification System (LPIS)". Three watersheds with high aquatic risk potential were identified. Here an in depth risk analysis was conducted at the level of stream segments.

The time varying concentration of the selected stream segments was analysed over the whole vegetation period considering the exposure pathways drift and run-off. A GIS evaluation on the entrance points and runoff-pathways from critical source areas was conducted. In combination with a run-off model the initial load of the stream segments by run-off was evaluated. Drift loads were assessed with a probabilistic approach. Stream segments which exceed a certain trigger concentration were identified and segments with specific concentration pattern in given time windows could be addressed. With this approach different scenarios of risk mitigation measures were analysed and compared, like the compliance to product specific drift mitigation requirements and the implementation of fixed non crop zones.

MO 361

Derivation of environmental quality standards for plant protection products: factors influencing the outcome

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This study aimed to clarify the technical aspects of environmental quality standards (EQS) derivation for active substances used in plant protection products. In case studies using six compounds relevant for Swiss surface waters, EQS were derived according to three currently used guidance documents: the Lepper guidance document (Lepper 2005), the Dutch guidance document for deriving EQS (van Vlaardingen and Verbruggen 2007) and the EU draft TGD for EQS (EU 2010). The active substances were: the herbicides Diuron, Mecoprop and Terbuthylazine; the fungicide Carbendazim; and the insecticides Diazinon and Imidacloprid. The EQS derivation consisted of three steps: (i) ecotoxicity data were compiled by database and literature search, (ii) the data were assessed separately for their reliability and relevance according to the guidance given in each of the three guidance documents, and (iii) EQS were derived separately according to each of the three guidance documents.

Although a review of the methods showed that the guidance documents are very similar, a few minor differences were detected, which led to small differences between the EQS values derived in the case studies. However, the differences never exceeded a factor of 3, when the same set of data was assessed by the same hazard assessor.

In contrast, the so-called "expert judgment"had a stronger influence on the EQS derivation. By comparing the derived values with publicly available EQS for the same substances, values differed by up to a factor of 20 due to different weighting of the data or due to the choice of a different assessment factor. Finally, the data validation was identified as a very critical step in the EQS derivation. The assessment of data reliability in all three guidance documents is performed according to the Klimisch scoring system, which allows extensive expert judgment. It is therefore recommended to revise or amend the Klimisch scoring system, since it does not seem to guarantee consistent validation between hazard assessors. It should also be mentioned, that the available data was insufficient for the derivation of an EQS based on the SSD approach for all active substances considered here. This is astonishing for such relatively well-studied PPP, and raises questions regarding the future relevance of the SSD approach in EQS derivation.

MO 362

Introducing the EcotoxTools Project: ecotoxicological tools for assessing agriculture associated environmental risks in Southern Europe big man-made freshwater reservoirs

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The prior objectives of the Water Framework Directive for 2015 converged on the need for implementation of risk management processes to achieve environmental quality objectives, i.e., the 'good ecological potential', also relatively to strongly modified water bodies. For this, it is necessary to characterize environmental risks and, afterwards, implement site-specific risk management processes. In this regard, ecotoxicological bioassays have been recognized as valuable tools for evaluating the ecological impacts of contaminants and the interactions between the biotic and abiotic environment. The aim of the ECOTOXTOOLS project is to implement, evaluate, and validate a battery of short-term bioassays that can be used as sensitive, rapid and simple (i.e., cost-effective) tools for the characterization of ecotoxicological risks due to pesticides and cyanobacteria toxins in Southern Europe big freshwater reservoirs adjacent to intensive agricultural areas, taking as case study one of the worst cases scenarios: the Alqueva reservoir (Guadiana river basin, Portugal). In fact, the high temperatures and low precipitation, with long dry periods, characteristics of the Southern Europe countries, especially those influenced by the Mediterranean climate, dictate slow water renewal and strong evaporation, enhancing the adverse effects caused by excessive inputs of nutrients and by intensive farming practices. The ECOTOXTOOLS project integrates a multidisciplinary team of researchers from Academia, that have been working on the evaluation of the main parameters responsible for the variability of water quality provided by reservoirs in southern Portugal, namely on nutrients, metals and pesticide residues, on incidence and toxicity of algal blooms, and on aquatic ecotoxicology, as well as a main stake-older and end-user: the company that manages the Alqueva reservoir (EDIA), which will be a permanent advisor.

MO 363

Development of regulatory testing procedures to study the metabolism of pesticides in farmed fish

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Information provided for the authorisation of a plant protection product (PPP) must be sufficient to permit an evaluation of the risks for man and (food producing) animals arising from residues of the active substance and relevant metabolites remaining in food and feed. Metabolism studies on food producing animals provide an estimate of PPP residues in edible products and must be performed when a PPP is used in crops from which parts or products are fed to livestock. Metabolism studies on ruminants and poultry are a current regulatory requirement. However, due to the increasing proportion of plant derived materials in aquaculture diets, metabolism studies on (freshwater) fish such as rainbow trout or carp will also be required when PPP of log-Kow >3 are used in crops fed to farmed fish. Metabolism studies are normally conducted with a suitable radiolabelled form of the active substance to identify the major components of the total residue and to show the efficiency of extraction procedures for these components. Following this initial assessment of the nature of the residues in food commodities, such as meat, milk and eggs, further livestock feeding studies are usually required. These use non-radiolabelled test material and a larger group of animals to provide the data necessary to establish maximum residue levels for food products of animal origin. Available guidance documents on metabolism and feeding studies for ruminants, poultry and pigs are available but these are not fully applicable to fish due to the differences in the animals' environment and husbandry conditions. More specific guidance documents for fish are therefore necessary.

A concept for fish metabolism is described and compared with the established guidance documents for other food producing animals. The results of a study on rainbow trout, designed to assess the practicality of the experimental design and to identify further technical challenges, are presented. The methodology used in the study is anticipated to meet the needs of new regulatory guidelines, which will require an understanding of metabolism and residues in fish farmed for human consumption.

RA07 - Monitoring data and post-registration studies for PPPs: generation, compilation and use in the environmental risk assessment and management

MO 367

Pesticide usage monitoring of Swiss farms

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Until recently trends of pesticide usage in Switzerland were derived from sales data. However, pesticide usage differs strongly between different crops, over the course of the year and between different regions. Such information can only be gained if data on the pesticide usage on farms are collected. Different approaches for pesticide usage data collection exist in Europe: farm visits by trained staff, postal or e-mail surveys, or compulsory returns of pesticide usage data.

In 2009 Switzerland implemented an approach to monitor pesticide usage based on the data collected by farmers in order get subsidies. The data are collected with Agro-Tech, a bookkeeping software. Agro-Tech was extended recently to include environmental indicators like nutrient balance, green house gas emissions, use of veterinary pharmaceuticals, use of pesticides, but also size and quality of ecological compensation areas. The data collected for pesticides give information on the amount of product and the dates of application for different cultures.

The data are valuable for a large number of questions, e.g., the evaluation of plant protection practice, the targeted chemical monitoring of surface waters and also the calculation of ecotoxicological risk indicators. As the data will be collected on a yearly basis, the approach should also allow the distinction of fluctuations due to pest pressure and long term trends due to changes in agricultural practice, a distinction which is much more difficult if surveys are conducted at longer time intervals. A critical issue is the number of participating farms. Ideas to estimate the required sample size for the different cultures are presented.

MO 368

Monitoring crop preferences of birds in arable farmland: importance of oilseed rape for Reed buntings (Emberiza schoeniclus)

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The decline of natural habitat in Europe has clearly increased the importance of farmland habitats for birds in recent decades. However, it is still not understood in detail if specific crop types, grown under modern and intensive agricultural practice, are favored as breeding and foraging habitat for different birds. In a long-term field monitoring study, we monitored bird populations at Top House Farm (Rawcliffe Bridge, Goole, East Yorkshire, UK) from 2003-2010. This 142-ha farm, located in intensively managed farmland, employing best practice agronomy, is managed using commercial agronomic techniques under a relatively high-input regime (detailed information on the yearly use of plant protection products is available) and is cropped as a wheat-based rotation. The crops occupying the study area were primarily: winter wheat, winter oilseed rape, spring beans and spring linseed. In each year, four to five bird surveys were carried out between April and July at approximately 3 to 4 week intervals. Here, we focus specifically on the Reed bunting (*Emberiza schoeniclus*), a bird that was on the UK 'red list' of conservation concern, although now moved to the 'amber list'. We show that oilseed rape is the crop in which the highest number of birds and the highest numbers of breeding territories were observed compared to winter wheat or the other crops. Notably, with the absence of oilseed rape at Top House Farm in 2008, the density of Reed buntings substantially declined in that year. Despite the relatively high-input and intensive agronomic regime used at Top House Farm, the densities of Reed buntings on Top House Farm were considerably higher than the UK average. This long-term study strongly supports recent short-term findings indicating that Reed buntings benefit from the inclusion of oilseed rape in the crop rotation and suggests that the inclusion of oilseed rape is an efficient management tool benefiting this bird species. In addition, this monitoring study provides an example of how simple survey methods can be used in monitoring programs to assess bird diversity in fields with standard use of plant protection products.

MO 369

Predicting input of pesticides to surface water via drains - comparing post registration monitoring data with FOCUSsw predictions

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The environmental risk assessment of pesticides in Europe is based on crop relevant exposure scenarios for the intended uses. A tiered FOCUSsw modelling approach (Step 1-4) is used to provide harmonised exposure data for the aquatic risk assessment. At FOCUSsw Step 3 exposure is modelled in different water bodies (pond, ditch and stream) in 10 scenarios representing geo-climate conditions across Europe. The model provides estimates of surface water concentration, based on the intended use, taking into account potential input routes (drift, drainage and run-off). Leaching and subsequent transport through the drainage system poses an important contamination pathway allowing rapid transport of pesticides to the surface water system. With FOCUSsw this input is modelled via the 1 dimensional root zone model MACRO allowing preferential transport to occur in the unsaturated zone. Although models (such as MACRO) are widely used within the registration process, their validation requires further work, not least because of the limited availability of field data.

The Danish Pesticide Leaching Assessment Programme (PLAP), an intensive monitoring programme which is used to evaluate the risk of leaching of pesticides under field conditions, aims to analyse whether pesticides applied in accordance with granted uses enter the aquatic environment in unacceptable concentrations. Within this programme a high resolution data set comprising 10 years of various pesticide concentrations in drainage from 3 field sites (1.7 - 2.3 ha, sandy loam) are available for model validation. Moreover, a "site specific"setup of the MACRO model already calibrated and validated with respect to water and non reactive solute transport is available from these field sites.

For a sub-set of pesticides representing a range of use patterns and inherent properties (e.g. sorption potential, persistency and toxicity) output from drain to surface water has been assessed from both measured and modelled data. The latter comprising output from the MACRO model parameterised either via the FOCUSsw setup (relevant scenarios) or the "site specific setup"applied at the PLAP sites. Output from monitoring and the two modelling approached are compared and uncertainties of the different approached are discussed. It is considered how monitoring data from post registration systems can be "optimised"in order to improve their usability in risk assessment (e.g. validation of modelling, output format).

MO 370

Constraints in monitoring environmental and health risk management studies - Post registration in INDIA

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Pesticides and certain other chemicals classified as dual-use chemicals are primarily regulated by The Insecticides Act., 1968. Registration process of such chemicals are granted after adhering to a stringent and exhaustive set of guidelines framed for various categories of registration. Incidentally, once a product has been granted registration, post-registration monitoring is practically missing. Review of such chemicals take place depending upon its use pattern and subject to any international concern. However, a well planned scientific and regulated post-registration is not mandatory, neither at the national or state level. This is primarily due to lack of enforcement of legislations and proper timely coordination. Communication of decisions and its implications are generally waived by various departments on the pretext of their own dedicated authority. Risk assessment in a broad sense is missing due to lack of responsibility and an commitment towards the society and nation as a whole. Interesting facts and case studies will be revealed in this paper and make an attempt to give an overview of the issues involved in country where legislations are in the plenty but enforcement and implementation are lacking.

MO 371

ASTERisk, a tool to assess the pesticide risk

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The planning of controls on pesticide retails and farms carried out by Regional Local Health Units in Italy is not homogeneous and is not tailored on local specificities. This entails a less than efficient use of time, human resources and budget, but above all, a less effective risk assessment and prevention for human health and the environment.

The International Centre for Pesticides and Health Risk Prevention (ICPS) supports the Health Directorate of the Region Lombardy, to achieve the sustainable use of pesticide by developing preventive and safety programmes and by collecting and managing pesticide sale datasets at sub-Regional scale.

ICPS developed a tool, a suite of different databases including information about pesticide loads as sold yearly in Lombardy Region, from certified European and National sources on toxicity, fate, ecotoxicity characteristics of pesticides, and data on geo-referenced crops and underground waters. This tool, called ASTERisk (the acronym stands for a Tool to ASsess the pEsticide Risk) is able to address prevention activities planned by the public administration by providing mainly:

- risk assessment of the general population and farmer exposure to the most sold pesticides in Lombardy Region;
- identification of the areas at higher environmental risk after agricultural use of pesticides.

In addition, ASTERisk may provide, through the use of some ad hoc qualitative indicators, some hints to assess the selection pesticide stores and in agricultural farms to be inspected and monitored.

From a normative point of view, ASTERisk meets the requirement of the Regional law N.8 "Disposizioni in materia di attività sanitarie e socio- sanitarie"(April 2007) requests, which aim primarily at ensuring a higher protection of the citizen's health right through an integrated system of prevention and control. In addition, ASTERisk is relevant also for the "Piano di controllo ufficiale su commercio ed impiego dei prodotti fitosanitari per il quinquennio 2009-2013", a recent legislative (April 2009) issued by the Conferenza permanente per i rapporti tra lo Stato, le Regioni e le Province autonome di Trento e Bolzano. In this case, the opportunity to have all data and information on pesticide characteristics, pesticide retails and farms in a unique tool allows the public administration to manage and easily comply with the legislative and bureaucratic requirements, about risk assessment of pesticide stores and users.

MO 372

Assessment of lambda-cyhalothrin in vegetables: case study of Nairobi City, Kenya

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Vegetables are essential in the diet for they provide, fibre, trace minerals, vitamins, folacin, carbohydrates and protein. However, pests, diseases, and weeds destroy vegetables. As such many pesticides have been developed to control pests. Most of them are poisonous to creatures besides those they are intended to kill and can be harmful to the human health. Although vegetables are widely consumed by almost everybody in Kenya, there is little work reported on the analysis of pesticide residues in them. In this study lambda-cyhalothrin was analysed in selected vegetables which included; kales, cabbages and tomatoes. Lambda-cyhalothrin is an insecticide which is commonly used on vegetables and other crops. Similar vegetables from different markets were then homogenized to give analytical samples. Lambda-cyhalothrin residues were extracted from the samples using organic solvents. The lambda-cyhalothrin residues were then determined by high performance liquid chromatography (HPLC). The data derived was analysed statistically using t-test and regression analysis. The lambda-cyhalothrin mean residue levels ranged from 0.0300±0.0100 to 0.3400±0.1100 mg/kg during the dry season and from non-detectable level to 0.0040±0.0090 mg/kg during the wet season. The study showed that, during the dry season lambda-cyhalothrin residue levels were significantly higher than during the wet season in the vegetable samples analysed. During the dry season the lambda-cyhalothrin mean residue levels in the vegetable samples were higher than the Acceptable Daily Intake (ADI) of 0.02 mg/kg, although most of them were lower than the Maximum Residue Levels (MRLs) of 0.2 mg/kg except in tomatoes. During the wet season the lambda-cyhalothrin mean residue levels were all lower than both the ADI and MRLs.

RA12 - Risk communication for environmental protection: Scientific and regulatory needs

MO 375

Tools of communication about chemicals in the contest of the REACH implementation

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For the implementation of the REACH Regulation (Registration, Evaluation and Authorisation of Chemicals), the Italian Ministry for the environment, land and sea has the task to give information about the risks of chemicals, involving also consumer and environmental organizations. Therefore, in July 2010, the publication of the e-bulletin "Sostanze chimiche - Ambiente e

Salute"was undertaken. It deals with activities and news related to REACH, other Regulations concerning chemicals and risk mitigation measures. The aim is to provide through a simple language adequate information for the public on risks and safe use of chemicals, dealing with specific items in each e-bulletin.
In addition to the main topic of the e-bulletin it has been prepared:
- an 'Events' section about meetings, seminars, workshops on technical legal issues relating to chemicals;
- a 'News from ECHA' section containing details of the main procedures of public consultation launched by ECHA (European Chemicals Agency), about proposals for restrictions and/or inclusion in the "Candidate List of Substances of Very High Concern";
- a "Link"section.
To manage the registration and sending the newsletter a dedicated mailbox is created. The address is sostanzechimiche@minambiente.it.
An e-mail with the first issue of the bulletin and the registration form to receive future issues was sent to:
- Environmental organizations;
- Consumer organizations;
- Regional Environmental Protection Agencies;
- Regions (Environment and Health Departments);
- National Technical Committee for the REACH implementation;
- Regional authorities of vigilance.
The Ministry should also promote activities to ensure public access to information on chemicals through the creation of databases that allow easy access to information on hazardous properties of substances.
For this purpose the database DESC (Database Ecotossicologico sulle Sostanze Chimiche) was created. It contains the main ecotoxicological and environmental information of more than 650 chemicals manufactured and/or imported into the European market. The database is structured to be implemented and to contain an unlimited number of substances. Moreover it's in Italian language, to facilitate consultation by not expert public.

MO 376
Magnifying Perceived Risk: how misconceptions about the importance of ethinyl estradiol (EE2) to the overall endocrine disruptor issue is driving misguided risk management decisions
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'Selective' reporting and exaggeration have resulted in undue concern over environmental presence of EE2. Inaccurate or snapshot field measurements used as 'environmentally-relevant' test concentrations in laboratory studies, biomarker detection (i.e., vitellogenin in male fish) incorrectly reported as effect, and field experiments using confined exposure (i.e., lake) being inappropriately extrapolated to surface water (river) risk assessments have all contributed to the misconception that EE2 exposure is of great consequence to wildlife and humans.
With the media always placing contraceptives at the top of the list when discussing the endocrine issue, the regulatory community is being swayed to take unwarranted risk management actions with potentially costly consequence. A detailed review of monitoring and effect data show that fish exposures are largely at or below sensitive predicted no effect concentrations and that human exposures are miniscule when compared to other estrogens in the diet. All of which support the contention that undue attention is being paid to EE2 and that overall estrogenic exposure is the appropriate determinant when considering this aspect of endocrine disruption.

MO 377
The Maximum-Allowable-Daily-Loss (MADL) Report: a site-specific manufacturing risk communication tool for industry
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Through the application of a database structure designed to organize information in a highly accessible, parsed format and an automated report generating protocol capable of gathering, filtering, and manipulating data we deliver a detailed, site- and substance-specific report describing inherent hazard , PBT profile, estrogenic potential, and risk-based MADLs for manufacturing facility consideration. The MADL incorporates site characteristics to transform the traditional PEC/PNEC assessment into a mass loss below which no adverse impacts are expected. Separate MADLs are calculated for WWTP function, focusing on bacterial inhibition and for local receiving waters, focusing on indigenous or representative organisms. In addition, a 'stoplight' risk analysis matrix is supplied to point out the parameter(s) driving the risk so that informed decisions can be made for future testing strategies.
The MADL report can be used to support site selection, WWT enhancements, or waste stream diversion. While useful for understanding individual compounds, future enhancements will consider coexisting substances to report combined risk.

MO 378
Challenges of the EU REACH regulation: a perspective from British American Tobacco (BAT)
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This poster presentation describes some of the challenges faced by BAT, in interpreting the new EU REACH regulation. Ingredients are added to the tobacco depending upon the product type which can be single substances on their own or in preparations. Under REACH, BAT is an importer and a downstream user (DSU), and not a manufacturer of such substances.
A cigarette is a "preparation in a container"with the container considered to be an 'article' under the scope of REACH. 'Articles' include non tobacco materials such as cigarette papers and filters, manufactured in (or imported into) the EU for use in BAT products. Substances in articles or preparations may require registration depending on the criteria defined in the regulation for example if they are present in quantities exceeding one tonne per producer or importer per year. REACH also identifies specific exemptions as defined in Article 2, Annex IV and Annex V.
A REACH evaluation screening process has been developed within Group Research & Development (GR&D) as a simple and effective method for identifying REACH compliance requirements for tobacco ingredients. Key elements of the REACH screen include 1) Annex IV exemption 2) Annex V exemption 3) Tonnage and 4) Registration and Pre-registration status. This process has been documented as a flow chart with decision codes and outcomes. Key learnings and experiences of implementing the REACH screen are presented in this poster.

MO 379
Environmental risk assessment outputs for socioeconomic analysis under REACH
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The outputs from environmental risk assessments (ERAs) are not the same as the "impact assessment"information required for socioeconomic analysis (SEA), making it difficult to develop Restrictions and Authorisations under REACH. ERA outputs are usually in the form of Risk Characterisation Ratios (RCRs), in which a PEC is divided by a PNEC. If the RCR is >1 then a potential risk is assumed. However, SEA requires the translation of an RCR or its underpinning data into a "value". Translating RCRs into an assessment of impact requires further, ideally quantitative, information on how severe and extensive any effects are likely to be in the real world, our level of certainty about these effects, and how different risk management measures might alter this risk. ECHA has identified the following additional problems in translating ERA outputs into SEA impacts: 1) a chemical's persistence is a key reason for concern about its presence in the environment, but this persistence makes it difficult to quantify any changes in impacts over time; 2) chemical persistence gives rise to trans-boundary issues, complicating both the quantification of effects and any attempts to value such effects ("whose values"should be assumed?); 3) an absence of environmental monitoring data (together with transport, fate and behaviour data in some cases) to establish the geographic extent of environmental concentrations above the no effects level; 4) difficulties in linking toxicity data for sensitive species to other species or ecosystem effects; 5) combinations of sources contributing to environmental concentrations, leading to difficulties in determining how restrictions on some uses would affect concentrations in the environment; and 6) significant variations across the EU in the processes used, the continuous or sporadic nature of use and emissions, and in existing levels of treatment. This poster presents examples of potential ways to translate ERAs into environmental impacts which can be practically used to inform SEA and eventual decision making under REACH. These approaches include: 1) exposure-based "proxies"of effect, such as changes in volumes of exposed media, as generated by probabilistic EUSES modelling; 2) use of species sensitivity distributions and the dose-responses of sensitive species; 3) read-across from similar substances for which there is more information; 4) Life-cycle Impact Assessment; and v) the ecosystem services concept.

MO 380
Communication - a tool to reduce the exposure of pharmaceuticals in the environment!
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Human pharmaceuticals mainly enter the environment via domestic wastewater. Although concentrations are usually very low, first impacts on aquatic organisms have been detected. One important step to reduce the entry of pharmaceutical residues into the environment is to improve the awareness of specific target groups within the health care system: the public, the physicians and the pharmacists respectively. For this reason the Federal Environment Agency, Germany (UBA) and the Institute for Social-Ecological Research (ISOE) prepared recommendations for a communication strategy. The current knowledge of the specific target groups and factors, affecting the handling of pharmaceuticals has been identified, revealing that the problem of pharmaceutical residues in the environment is widely unknown. Therefore the first objective was to create profound knowledge. This sensitisation is the basis for implementing further strategies leading to raise awareness and initiate behavioural changes. Modifications will affect daily routines concerning medication such as prescription, compliance, non-medicamentous prevention as well as patient-doctor-interaction. Moreover the results of the project clearly reveal that the knowledge, the handling and the reflection of the subject pharmaceuticals in the environment varies not only between, but also within the individual groups. This means that a promising communication strategy has to be tailored to the individual needs in the specific target groups. This strategy also contains the communication of a proper medication disposal and is intended to promote the return of all unused medication by implementing a feasible take-back scheme. The strategy is also to emphasise that under any circumstances pharmaceuticals should not be flushed down the toilette or the sink and by this get into the water circle. Based on an analysis of typical shortcomings in available publications, the project compiled recommendations for conceptualizing information material.

MO 381
Chemical risk communication from the ecodesign perspective: legislative preconditions and needs for more information than required by law
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Ecodesign or design for the environment is a systematic design approach to reduce environmental impacts of products and to achieve higher protection standards than set by law.
The chemicals legislation of the European Union is seen as one of the most advanced in the world regarding protection of human health and environment. REACH - Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals enhances risk communication on the supply chain and makes a ground for generation of chemicals safety information, e.g., feeds the information needed for communicating chemicals' risks down the supply stream including classification, labeling and safety data sheets as required by the regulation on CLP (Classification - Labelling - Packaging) according to GHS (Globally Harmonized System of Chemicals). The main responsibility about chemicals' safety is placed on the industry; however, the most dangerous chemicals are restricted and banned in a transparent and systematic way. Are the risk assessment measures upstream sufficient for the ecodesign? Is it needed to judge upon chemical risks of the non-chemical products during design phase to achieve higher standards for the environmental and human health protection?
This study indicates the benefits from the REACH system for the ecodesign, as well as highlights the needs for an enhanced risk communication on the supply chain compared to the current legislative requirements, especially regarding the materials: intended content of chemicals and residues /impurities. For example, in order to demonstrate compliance with environmental labelling criteria, very detailed information about the chemicals contained in materials is required.

MO 382
A scheme for the analysis of the results and dissemination and exploitation activities of in silico models
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ORCHESTRA (Organising dissemination on results of projects on Chemical evaluation, spreading techniques for Risk Assessment) is a project funded by the European Commission,

aimed to disseminate and exploit the European research activities dealing with computer models for the environment. Nine EC funded projects have been chosen as case studies to develop and apply a dissemination/exploitation strategy for in silico models promotion. Each of these projects produced (or is producing) results and developed a dissemination and exploitation plan. In order to improve the dissemination and exploitation strategies, the selected projects have been analysed, from the point of view of the goodness and importance of the results produced and of how they have disseminate these results. Then, the outcome of this analysis has been used to try to evaluate and explain the impact obtained by the considered research activities.
Thus, the target of this is not to identify a winner project, regarding results or dissemination strategy. On the contrary, the target is to identify good results and, on the basis of these examples, learn from success stories, but also on not optimal experience done within these nine projects. The lesson we will get will be obtained on the basis of the comparison of what has been achieved. The overall, improved, strategy for dissemination is the final goal. On the basis of this analysis, the ways which are more suitable to achieve a broad dissemination and use of in silico models are identified. This involves suitable communication ways and strategies, but also evaluation of the user's need. We will describe the in silico tools which appeared more promising for this, and the reasons which produced the higher possibility of dissemination.

MO 383
Stakeholder perceptions of opportunities and challenges for the use of in silico methods in Europe
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Europe's 2007 REACH regulation (Registration, Evaluation, Authorisation and Restriction of Chemical substances) has created an urgent demand for testing large numbers of chemicals for toxicity. *In silico* methods may be of significant help in meeting that demand. *In silico* methods rely on computer modelling. Results from existing *in vivo* or *in vitro* tests are used to model the ways in which a chemical behaves in the body and in the environment. The toxicity of a particular chemical used in a particular setting can be predicted and assessed without further tests on animals or living cells.
Development of *in silico* models may generate a more sophisticated and more reliable understanding in the future of how a chemical may affect humans and the environment. *In silico* methods inevitably have limitations as well as potential. The limitations must be clarified in order to ensure that these methods are applied wisely and are adequately supervised and regulated.
The EU has funded the ORCHESTRA project (2009-12) to promote wider understanding, awareness and appropriate use of *in silico* methods. ORCHESTRA will support good practice and regulation by bringing together EU research on *in silico* methods and practical feedback from their use. The project web portal will become a central resource for sharing of knowledge and experience between professionals who are developing and using *in silico* models. For industry users and regulators, it will also provide downloadable software for *in silico* models reviewed by the project.
Experience of applying *in silico* methods to respond to regulatory requirements is built up on a case-by-case basis. Both industry and regulators are learning by doing. Dialogue is needed between researchers, stakeholders and regulators to identify zones of uncertainty and success. ORCHESTRA contributes to this clarification. In September 2010, ORCHESTRA invited 234 regulators and industry stakeholders to input using a specialist questionnaire (view and fill out online at www.in-silico-methods.eu).
Responses provide insight into current:
1. Awareness of and interest for *in silico* methods
2. Perceived benefits and attractions of *in silico* methods
3. Barriers to the use of *in silico* methods
4. Professional and policy needs
5. Stakeholders' sources of information on methods in toxicology.

The poster reports and analyzes responses received until April 2011, and highlights responsive actions taken in the context of ORCHESTRA.

MO 384
IFRA environmental standards: risk and hazard assessment update for 2011
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The International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. The development of this program was reported previously at SETAC (Seville, 2010). To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the developing IFRA Environmental Standards, reported here are the results of these additional tests (for both risk and hazard assessments). These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

MO 385
Complexity of regulatory enforcement under various legislations and effective communication of possible environmental impact and hindrances in addressing public awareness
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INDIA, is considered as an advanced developing country, having great potential for maintaining a meaningful global presence. Legislations have been enacted in the plenty to practically satisfy all stakeholders in every situation. Unfortunately as and when a situation demands a new legislation is enacted or framed, with a little understanding of the already existing regulations in enforcement. A quos is created and a lack of clarity of the exact regulations required create a confusion in manners which are often devastating. The paper attempts to reflect the complexity of understanding all legislations involved and regulated through various ministries at a political level and relevance of scientific opinion. The complex network of such legislations being enforced in a bureaucratic and scientific conflict, leaving the stakeholders vulnerable to an outcome which is usually unpredictable and a matter of grave concern.

MO 386
Risk mitigation measures for biocidal products - how to develop a harmonised approach for product authorisation?
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In assessing the impact of biocides on the environment, specific measures to reduce a risk to the environmental compartments may be required. The evaluation of biocidal active substances up to now showed that the Rapporteur Member States have followed different approaches of risk mitigation measures (RMM). For the environmental risk assessment a harmonisation of possible RMM is missing.
This research project contributes to establish RMM required for a harmonised assessment of biocidal products in the EU and compiles appropriate solutions to prevent/reduce the identified risks for the environment. This is elaborated exemplarily on biocidal products used as wood preservatives and for the control of insecticides. Therefore RMM proposed by producers, industrial/professional users, and authorities are collected and critically evaluated in terms of practicability and efficiency. This project focuses on the mitigation of environmental risks.
The RMM proposed in Competent Authority Reports and considered in the Inclusion Directives are based on the intended uses and a representative biocidal product, which might not cover all applications. Product integrated RMM implemented during product development, design and distribution are immediately practicable while those referring to the user of biocidal products mainly depend on the communication of risks, risk awareness, education, specific training and on the information availability. However, apart from RMM being addressed in regulatory decisions, a broader strategy of sustainable use of biocides is required. Further, little information is available on the efficiency of RMM since e.g. only few monitoring data for active substances in the receiving environmental compartments exist. More specific RMM may be included in the authorisations of biocidal products when full information on the formulation and the conditions of use are available.

RA14 - Waste fluxes around the world and the associated risks

MO 389
Assessing the potential for the upstream control of contaminants present in materials spread to land
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MO 390
Socioeconomic aspects on emissions of chemicals from the flow of global e-waste
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The purpose of this study is to start outlining aspects of the socio-economic negative impacts of the global WEEE flows in general and hazardous substances contained in this WEEE flow in particular. This may ultimately form a framework for the socio-economic assessment of the costs and benefits of chemicals in products. The present study will focus on literature data regarding the costs for damages, on human health and the environment, which can be assigned to hazardous chemicals present in WEEE flows.

MO 391
Risk-based management of chemicals and products in a circular economy at a global scale
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Products undergo a recycling process and make their ways into a recovered material with unpredictable and not foreseen health and safety problems. The primary aim of RISKCYCLE is to identify future R&D needs required to establish a risk-based assessment methodology for chemicals and products that will help reduce animal testing while ensuring the development of new chemicals and product management pattern leading to minimized risks for health and the environment. The project is focussing on consequences due to the behaviour of chemicals and their release during recycling of the six fractions: paper, electronics, leather, lubricants, plastics, textiles. The critical points throughout the products life cycle for the release of chemical substances and the hazardousness of the material set free will be evaluated. Beyond this it is also important to know if the effects caused by the chemicals have a global or only local meaning and if the release of specific substances in the circular economy is an actual risk or a perceived risk.
The current results of the project will be presented within this poster.

MO 392
A new tool to assess uncertainty in risk assessment
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The characterization and quantification of uncertainty and variability in health risk assessment are important to prevent erroneous inferences in multimedia modelling and exposure assessment, which may lead to major environmental policy implications. Health risk assessment calculations require a better understanding of exposure variables and uncertainty associated with them. Although there are many sources of uncertainty in system models, two basic kinds of parametric uncertainty are fundamentally different from each other: natural/stochastic and epistemic uncertainties. However, conventional methods such as standard Monte Carlo Sampling (MCS), which assumes vagueness as random property, may not be suitable for this type of uncertainty analysis. An improved systematic uncertainty and variability analysis can provide insight into the level of confidence in model estimates, and it can aid in assessing how various possible model estimates should be weighed. The main goal of the present study was to introduce Fuzzy Latin Hypercube Sampling (FLHS), a hybrid approach for incorporating epistemic and stochastic uncertainties separately. An important property of this technique is its ability to merge inexact generated data of the LHS approach to increase the quality of information. The FLHS technique ensures that the entire range of each variable is sampled with proper incorporation of uncertainty and variability. A fuzzified statistical summary of the model results produces a detailed sensitivity analysis, which relates the effects of variability and uncertainty of input variables to model predic-

tions. The feasibility of the method has been tested with a case study, analyzing total variance in the calculation of incremental lifetime risks due to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) for the residents living in the surroundings of a municipal solid waste incinerator (MSWI) in Spain. The results showed that FLHS clearly separates controllable and uncontrollable uncertainty associated with models, which helps the models /and decision makers to identify the priority area in order to improve the results.

MO 393

Local laboratory reactant exchange network

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The experimental work performed in laboratories use many chemicals with different characteristcs. Some of them presente several dangers that can affect people that work in the laboratory and also the environment if they are not carefully used/managed. The wastes produced can also be dangerous and demand careful attention.
In many laboratories there are reactants that are rarely used and that become out of date and create a problem of waste management since they must be correctly disposed. In order to prevent these problems we developed a computational application (website) for promoting the exchange of reactants between laboratories.
This Local Laboratory Reactant Exchange Network is useful to perform a sustainable management of a laboratory since it allows the adoption of suitable strategies to deal with reactants and wastes assuring the safety of the people that work in the laboratory but also some more general environment and social advantages. In spite of the quantities are relatively small the risk associated with some of the reactants/wastes raises the problem of its correct management. A good management will contribute to the ecosystems protection. In this way it will be possible to maintain or increase the quality of life since it decreases with the degradation of the ecosystems.
The development and implementation of this computational application is important to achieve a sustainable management of laboratories since it improves the laboratory operational, economical, social and safety performance. It will allow a better use of resources and will contribute to the prevention of chemical waste generation.

MO 394

Panorama of the lubricating oil sector and recycling in Brazil

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The Brazilian market for basic Lubricants oils is the world's sixth largest. The present paper analyzes this sector and succinctly presents its current panorama, identifying the market structure, productive chain, actors and balance of production versus recycling. The feedstock oils in the sector come from three streams: the basic oil produced at three of the refineries operated by Petrobras, the state-controlled oil company; the basic oil imported by the 41 companies authorized for this by the National Petroleum Agency (ANP); and the oil re-refined from used oil collected in the Brazilian market, at 19 refineries. At present, about 15% of the lubricants Oils sold in Brazil is from re-refining ANP (2010). Over the period from 1991 to 2006, demand for lubricants grew by an average of 4.5% a year. In 2009, Brazil consumed some 1.2 billion liters of lubricant oil. Used lubricants are dangerous to the environment: they contain engine metal particles (lead, chrome, barium and cadmium) and various contaminants that harm the soil, water and air. Of these, water pollution is the most severe problem. According to the U.S. Environmental Protection Agency, a liter of lubricating oil has the potential to contaminate up to a million liters of potable water. Although good progress has been made in this respect in Brazil, there is still room for further improvement, particularly regarding quantitative and qualitative monitoring of the lubricant market. There is a need to provide consolidated official data representing the entire market. In this sense, it is necessary to invest in research to develop and consolidate a method for life cycle assessment of lubricants, to allow obtaining consistent and reproducible results.

MO 395

Methane-bearing rock waste

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Waste rock in coal mining is the overburden material that is excavated and disposed of in order to access valuable coal seam. In particular, such rock waste as natural stone (sandstone and limestone) is used as bulding material and, therefore, it can be exported to other countries. The sanstone and limestone are applied as aggregates, ornamental and dimension stones. Some sandstones and limestones are methane-bearing. After methane-bearing waste rock has been excavated from its original location, it is important to evaluate the methane emission from the waste to the atmosphere. Methane is greenhouse gas that remains in the atmosphere and is over 20 times more effective in trapping heat in the atmosphere than carbon dioxide. Methane poisoning, fire and explosion can occur when methane concentration on the ground surface reaches a potentially dangerous value. In this study, duration of methane emission from excavated gas-bearing waste (waste rock pile), gas volumetric flow rate from rock waste, iniatial gas content of rock before the rock was excavated, height and volume of waste rock pile, and number and dimensions of large and unconsolidated rock fragments in waste rock pile were observed. Observations show that the duration of gas emission from waste rock pile can achieve 60-84 months, and maximum gas volumetric flow rate is between 0.003 and 0.011 cubic meter /min from 1 cubic meter of waste rock pile. It is established that the duration of methane emission from waste rock pile increases exponentialy with increasing initial gas content of waste and volumes of waste rock pile and rock fragments in the pile. It is revealed that increase in number of unconsolidated rock fragments in waste rock pile leads to decrease in duration of methane emission from the waste pile. It is found that gas volumetric flow rate from 1 cubic meter of rock waste and duration of gas emission can be presented as double exponential functions of iniatial gas content of rock, ratio between volume and height of waste rock pile, and number of rock fragments in waste rock pile. These double exponential functions suggest that the time-zero is time when rock waste was extracted from coal mine to the ground surface.

CS03 - New approaches in the simulation of realistic multistress and multispecies scenarios

TU 002

Emergence in stream mesocosms before and after repeated short-term insecticide pulses

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In contrast to established biotests for assessing the ecotoxicological effects of chemicals, contamination of lotic surface waters by spray drift and run-off events generally occurs in multiple short pulses. Besides other endpoints like mortality and organismic drift, effects on the timing and the extent of insect emergence may be relevant endpoints. In 2009, a comprehensive experiment with the insecticide imidacloprid was carried out in the indoor stream mesocosms of the Federal Environment Agency in order to exemplary investigate if the endpoint insect emergence besides other parameters can be used for risk assessment. 8 artificial streams were filled with sand and groundwater and equally stocked with macrozoobenthos from a reference creek. Organic straw was introduced as substrate and served as both hiding place and food for the invertebrates. After a short period of establishment in spring, 4 of the 8 streams were contaminated three times with 12 µg/L Imidacloprid in the evening at weekly intervals. Both the contaminated streams and the 4 control streams were flushed with uncontaminated water 12 h later. This scenario was repeated 4 weeks later in summer. Emergence was determined in the weeks before and after the pulses by means of 4 emergence traps which covered about 10% of the entire water surface in each stream. Repeated macrozoobenthos sampling allowed for the determination of the emergence relative to the corresponding population size. Direct and indirect (drift mediated) effects on insect emergence were detected.

TU 003

Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem

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In the present work a small-scale terrestrial ecosystem ("STEM") containing a Mediterranean agricultural soil was used to survey the effects of the combination of the herbicide glyphosate and the insecticide dimethoate, mimicking real scenarios of exposure. Earthworms (Eisenia andrei), isopods (Porcellionides pruinosus), turnip seeds (Brassica rapa) and bait-lamina strips were placed in the STEM. The results showed that the application of the recommended field dose (ecological relevant concentration) of both pesticides did not cause any effect in their growing ability of both earthworms and plants. The application of the herbicide (even at 5 and 10 times the field dose) increased feeding activity (bait-lamina test) in soil, although the application of dimethoate led to a decrease in feeding activity in all concentrations tested. The binary mixtures performed showed that according to the independent action model, an antagonistic deviation (smaller effect than expected from the single exposures) was observed in both the shoot length and fresh weight of B. rapa. There was a decrease in the earthworm's weight in all concentrations tested, although no statistical differences were observed in any of the treatments made. Regarding the depth distribution of E. andrei in the field dose and five times the field dose of the two pesticides, antagonism was observed whereas in the highest concentrations tested (10 times the field dose of both pesticides) synergism took place (worms escaped more than predicted from the single exposures made). In all the binary mixtures performed a decrease in the feeding activity (bait-lamina consumption) of the soil fauna was observed. Biomarkers (Catalase, AChE, GST) assessed in the isopod led to a decrease in the enzymatic activity in the animals exposed to the highest concentrations of dimethoate and to the binary mixtures performed.

TU 004

Applying a novel experimental system for the independent control of chemical and drought stress

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In their natural habitat, soil organisms are not only exposed to different contaminants but also to a wide range of physical, chemical and biological stressors. The physical and chemical conditions in soil can greatly affect the toxicity of the contaminants present, and new experimental systems are therefore required in order to be able to test effects caused by multiple stressors. The aims of this study were (i) to develop a novel experimental system for the independent control of both chemical and drought stress and (ii) to apply it in two-factor experiments with both stressors. The three polycyclic aromatic hydrocarbons (PAHs) naphthalene, phenanthrene and pyrene were selected as test contaminants. (i) The experimental system; Exposure to the contaminants was controlled by passive dosing in 5-mL glass vials with PAH loaded silicone in the bottom. Three open passive dosing vials were placed inside a closed 120-mL glass jar with aqueous saline solution in the bottom which controlled the humidity within the entire jar. In this way, the test organisms are exposed to controlled chemical stress from below, while the drought stress is controlled from above. (ii) Application in a two-factor experiment; The terrestrial springtail Folsomia candida was selected as a test organism. The PAH exposure parameter was chemical activity (unitless, [0-1]), and the humidity was expressed in terms of water activity (unitless, [0-1]). The springtails were exposed to several combinations of chemical and drought stress in order to determine their combined effects on springtail survival (7 days).

TU 005

Results of a 21-day fathead minnow fecundity study using a mixture of an EDC , ehtynylestradiol and ammonia.

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Aquatic organisms are exposed to a multitude of contaminants and stressors and to fully understand impacts of multiple stressors on fish populations, we must understand the mechanism of action of each different toxicant on an individual and how the combined effects manifest at the level of the individual. The synthetic estrogen, 17α-ethinylestradiol, is a common ingredient in oral contraceptives and is extremely difficult to completely remove from wastewater. This toxicant has been known to cause adverse reproductive effects in fish, including reduced fecundity and fertility, intersex and sex change by mimicking naturally produced estrogen at low concentrations. Ammonia is another common pollutant in aquatic systems and has also been shown to cause adverse reproductive effects and mortality in fish populations through attacks on the central nervous system. This study used a 21 day flow through diluter system to test the individual effects of these two contaminants, as well as their mixtures, on fathead minnow reproduction. We also wanted to determine if the mixture of these two contaminants at their respective environmentally relevant concentrations would result in adverse reproductive effects. Significant findings from this study indicate that the current U.S. EPA water quality criterion for unionized ammonia in waters where non-salmonids, such as fathead minnows, are present (8.4mg/L) is above the lowest observable effect concentration for fathead minnow reproduction. The lowest unionized ammonia concentration (5mg/L) tested during this study resulted in decreased fecundity. The mixture

of both 17α-ethinylestradiol and ammonia at their respective environmentally relevant concentration resulted in increased mortality, however, did not to show adverse effects on reproduction. This study demonstrated the need for toxicity testing with multiple stressor scenarios and the importance of re-evaluating current U.S. EPA water quality criteria.

TU 006

Effects of ultraviolet radiation on terretrial isopod Porcellionides pruinosus (Crustacea, Isopoda) in three simulated environments

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Nowadays climate changes are introducing extra stress to the environment. UV radiation reaching ecosystems is increasing due to ozone depletion and deleterious effects are expected to happen to living organisms. In this work, the effects of high doses of ultraviolet (UV) radiation to the terrestrial isopod Porcellionides pruinosus were investigated in three simulated environments. Organisms were exposed to two hours of visible light followed by eight hours of UV radiation and another four hours of visible light. After that, they were kept for recovery in a climatic chamber at natural light and temperature conditions. To evaluate isopod stress levels, the activity of the biomarkers acetylcholinesterase (AChE), glutathione S-transferases (GST), catalase (CAT), glutathione peroxidase (GPx), lactate dehydrogenase (LDH), lipid peroxidation (LPO), oxidized glutathione and reduced glutathione (GSH/GSSG) ratio was analyzed at the end of exposure, and after 24h, 96h and 7 days. Despite the behavioral responses observed during the exposure, no significant mortality was registered. Significant differences in biomarkers results were obtained, mainly on those related with oxidative stress (e.g. LPO increased during the recovery time). The results obtained highlight the value of biomarkers to assess the levels of stress on sub-lethal exposures to natural stressors, including UV radiation.

TU 007

Potential of the European bullhead (Cottus sp.) to assess the effects of pollution using a set of biochemical biomarkers

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Multi-biomarker approach is extensively used to characterize the effects of ecosystem contamination on fish health. In order to measure multi-biomarker responses in freshwater ecosystems, numerous wild fish species have been used. However, the geographical distribution of these extensively characterized fish species did not allow a large sampling for ecotoxicological application and justifies the research of novel sentinel species for biomonitoring. The aim of this study is to characterize the potential of the European bullhead (Cottus sp.) as sentinel fish species to assess the effects of pollution using a set of biochemical biomarkers. This fish is widespread throughout Europe, and it's sedentary fish that allows assessing local disturbances conversely to migratory or mobile fish species. The selected biomarkers included biotransformation enzyme (i.e 7-ethoxyresorufin-O-deethylase, EROD), oxidative stress (i.e glutathione peroxidase, GPx and lipoperoxidation, TBARS) and neurotoxic parameters (i.e acetylcholinesterase, AChE). To evaluate the potential of European bullhead for biomonitoring, laboratory and field experiments were conducted. Laboratory investigations were performed to characterize chemical-induced variations of biomarkers using reference chemical compounds. For this purpose, bullheads were exposed to β-naphtoflavone (BNF), fenitrothion and copper in order to evaluate respectively EROD, AChE and GPx activities as well as TBARS data. BNF generated a rapid induction of liver EROD activity in bullhead. Fenitrothion had no effect on the AChE activities. In field, wild bullheads were sampled, in the North of France, between September and October 2008 at seven sites considered as benchmark by the Water Framework Directive, and five contaminated sites. The selected set of biomarkers was measured in fish from sites exhibiting various contamination levels to evaluate discriminant power of this tool and to characterize response profiles in a multi-contamination context. The response profile of AChE allowed a good discrimination between reference area exhibiting low AChE levels and contaminated sites with high AChE levels.

EC04 - Environmental fate and bioaccumulation of organic pollutants in aquatic systems

TU 010

Impact of organotin compounds at Brazilian Estuarine Systems: studies before and after banning

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Because of their wide use in many human activities, organotin compounds (OTs) were considered as the most deliberately introduced contaminant into the environment and, despite their ban in 2008, they can be found in all aquatic compartments. In Brazil, since 2001, many studies are developed involving abiotic and biotic matrices and the results still worrisome. Because of their hydrophobic behavior in aquatic systems, OTs are mainly found in sediment and are particularly available for organisms in direct contact with the bottom. However, due ressuspension and transport process in environment, the study of suspended particulate matter (SPM) can be considered important to estimate the risk of all system, even in areas without punctual sources. Studies with organisms demonstrate a bioaccumulation thought sediment feeding or even by thropic chain bring a serious ecological risk for these communities. Paranaguá Estuarine Complex is located on south coast and has the greatest harbour for grains export in Latin America. This area was previously OTs monitored, in 2006, and the contamination was founded in all sediment samples (250 to 3019 ng Sn.g-1 d.w) and SPM from maximum turbidity zone (14 to 700 ng Sn.L-1) located next to Paranaguá Harbor. Three years after, a new sampling was made in three regions of the estuary. The OTs analysis was carried using the same analytical procedure, involving GC-PFPD analysis. In sediments, butyltin concentrations range from <LD (LD=3.3, 8 and 12.5 ng Sn.g-1 for TBT, DBT and MBT respectively) to 1053 ng Sn.g-1. For SPM we have found these compounds in all samples ranging from 0.01 to 0.84 ng Sn.L-1, below the results detected on 2006. However, this difference can be caused by the different sampling points and beside this concentration, the impact cannot be unconsidered. The results has shown that even after ban, these compounds can be detect mainly in sediments of estuarine complex and the distribution pattern of BTs by ressuspension can also contribute for the impact acting on local biota and communities.

TU 011

Occurrence of antifouling biocides in two Italian marine systems and comparison with previous monitoring surveys

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Antifouling (AF) paints are used to prevent the attachment of living organisms to submerged surfaces of ships, boats and aquatic structures, usually by release of biocides. The serious environmental problems caused in aquatic ecosystems by extensive use of organotin compounds (e.g. tributyltin, TBT) drove to a global ban of TBT-based paints for all vessels. Alternative products were developed by paint manufacturers and usually contained Cu(I) compounds as main biocide, but this component was ineffective against some algae and diatoms; hence AF properties were enhanced by the addition of "booster"biocides.

Recently in some European countries (UK, Denmark, Sweden and the Netherlands) legislative restrictions have been introduced concerning use of two popular biocides irgarol 1051 and diuron in vessel paints, due to the adverse environmental persistence and toxicity. This measure did show a positive effect in decreasing levels of these antifoulants found in sensitive UK coastal areas. In Italy no regulations about their presence in AF paints have been enforced, but an indirect effect of these control measures on Italian market of AF paints cannot be ruled out because of the production at multinational level.

This study was designed to sample locations previously investigated inside the Gulf of Napoli to determine whether environmental concentrations of four common booster biocides (irgarol 1051, diuron, dichlofluanid and chlorothalonil) had significantly altered over a five year period. Analytical results showed that actual concentrations of diuron declined substantially on average to 10% compared with those recorded during 2005 monitoring survey, on the other hand concentrations of irgarol 1051 were found similar to those detected in the previous survey. Monitoring data were interpreted on the basis of active ingredients contained in the paints on sale in Italy. Spatial and temporal patterns of these compounds in seawater were investigated. Other booster biocides we screened for (dichlofluanid and chlorothalonil) were below limit of detection in all samples and this concurs with their environmental fate.

The presence of organotin compounds in the aquatic environment of Gulf of Napoli were also investigated, and the low concentrations we found suggested that these banned compounds were not being used for vessel's hull protection.

Moreover a parallel survey on alternative biocides and TBT was carried out in a different marine system that is Gulf of La Spezia.

TU 012

PCBs in the Mondego Estuary (Portugal): a preliminary survey

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The Mondego River flows along 227 km, draining the largest hydrological basin entirely comprised in the Portuguese territory. During its course, it runs through rural as well as highly urbanized and industrialized areas before reaching its 1,600 ha estuary. The Mondego River basin supports over half a million inhabitants and as a result the estuary has consequently been subjected to a strong anthropogenic pressure. Over the last 15 years, applied research has been conducted in the Mondego Estuary, providing a comprehensive dataset on several areas and an insight into the pollution levels of the ecosystem. However, in spite of the environmental persistence and toxicity of PCBs, the information available on their levels in the estuary is scarce and limited to sediment samples. Therefore, the present study aimed to report the occurrence of the twelve PCB congeners designated as toxic by the World Health Organization (non-*ortho* PCBs 77, 81, 126, 169; mono-*ortho* PCBs 105, 114, 118, 123, 156, 157, 167, 189) and the six indicator congeners (PCBs 28, 52, 101, 138, 153, 180) in sediment and biota from the Mondego Estuary.

PCB levels were measured by a high resolution gas chromatograph - high resolution mass spectrometer (HRGC/HRMS) in samples collected in November 2009 from the Mondego Estuary. Concentration in sediment on a dry weight basis was 199.23 $pg.g^{-1}$ dw for the sum of dioxin-like PCBs (∑dl-PCBs) and 1127.25 $pg.g^{-1}$ dw for the sum of indicator PCBs (∑iPCBs). Regarding biota, ∑dl-PCBs concentration on a wet weight basis ranged from 6.15 to 2807.97 $pg.g^{-1}$ ww in brown algae *Fucus* sp. and eel *Anguilla anguilla*, respectively. ∑iPCBs values ranged from 0.04 $ng.g^{-1}$ ww in *Ulva* sp. to 19.05 $ng.g^{-1}$ ww in *A. anguilla*. Lipid-normalised concentrations showed a different outcome: ∑dl-PCBs ranged from 3.62 to 29.25 $ng.g^{-1}$ lipid in macrophyte *Spartina maritima* and red algae *Gracilaria* sp., respectively, and ∑iPCBs ranged from 14.62 $ng.g^{-1}$ lipid in *S. maritima* to 132.86 $ng.g^{-1}$ lipid in sea bass *Dicentrarchus labrax*. Fish samples (*A. anguilla*, *D. labrax* and common sole *Solea solea*) presented higher lipid-normalised PCBs levels than those of benthic invertebrates (cockle C*erastoderma edule*, clam *Scrobicularia plana* and ragworm *Nereis diversicolor*). In general, the dl-PCBs profiles of the different matrices showed a predominance of the mono-*ortho* PCB 118 and PCB 105. Concerning iPCBs, PCB153 and PCB 138 accounted for the highest percentage of total iPCBs concentration.

TU 013

Trend of surface water concentrations of nonylphenol and its ethoxylates in an impacted basin: the Lambro river (Northern Italy)

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According to the Water Framework Directive (2000/60/EC) and its Daughter Directive on Priority Substances (2008/105/EC), nonylphenol has been inserted in the list of the priority hazardous substances (PHS) which should be phased out within 20 years, by applying the appropriate measures.

Chemical quality of the main Italian river, river Po, is strongly influenced by its tributary, Lambro river, which flows through the most urbanised and industrialised area of Italy, collecting discharge water also from the area of Milano. In the last 5 years new wastewater treatment plants, which treats the whole of Milano wastewater, became operative in order to improve the quality of the river.

The aim of this work was to compare the concentrations of nonylphenol and its ethoxylates in the river Lambro basin (Northern Italy) measured in two monitoring campaigns in 2004/05 and 2009/10, respectively before and after the WWTP building.

Trend of concentrations has been followed in different stations of the basin on Lambro river and on its main affluents (Seveso, Olona and Lambro Meridionale). Along the course of the river Lambro two stations, sited up- and downstream Milano, were monthly monitoring in order to understand the effectiveness of the treatment plants.

Comparison between the annual averages of 2004/05 and 2009/10 shows that the concentra-

tions of nonylphenol and its ethoxylates were reduced of more than 70% in all the measured stations, included stations upstream Milano. Therefore this reduction is attributed to the substitution of alkylphenols in detergents and formulates and not only to the new WWTPs. As an example, at the basin closure (Oriolitta station) we measured average concentrations of nonylphenol of 0.61 and 0.15 µg/l, respectively in the two annual campaigns. Average concentrations in 2002 and 2003 measured in the same station were even higher (1.82 and 1.02 µg/l respectively). The only peak of concentration (> 2 µg/l) in the last year was measured after an intentional oil spill event in Lambro on February 2010.
Nevertheless the effect of the new WWTPs in Milano is highlighted by the reduction of the nonylphenol fraction adsorbed on particulate which decreased from 35 % to 1%. This reduction is not observed in the station upstream Milano, which is located downstream an already operating WWTP. The mechanism of partitioning and transport of nonylphenol compounds in an impacted river is also discussed.

TU 014

Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in swordfish (Xiphias gladius) and bluefin tuna (Thunnus thynnus) from the Mediterranean Sea

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The swordfish (Xiphias gladius) and the bluefin tuna (Thunnus thynnus) are top predators of the Mediterranean Sea. They are important species from both ecological and economic point of view. These species bioaccumulate contaminants in their tissues and being largely consumed by humans, they may be responsible of transferring pollutants to humans. Polybrominated diphenyl ethers (PBDEs) are a class of chemicals extensively used in the last decades as flame retardants. Recently, increasing concentrations of PBDEs are reported in the environment and in animal and human tissues. Polychlorinated biphenyls (PCBs) are compounds that have been widely produced and used in the past. PBDEs and PCBs are persistent and they tend to bioaccumulate trough the food web. The aim of this study was to evaluate PBDE and PCB levels in the muscle of X. gladius and T. thynnus from the Mediterranean Sea. PBDE concentrations were higher in tuna than in swordfish (980 and 70 pg/g w.w., respectively), and also PCB levels were higher in tuna (46 ng/g w.w.) than in swordfish (27 ng/g w.w.). The BDE accumulation pattern varied between species and it was BDE47 > BDE154 > BDE100 in swordfish and BDE47 > BDE100 > BDE154 in tuna fish. In both species, the tetra-brominated isomers prevailed (48% in swordfish and 49% in tuna fish). Regarding PCBs, congener no.153 was always the most abundant (11% in swordfish and 14% in tuna fish) followed by congeners no. 138 and 105 in both species. Hexa-CBs, penta-CBs and tetra-CBs were the most abundant classes of PCB isomers and together they made up more than 88% of the total PCB residue in both species. Moreover, swordfish and tuna fish were analyzed according to the sex of specimen and PBDE concentrations were higher in female than in male: PBDE were 75 pg/g w.w. in female swordfish and 49 pg/g w.w. in male swordfish, while they were 1443 pg/g w.w. in female tuna and 714 pg/g w.w. in male tuna. In contrast, PCB levels were higher in male than in female and in swordfish PCB concentrations were 52 ng/g w.w. in male and 13 ng/g w.w. in female, while in tuna they were 47 ng/g w.w. in male and 34 ng/g w.w. in female. Although PBDE and PCB levels found in these specimen were lower than those evaluated in other fish from the Mediterranean Sea and from other European seas, these data are important from an ecological perspective and from the point of view of human health as swordfish and tuna fish are important in the diet of the Mediterranean populations.

TU 015

Chlorinated chemicals in Boreogadus saida from the Greenland Sea

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Boreogadus saida (Polar cod) is one of a key-species in the Arctic ecosystems being a trophic link between producers and upper trophic levels. It is fished in the North Atlantic Ocean due to its commercial value for human consumption of its fillets and liver oil. Therefore it is important to evaluate the presence of toxic contaminants in their tissues. Samples of muscle tissues were excised from specimens collected in the Greenland Sea in order to evaluate the presence of some chlorinated persistent contaminants: polychlorobiphenyls (PCBs), dichloro diphenyl trichloroethane (DDT) and its isomers, hexachlorocyclohexannes (HCHs) and its isomers, hexachlorobenzene (HCB), polychorodibenzodioxins (PCDDs) and polychorodibenzofurans (PCDFs). Gas-chromatographic analyses revealed low concentrations of the pollutants. ∑PCBs (7.67±0.06 ng/g wet wt) made up most of the residue, followed by ∑HCHs (2.63±0.02), ∑DDTs (1.21±0.01 ng/g wet wt), and HCB (2.77±0.01 ng/g wet wt). The p,p'-DDE was he most abundant isomer of DDTs; it was 41% of ∑DDT. The PCDDs/Fs were found in some sample and were often below the limits of detection (LOD = 0,1 pg/g wet wt).
The levels of PCB and organochlorine pesticides measured in NE Greenland are of the same order of magnitude of those reported in other fish from remote areas like Antarctica. Despite low levels of chlorinated persistent contaminants have been detected, their potential transfer to higher trophic levels might be expected, due to the key position of the species in the food webs.

TU 016

Assessment of representative and priority pesticides, in surface water of the Alqueva reservoir (South of Portugal)

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Surface waters located in intensive agricultural areas are more vulnerable to the pesticides contamination, which is a major concern if the water is intended to be used for human consumption. The aim of this study was to evaluate the presence and the distribution of pesticides in the Alqueva reservoir, an important source of water supply (South of Portugal), considering their representativeness in the agricultural practice of the area. For the analysis of pesticides risk impact we used the environmental quality standards (EQS) in the field of water policy proposed by the European Commission. The pesticides belonging to the classes of phenylureas, triazines, chloroacetanilides, organophosphorous and were analysed by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. The pesticides more frequently detected were atrazine, simazine, diuron and terbuthylazine. The highest levels of these pesticides were registered in spring, after pesticides treatment, namely in olive-tree and vine crops. The priority pesticides atrazine and diuron reached values above the AA proposed in the EU legislation. The herbicide atrazine reached values that surpassed the proposed MAC (2 000 ng L-1). The sampling stations most affected by these pesticides were Sra. Ajuda, Lucefecit and Alcarrache, located in the northern part of the reservoir, closer to Spain where the agricultural activity is more intensive.

TU 017

DDT bioaccumulation in fish tissues of the Lake Maggiore

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The research is focused on actual DDT contamination of the Lake Maggiore basin and its effects on aquatic living organisms. The level of DDT contamination has been measured in three fish species representative of different trophic levels. Fish sampling of twait shad (Alosa fallax lacustris), lake whitefish (Coregonus lavaretus) and common roach (Rutilus rutilus) has been carried out seasonally from March 2008 to December 2010 in the Lake Maggiore. Fish were age-selected in order to obtain a homogeneous age-class (2-3 years old) representative of relative young individuals. The muscle of the selected fish has been analysed from 2008 to 2010, whereas the DDT gonad concentration was calculated only in 2008 samples. The matrices were freeze-dried, extracted in a hot Soxhlet apparatus, concentrated and finally purified for the GC-MS analysis of DDT and its isomers and metabolites.
The results showed that the highest concentrations of contaminants were mostly observed in the period before spawning. Generally, the highest concentrations of DDT were detected in twait shad muscles, with values near or greater than the threshold levels for fish consumption, and in the lake whitefish gonads. In 2010, the lake whitefish muscles were reaching the limit concentrations and also the levels of contamination of common roach tissues were increasing. A significant correlation between DDT concentrations and the muscle lipid content was observed only for the twait shad, whereas no correlation was evident for other species.
Analyses of fish tissues on the DDT concentration levels from 2008 to 2010 showed an inverse trend of contamination than in the past, requiring further investigation on the contamination source in the lake basin.

TU 018

Monitoring the effect of tides in the distribution of polar organic contaminants in the East River (NY)

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The aim of this work was to determine the concentrations of a wide group of polar organic contaminants in surface waters from the East River (NY), their partitioning between dissolved and particulate phases, and the tidal effect on their distribution. Two different classes of contaminants were selected: synthetic surfactants (LAS, linear alkylbenzene sulfonates, NPEO, nonylphenol ethoxylates, and AEO, alcohol ethoxylates) and pharmaceutically active compounds (PhACs, a selection of 70 substances including different types of analgesics, antibiotics, beta-blockers, lipid regulators, psychiatric drugs[3DOTS]). Analysis of surfactant degradation metabolites such as SPC (sulfophenyl carboxylic acids), NPEC (nonylphenol ethoxycarboxylates), NP (nonylphenol) and PEG (polyethylene glycols) was also carried out. A multi-residue analytical methodology was employed for the analysis of this wide range of compounds, using a combination of time-of-flight and triple-quadrupole detectors for their identification and quantification. Surfactant concentrations in filtered water ranged from 0.01 to 1.20 ppb, while their degradation metabolites showed values higher than 10 ppb. Concentrations in suspended solids were significantly higher, from 3320 to 25327 ppb depending on the surfactant considered. Partitioning coefficients (log K) were calculated from this data, being in the range between 3.9 and 5.4, and lower for degradation products (log K < 3) due to their higher polarity. On the other hand, and in spite of the low detection limit values (typically <1 ppb), 90% of target pharmaceuticals were hardly detected in suspended solids and/or they were below quantification limits. This is mainly due to their lower volume of use compared to surfactants, their relatively poor sorption capacity and/or fast degradation in the water column. Only those compounds having the highest hydrophobicity values and/or production volumes (e.g., ibuprofen) could be detected. Overall, the occurrence and distribution of these contaminants in the sampling area were directly related to the existence of wastewater discharges coming from NY City, as the highest concentrations were measure during the flooding, when the water flow comes from urban areas, and the lowest values during the ebbing, when the tidal wave enters the East River from the open ocean.

TU 019

Monitoring sewage derived contamination in Guadalete River (SW, Spain) by analysis of synthetic surfactants

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The presence and longitudinal distributions of some of the most world wide used synthetic surfactants, alkyl ethoxysulfates (AES), alcohol polyethoxylates (AEOs), nonylphenol polyethoxylates (NPEOs), linear alkylbenzene sulfonates (LAS) and their carboxylated metabolites (SPCs) have been studied in water and sediment grabs from Guadalete river (SW, Spain). In general, concentrations values in water ranged from 14.62 to 2765.93 ppb for LAS, from 3.53 to 72.19 ppb for AES, from 5.27 to 49.26 and from 0.23 to 1.89 ppb for AEOs and NPEOs respectively. Concentrations values in sediment grabs ranged from 0.11 to 242.55 ppm for LAS, from 0.20 to 0.59 ppm for AES, from 0.97 to 3.04 and from 0.12 to 0.47 ppm for AEOs and NPEOs respectively. Urban wastewaters discharges were identified as the main contamination sources for these compounds and their metabolites. The highest concentrations of synthetic surfactants were found in water and sediments grabs associated to untreated wastewaters discharges from small urban areas of Jerez de la Frontera, farms and individual households. The relative distribution of LAS, AEOs and AES homologues in sediments showed higher percentages for those having longer alkyl chain due to differential sorption and degradation processes. SPCs concentrations values in water ranged from 163.49 to 486.63 ppb, being measured the highest concentrations near a WWTP effluent discharge outlet. The general trend shows a progressive shortening in the alkyl chain of SPCs homologues, predominance of short-homologues (C6SPC and C7SPC) compared with long homologues (C11SPC, C12SPC and C13SPC) in each sampling point.

TU 020

Contaminant distribution in harbour porpoises, Phocoena phocoena, stranded along the Dutch coast.

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In the past decennium an increasing number of harbour porpoises, Phocoena phocoena, beached on the Dutch coast. In winter time, beached animals contained a thick blubber layer and seemed to be victims of accidents or bycatch of fishery. In summer time, beached porpoises were mainly found emaciated, predominantly without visible physical injuries. To assess redistribution processes of organic contaminants due to starvation, the liver and blubber of 36 beached harbour porpoises were analysed for PCBs, PBDEs, HBCD, PFCs and organotin compounds. No differences were found for concentrations of non-lipophilic compounds (PFCs) between summer and winter. Concentrations of PCBs and PBDEs in both liver and blubber on lipid weight were on average higher in emaciated porpoises (beached in summer) when compared to porpoises with a good nutritional status (beached in winter), and showed slightly deviating profiles in liver. This may point at remobilisation of lipophilic contaminants during emaciation. Our data indicate that concentrations and profiles of organic contaminants in marine top predators, such as harbour porpoises, will not only be influenced by common bioaccumulation processes such as e.g. uptake from food and metabolism, but also by starvation.

TU 021

Occurrence and fate of organic pollutants in aquifer systems of Jerez de la Frontera region (Cádiz, Spain)

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Because organic compounds are now used so extensively, it is necessary to evaluate their presence and distribution in various different environmental compartments. In this work, polycyclic aromatic hydrocarbons (PAHs), polychlorinated byphenyls (PCBs) and pesticides (carbamates, pyrethroids, organochlorinates, organophosphorus and organonitrogenates) have been monitored in two aquifers, those of Jerez de la Frontera and the Alluvial Guadalete, also including samples from the Guadalete River. PAHs and some pesticides were detected at significant concentrations at specific points. In fact three pollutants (chlorfenvinphos, chlorpyrifos and prometryn) were detected at more than 100 ng L-1 in groundwater samples, and endosulfan sulfate, terbutryn, terbuthylazine, alachlor, prometon, pyrene and fluoranthene were found at low concentrations (1 to 50 ng L-1). The chemicals found in the soil matrix were the most hydrophobic pollutants, including apolar pesticides and high molecular weight PAHs, and at higher levels (µg L-1) than those present in groundwater samples.

TU 022

Air-sea exchange of current-use pesticides in the North Sea

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Increased public and regulatory attention during the 1970s and 1980s resulted in bans for many legacy pesticides and led to the development and licensing of new pesticides with less persistence. This group of newer compounds is called current-use pesticides (CUPs). High usages and new concerns regarding the prevalence and effects of these compounds in the environment resulted in CUPs becoming a significant issue for environmental and toxicological studies. Recently, increasing levels of some current-use pesticides(CUPs) have been found in remote areas, e.g. in the Arctic and in some elevated mountains, however, compared with the legacy organochlorine pesticides, data of CUPs in the marine environment are relatively sparse.
Sampling in the German Bight of the North Sea took place during three sampling cruises with the German Research Vessel RV Heincke in March, Mai and September 2009. Air samples were collected with a high-volume air sampler equipped with a glass fibre filter (GFF) connected with a glass column packed with PUF/XAD-2 resin. Water samples were pumped through a GFF followed by a glass column packed with PAD-2.
Atmospheric and aquatic concentrations of CUPs in the North Sea have been determined by GC/MS-system. The seasonal distribution and partitioning between different phases of CUPs in air and seawater have been investigated. Air-sea exchange fluxes of CUPs were estimated using a two-film model. Varying fluxes indicated that air-sea exchange is an important process which intervenes in the mass balance of CUPs in the North Sea.

TU 023

Benthic Degradation and sorption to soot carbon are key processes controlling the occurrence and fate of Polycyclic Aromatic Hydrocarbons in Lakes

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Semivolatile organic pollutants have potential for long range atmospheric transport and thus can reach pristine lakes by atmospheric deposition. While the environmental fate and occurrence of persistent organic pollutants (POPs), such as polychlorinated biphenyls, in lakes has been assessed and modeled previously, there is still a poor knowledge of the main factors controlling the fate of non-persistent organic pollutants, such as polycyclic aromatic hydrocarbons (PAH), in the environment. Here we show a soot-inclusive environmental fate model for PAHs in lakes and its validation/application to Lake Redó in the Pyrenees Mountains. A model for the refractory soot carbon has been coupled to the general organic carbon based POP model. In addition, degradation in benthic waters and sediments has also been included in the model. This model allows to predict water and sediment concentrations of pollutants from knowledge of atmospheric concentrations and Lake characteristics. It is shown that only when soot carbon and degradation processes are included in the model, it is possible to predict similar PAH concentrations in the sediment to those measured in Lake Redo. The extend of the degradation sink due to benthic degradation is quantified and compared to the reservoir of PAHs accumulated in the sediment and other cycling fluxes in the lake.

TU 024

Occurrence and trends of POPs in the Southern Ocean and Antarctic region

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POPs transport and occurrence in Polar Areas have been focused in the North Hemisphere (Ockenden et al., 2001). Few Studies have reported seawater, atmospheric and biota POPs cocentrations in the southern Ocean and Antarctic area (Dickhutt et al., 2005; Cincinelli et al., 2009). Gas phase concentration data were the most studied in the area (Oeckenden et al., 2001; Cincinelli et al., 2009).Seawater concentration of POPs have been reported for surface microlayer in 2001 by Fuoco et al. and for surface seawater in 2009 by Cincinelli et al. In the case of biota a lot of studies have reported concentrations of POPs for mammals, fish, and Krill (Chiuchiolo et al., 2004). But none of them mentioned Phytoplankton concentrations and possible implications of the role drived in the cycling of POPs and the keyrole of the Biological Pump in Polar Areas. None of the published studies have mentioned concentration for the particulate and aerosol concentratios. In the present work we show concentration and trends of Chlorinated and Polycicliic Aromatic Hydrocarbons in the Gas, Aerosol, Particulate, Seawater, Phytoplankton and Krill for the first time.
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TU 025

Urban tributaries as a pathway of PCBs, PBDEs, PAHs, and polycyclic musks to adjacent Lake Ontario, Canada

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Urban rivers continue to deliver contaminants such as the legacy polychlorinated biphenyls (PCBs) and current/recent-use chemicals to receiving waters like the Great Lakes where they may persist, bioaccumulate, and impact biota. To investigate the relative roles of urban contaminant fate pathways (atmospheric deposition, tributary, municipal wastewater) to adjacent waterbodies, using Toronto, Canada and Lake Ontario as examples, we measured concentrations for a suite of organic compounds of varying uses and sources and estimated stream loadings. This presentation highlights results for the urban tributary pathway and its contributions relative to atmospheric deposition and municipal wastewater discharges.
Bulk water concentrations of PCBs, polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenylethers (PBDEs), and polycyclic musks (PCMs) were determined in dry and wet weather samples collected from 10 sites in 6 watersheds across the Toronto area in 2007 through 2009. Wet weather events were intensively sampled on 3 occasions to provide an indication of the variability with hydrological change. For all analytes, concentrations were greatest at the downstream sites located in intensively developed urban areas. Median (maximum) concentrations across the downstream sites ranged from 2.2-6.7 (19-144) ng/L for ΣPCBs, 100-950 (1700-19300) ng/L for ΣPAHs, 5.1-6.6 (25-46) ng/L for ΣPBDEs, and 29-1800 (53-5800) ng/L for ΣPCMs. BDE-209 was the dominant PBDE, while PCMs consisted mainly or HHCB and AHTN. Concentrations of PCBs, PAHs, and PBDEs were higher during wet weather when streams were much more turbid. However, PCM concentrations tended to decline during wet weather, suggesting sewer cross-connections may be a source of PCMs. PCM concentrations were considerably higher in the Don River which receives municipal wastewater discharges.
Stream contaminant loads to Lake Ontario were calculated using USGS's LOADest for the 6 downstream sites. Estimated average annual loads were 2200 kg/yr for ΣPAHs, 42 kg/yr for ΣPCMs, and 8 kg/yr each for ΣPCBs and ΣPBDEs. On a mass basis, the tributaries were the most important pathway for ΣPAHs, contributing 60% of loads compared to atmospheric and wastewater pathways from urban Toronto. In comparison the tributaries were estimated to contribute 40% for ΣPBDEs, 20% for ΣPCBs, and 4% for ΣPCMs. Urban tributaries are important contributors of compounds with urban sources to adjacent aquatic environments.

TU 026

Bioaccumulation and biomagnification of chlorinated and perfluorinated persistent organic pollutants in a pelagic food web from the Mediterranean Sea

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Mediterranean swordfish (Xiphias gladius), bluefin tuna (Thunnus thynnus) and longfin tuna (Thunnus alalunga) are large pelagic species heavily caught for commercial purposes, being an important resources for fish markets. The anthropic impact is heavy on these species due to their top position in the trophic webs, thus it is very important to know their health status and evaluate the toxicity for humans. To these purposes, the presence and amount of persistent organic pollutants were determined in their tissues and their preys: polychlorobiphenyls (PCBs), perfluorinated compounds (PFCs), chlorinated pesticides (HCB, HCHs, DDTs, dieldrin, eldrin), polychlorodibenzodioxins (PCDD), and polychlorodibenzofurans (PCDFs) were determined. Moreover, the 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalents (TEQs) were also calculated to assess risk for humans due to fish consumption.
The contaminant trend was PCBs>DDTs>HCHs>HCB in all samples; concentrations were in the ranges 15-27 ng/g wet wt of PCBs, <0.001-0.3 ng/g wet wt of HCB, 0.004-0.3 ng/g wet wt of HCHs, and 0.2-62 ng/g wet wt of DDTs in predators and lower in preys. Concentrations in males were higher than in females of tuna and swordfish, and were lower than those reported earlier, likely due to the young age of specimens. Penta- to hepta-PCBs made up most of the PCB residue.
Coplanar PCBs were below detection levels in most samples, except in a few specimens of tuna and swordfish (PCB77>PCB81>PCB126>PCB169). Concentrations of PCDDs and PCDFs
were mostly <2 pg/g wet wt and <1.2 pg/g wet wt and very few congeners were found in the samples analyzed. TEQs were between 2.6 and 17.1 pg/g wet wt ; bluefin tuna muscle samples showed the highest values.
p,p'-DDE was the most abundant DDT isomer in predator and prey samples.
PFCs were below detection limits in most of the samples of predator and preys. PFOS and PFOA were above the detection limit (0.5 ng/g wet wt) in 9 of 61 and 2 of 61 samples, respectively.

TU 027

Organohalogenated compounds in Franciscana dolphin from Brazilian shore: a case of an endangered species in South Atlantic

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Organohalogenated compounds are one of the main pollutant groups that affect the ecosystems due to their persistence and toxicity. There are many studies concerning these compounds in marine mammals around the world, nevertheless the South Atlantic Ocean remains poorly known. Franciscana dolphin, Pontoporia blainvillei, is the most impacted cetacean off the eastern coast of South America by human activities. The Santos Estuary (23º55'S, 46º20'W) in São Paulo State, southeastern Brazil, South Atlantic Ocean, represents the most important Brazilian example of environmental degradation by hydric and atmospheric pollution from industrial origin in coastal areas. The aim of this work was evaluate the occurrence of organochlorine and organobrominated compounds in samples from 87 livers, 3 milks, 2 placentas and 1umbilical cord of franciscana dolphins, collected in 4 States along the southwestern and southern off Brazilian coast (Espírito Santo, n = 12; São Paulo, n = 42; Santa Catarina, n = 07; Rio Grande do Sul, n = 26). Among organochlorines, PCBs were the predominant compounds on lipid weight basis (0.3 - 42.2 ug g-1), followed by DDTs (0.1 - 7.2 ug g-1), HCB (< 0.002 - 0.15 ug g-1) and HCHs (n.d. - 0.05 ug g-1). PCBs concentrations in São Paulo coast presented the major values to franciscana in all of its distribution (Brazil, Uruguay and Argentina), showing the high industrial influence on this part of Brazilian coast. PCBs and HCB values found in this study were comparable to the values found in coastal cetaceans from developed countries in north hemisphere. Among the organobrominated, the higher concentrations observed came from the natural compounds, mainly 2'-MeO-BDE 47 (n.d. - 792 ng.g-1) and 6-MeO-BDE 68 (n.d. - 268 ng.g-1). PBDEs concentrations were also higher in the samples from São Paulo coast (n.d. - 450 ng.g-1). Higher contributions of naturally-produced organobrominated compounds are an indication of the presence of sponges, algae or associated organisms. The concentrations obtained for cetaceans from Brazilian waters are among the highest detected to date in marine mammals.

TU 028

24 POPS - Comprehensive analysis of all Stockholm convention and candidate POPS in marine biota

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In the last few decades, there has been a tremendous increase of knowledge about the adverse effects of persistent organic pollutants (POPs). Their persistency, accumulation and transport within the ecosphere together with risks for humans as well as for animals became obvious and lead after first agreements on a European scale to the internationally recognised Stockholm Convention in 2001. With its ratification in 2004, a ban and/or restriction of a first group of purposefully produced or unintentionally synthesised substances came into force on a global scale. Since then, additional compounds have been added to the first list and further chemicals are under review or discussed. All together, there are 12 substances from the original list (2001), 9 others which have been added in 2009 and actually 3 candidates, in total up to now 24 substances which we understand here as "Stockholm POPs / candidates".

The present paper tries for the first time a comprehensive analytical approach in order to get complete data sets for the whole range of POPs and to set up an analytical strategy for this approach. The presented study demonstrates the methodological feasibility for the whole range of the Stockholm Convention-/candidate POPs as well as giving for a first time complete data sets for certain marine biota. We see it as a starting point for an overall view on the most common contaminations in the field of POPs. The present data reflect a first step into this effort, focussed onto high resolution mass spectrometry as one of the most capable and sensitive techniques for production of unambiguous results for scientific purposes. Also, for some of the analysed compounds only very limited data has been available up to now, e.g. for SCCP or for fluorinated compounds in cod liver.

TU 029

A yearlong survey for diverse classes of estrogenic disrupting compounds suggests significant pollution in the estuary of the Ave River and nearby seacoast, Portugal

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The Ave River rises in North-Portugal at the Cabreira Mountain and reaches the Atlantic Ocean at Vila do Conde. During its course of ca. 85 km it crosses agricultural fields and intense industrial areas. In spite of the visible local pollution, this study is the first that investigates the presence of endocrine disruptor compounds (EDCs) from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins in this area. For this purpose, water samples were collected during one year, every two months, at 8 sampling stations located at the river, at the estuary and at the sea. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. Preliminary data showed the presence of FORM (ca. 2,000 ng/L), DAID (ca. 200 ng/L), GEN (ca. 200 ng/L), BIO-A (ca. 350 ng/L), SITO (ca. 7,000 ng/L), E1 (ca. 100 ng/L), EE2 (ca. 150 ng/L), OPs (∑= ca. 200 ng/L), NPs (∑= ca. 1,000 ng/L), APEOs (∑= ca. 7,000 ng/L) and BPA (ca. 150 ng/L) in both river and seacoast. Facing reference literature values, the current data strongly suggests that this area in under significant impact by estrogenic EDCs and needs urgent depollution measures; nonetheless further chemical and biological monitoring analyses are being undertaken.

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TU 030

Climate-induced fate of biocides in sediments

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Climate-induced fate of biocides in sediments

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In recent years, biocides and UV-filters have gained increasing interest as so called emerging contaminants since they are ingredients of various products used in every day life such as personal care products (PCPs), cleaning agents and paints and coatings. Biocides are biological active compounds applied to destroy or to inhibit the growth or action of organisms, even low environmental concentrations might have negative impacts on the aquatic environment. For example, triclosan has been shown to induce changes in the thyroid hormone-mediated process of metamorphosis of the North American bullfrog Rana catesbeiana and to cause a significant shift in the community structure of a natural river algae community at environmental relevant concentrations as low as 30 and 15 ng Lð'1, respectively.

The fate of biocides in the aquatic environment, particularly in river and stream sediments, remains poorly understood. This is at least in part due to the lack of analytical methods for quantification of these drugs in sediment and water samples. Concentration data in sediments needs to compliment data on aqueous phase concentrations, particularly in light of the high susceptibility of biocides to microbial (and abiotic) degradation.

The aim of the study is to examine the influence of climate change on the sorption and degradation behaviour of biocides in sediment. Therefore, an extensive data set on the sorption and degradation kinetics of selected biocides to sediments and the concentration dependence of sorption are collected. Batch experiments according to OECD 106 and 308 are performed to determine the sorption and degradation kinetics of selected biocides, e.g. triclosan. Sorption isotherms are also constructed. Isotherms spanned 2 orders of magnitude in initial analyte concentrations (i.e., 1, 3.2, 10, 32 and 100 ng/mL). The systems are equilibrated for 24 h, which was long enough to attain apparent uptake equilibrium based on the results of the kinetic experiment. At 24 h, biocides concentrations are quantified in the sediment and the water phases in duplicate batch reactors. To determine the influence of a possible climate chance, all experiments are performed at different temperatures ranging from 4 to 36°C.

TU 031

Chemical monitoring of sixteen polycyclic aromatic hydrocarbons reveals a high sediment pollution load in the Douro River estuary, Porto, Portugal

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Recent findings in fish caught in Douro River estuary and nearby Porto sea coast point to the presence of polycyclic aromatic hydrocarbons (PAHs) in Porto region. Because these compounds are extremely toxic, from aquatic animals to humans, and are highly lipophilic, their evaluation in coastal sediments are of the most interest. Their eventual appearance is always of great concern for both environmental and public health. Thus, we developed an analytical method based on microwave-assisted extraction (MAE) of sediments, followed by solid phase micro-extraction (SPME) and gas chromatography mass spectrometry (GC-MS) to simultaneously quantify sixteen PAHs in marine and estuarine sediment samples. The analysed compounds were those included in the US Environmental Protection Agency (US EPA) priority list which includes naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[k]fluoranthene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene and some potentially carcinogenic for humans; in particular benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene. Method detection limits were between 0.07 and 0.76 µg/kg. Sediment samples from the Douro River estuary and Porto marine sea coast showed an average load of all above mentioned PAHs of ca. 100 µg/kg and ca. 55 µg/kg, respectively, demonstrating that both studied areas are strongly impacted by this type of pollutants. The data agrees with former findings reporting bioaccumulation in local fish, with unknown impacts for them and consumers.

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TU 032

Annual monitoring of seventeen endocrine estrogenic disrupters reveals a high load of anthropogenic pollution in the Douro River estuary and nearby seacoast, Porto, Portugal

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The Douro River flows along ca. 900 km, from its source near Duruelo de la Sierra (Spain) to its estuary at Porto (Portugal). Due to its dimensions and huge watershed (98, 000 km2), this river passes throughout many different regions that include densely inhabited areas, industrial poles, and large agricultural fields, which, as a whole, contribute to its pollution. At the present, beyond the construction of one dam, the Crestuma-Lever, inside the innate estuary of the Douro River, the drainage rates of the river to the Atlantic Ocean are also affected by the recent construction of jetties at the mouth of the estuary. In a previous survey with HPLC based methods we detected several estrogenic chemicals. The data called for further and wider monitoring, eventually using more sensitive methods. Thus, this study reports the simultaneous evaluation of seventeen potential endocrine disruptors from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins in this area. For this propose, water samples were collected during one year, every two months, at 8 sampling stations located at low and upper estuary and at the seacoast located nearby. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. Briefly, the results showed the presence of FORM (ca. 220 ng/L), DAID (ca. 20 ng/L), GEN (ca. 90 ng/L), BIO-A (ca. 800 ng/L), SITO (ca. 2,500 ng/L), E1 (ca. 25 ng/L), E2 (ca. 2.5 ng/L), EE2 (ca. 6 ng/L), OPs (∑= ca. 60 ng/L), NPs (∑= ca. 500 ng/L), APEOs (∑= ca. 2,000 ng/L) and BPA (ca. 100 ng/L) in this area. The data confirmed the estrogenic prone scenario we previously reported. Thus, we conclude that conditions for endocrine disruption risk by estrogenic exposure, mainly from industrial origin, exist in this area. Biomonitoring is needed to evaluate effects.

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TU 033

A one year survey for seventeen estrogenic pollutants reveals endocrine disruption risks in the estuary of the Lima River and nearby sea coast (Portugal)

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The Lima River rises in Galicia (Spain) and reaches the Atlantic Ocean near Viana do Castelo (North of Portugal). During its course of ca. 100 km it crosses agricultural fields, but the estuary receives effluents from both urban and industrial discharges suggesting the possible presence of endocrine disruptors compounds (EDCs) in this area. Despite that, the river is still the natural habitat of important commercial fish species (e.g., trout). So, we evaluated the levels of seventeen EDCs from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins at upper and lower estuary and seacoast. For this propose, water samples were collected during one year, every two months, at 8 sampling stations. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. The results showed the presence of FORM (ca. 500 ng/L), DAID (ca. 80 ng/L), GEN (ca. 200 ng/L), BIO-A (ca. 350 ng/L), SITO (ca. 600 ng/L), E1 (ca. 30 ng/L), EE2 (ca. 20 ng/L), OPs (∑= ca. 150 ng/L), NPs (∑= ca. 500 ng/L), APEOs (∑= ca. 2,000 ng/L) and BPA (ca. 1,000 ng/L) in both estuary and seacoast. Facing the values and literature data on the effects of those chemicals, we conclude that endocrine disruption risks by estrogenic effects likely exist in this area but under a vaster context, and comparatively with other habitats we studied in Portugal, the Lima area is nevertheless one of the less polluted.
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TU 034
Quantification of estrogenic endocrine-disrupting chemicals in a supposedly reference ecosystem - the Mira River, Alentejo, in Southwestern Portugal
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The Mira River rises in the northern slopes of the Serra do Caldeirão and pursues a southeast-northwest course of 145 km with a generally mild inclination to the Atlantic Ocean, where it discharges through a small calm delta, near the town of Vila Nova de Milfontes, 115 km south to Lisbon. It is one of the two only rivers in Portugal with a chiefly south-north orientation (the other being its neighbour Sado) and one of the few rivers in Europe with this orientation. Mira basin borders Sado River basin, at north, and Guadiana River basin eastwards. Until the moment it is considered a reference river in ecotoxicology due to its low levels of pollution. However, no local surveys for estrogenic endocrine-disrupting compounds (EDCs) were undertaken to this date. To evaluate whether this river is as a valid reference also for several classes of EDCs, this study reports the evaluation of EDCs from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins. For this propose, water samples were collected during one year, every two months, at 6 sampling stations located at the river estuary and at the sea. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations of the above referred pollutants were evaluated by GC-MS. Briefly, the results showed the presence of FORM (ca. 600 ng/L), DAID (ca. 18,000 ng/L), GEN (ca. 2,000 ng/L), BIO-A (ca. 1,000 ng/L), SITO (ca. 20,000 ng/L), OPs (∑= ca. 7,500 ng/L), NPs (∑= ca. 350 ng/L), APEOs (∑= ca. 2,600 ng/L) and BPA (ca. 300 ng/L) in this area. Facing the diversity and the amounts of the chemicals, we conclude that the Mira River must be faced with caution as to be considered a reference when comparing estrogenic effects in biomonitoring. Actually, facing the literature the amounts we found may even pose a local toxicological risk.
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TU 035
Evidence of estrogenic compounds, mainly phytoestrogens, in the Mondego River estuary and nearby sea coast, Portugal
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The Mondego River rises in the centre of Portugal, at the Mountain Range of Serra da Estrela and reaches the Atlantic Ocean at Figueira da Foz. During its course of 227 km it runs through rural as well as highly urbanized and industrialized areas before reaching its 1,600 ha estuary, where it becomes visibly polluted. Its main pollution sources are waste waters, different types of industries, and the agricultural run-off; end result of 15,000 ha of cultivated land (mostly rice and corn fields) located upstream. In a previous survey with HPLC based methods we detected a few estrogenic chemicals. The data called for further and wider monitoring, eventually using more sensitive methods. Thus, this study reports the simultaneous evaluation of seventeen potential endocrine disruptors from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins in this area. For this propose, water samples were collected during one year, every two months, at 8 sampling stations located at the river, at the estuary and at the sea. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. Briefly, the results showed the presence of FORM (ca. 2,000 ng/L), DAID (ca. 1,500 ng/L), GEN (ca. 1,700 ng/L), BIO-A (ca. 14,000 ng/L), SITO (ca. 1,500 ng/L), E1 (ca. 150 ng/L), EE2 (ca. 50 ng/L), OPs (∑= ca. 800 ng/L), NPs (∑= ca. 800 ng/L), APEOs (∑= ca. 5,000 ng/L) and BPA (ca. 50 ng/L) in this area. The data confirmed the estrogenic prone scenario we previously reported. Thus, we conclude that conditions for endocrine disruption risk by estrogenic exposure, mainly from vegetal origin, exist in this area. Biomonitoring is needed to evaluate effects.
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TU 036
Occurrence of estrogenic pollutants in the Ria de Aveiro Lagoon (Portugal)
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The Aveiro Lagoon is located on the Atlantic coast in North Portugal. Its average area covers approximately 75 km^2 and is the local where four small rivers (Vouga, Antuã, Boco and Fontão) launch their final flow. Due to the strategic location of this area several cities are located here, being the Aveiro city the most important urban centre. Beyond fisheries, this zone also holds an important industrial sector and some protected areas where saltmarshes and seagrass meadows are present. To help evaluating local toxicology risks for the biota, this study aims to investigate, as exhaustively as possible, the presence of estrogenic endocrine disruptors, from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins. For this propose, water samples were collected during one year, every two months, at 8 sampling stations covering all area of the lagoon. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. The obtained results showed the presence of FORM (ca. 150 ng/L), DAID (ca. 120 ng/L), GEN (ca. 50 ng/L), BIO-A (ca. 1,000 ng/L), SITO (ca. 4,000 ng/L), EE2 (ca. 40 ng/L), OPs (∑= ca. 100 ng/L), NPs (∑= ca. 1,000 ng/L), APEOs (∑= ca. 9,800 ng/L) and BPA (ca. 5,300 ng/L) inside the lagoon and in the seacoast. Considering the toxicological effects reported in the literature for natural and experimental exposures, within those concentration magnitudes, we conclude that endocrine disruption risk by estrogenic exposure may exist in this area; anyway, further analysis are being done.
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TU 037
Off-site environmental impacts of wildfires: evaluation of the toxicity of runoff from a burnt area on freshwater aquatic species
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Wildfires have been found to increase the levels of polycyclic aromatic hydrocarbons (PAHs) in surface water bodies within and downstream of recently burnt areas. Whilst PAHs are well-known for their potential toxicity, environmental persistence and tendency to bioaccumulation, the potential toxicity of the runoff from burnt areas on aquatic species has received little research attention. The present work addresses this knowledge gap by laboratory assays with species from different taxonomic groups representing distinct trophic levels.
In the framework of EROSFIRE-II project, post-fire runoff generation and erosion has been monitored in a forest area in Góis municipality, central Portugal. Following a moderate-severity wildfire in August 2008, runoff samples were collect from a slope-scale erosion plot in a eucalypt stand on two occasions, namely in October 2008 after the first rains and about one year later, in September 2009. The concentration of the sixteen PAHs identified by US EPA as priority contaminants were determined for the particulate and dissolved phases of both runoff samples. The ecotoxicological assays were carried out with the following aquatic species: the bacteria (Vibrio fischeri), the green alga (Pseudokirchneriella subcapitata), the macrophyte (Lemma minor), and the invertebrate (Daphnia magna).
The PAH contents in the particulate as well as the dissolved phase of both runoff samples were dominated by compounds of the low (2-3 rings) and medium (4 rings) molecular weight. In general, the different PAHs revealed higher contents in the particulate phase than in the dissolved phase. The PAH contents differed for the two sampling occasions but not in straightforward manner. Namely, some PAHs revealed higher contents immediately after the fire, whereas others did in September 2009. These temporal patterns agree with the known persistence of the various PAHs.
The four species revealed different responses to the two runoff samples. Both samples were highly toxic to V. fischeri, P. subcapitata and L. minor, whereas they did not affect D. magna in a statistically significant manner. Furthermore, the non-filtered samples tended to produce greater effects than the filtered samples. The observed toxic effects can be explained well by the samples' PAHs contents.
This study underlines the importance of furthering knowledge of the potential deleterious impacts of wildfires on the aquatic species as a result of the PAHs production.

TU 038
Natural and man-made estrogenic endocrine-disrupting chemicals exist in superficial waters of the Ria Formosa Lagoon, a natural park in Algarve, South of Portugal
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Ria Formosa is a shallow mesotidal lagoon in the south coast of Portugal, with natural biogeochemical cycles essentially regulated by tidal exchanges at the seawater boundaries and at the sediment interface. By law, this area is a protected zone but, in reality, many focuses of pollution exist and several reports in local media have been made, denouncing the presence of direct discharges from urban, industrial and even naval sources. Thus, we anticipated that despite the law enforcements the area may be subject to pollution by estrogenic compounds. This study reports the evaluation of endocrine disruptors from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins. For this propose, water samples were collected during one year, every two months, at 9 sampling stations located in the lagoon and close to the sea. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations and identities of the above referred pollutants were evaluated by GC-MS. Seventeen compounds were screened. Briefly, the results showed the presence of FORM (ca. 2,300 ng/L), DAID (ca. 7,000 ng/L), GEN (ca. 10,000 ng/L), BIO-A (ca. 300 ng/L), SITO (ca. 12,800 ng/L), EE2 (ca. 50 ng/L), OPs (∑= ca. 6,000 ng/L), NPs (∑= ca. 2,000 ng/L), APEOs (∑= ca. 13,800 ng/L) and BPA (ca. 40 ng/L) in this area. Considering the literature data on effects on the range of concentrations found, we conclude that endocrine disruption risk by estrogenic exposure may exist in this area. Facing the particular law status of the area, the data calls for a continuous local monitoring, both with analytical and biological

approaches.
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TU 039

Chemical monitoring of seventeen estrogenic endocrine-disrupting compounds expose risk for the biota in the Portuguese 'dolphin estuary' (Sado River)

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The Sado River, which is located in the West of the Iberian Peninsula, in Portugal, flows from the Serra do Caldeirão to the Atlantic Ocean. It is one of the few European rivers flowing South to North. During its course of 175 km, it runs mainly through rural areas before reaching its estuary that is highly industrialized. It is most famous for harboring a permanent (and slowly declining) community of about 30 bottlenose dolphins to this date. For these species and other local biota endocrine disruption phenomena is a potential risk. Suspecting that there is ongoing exposure to waterborne disruptors, this study aimed the evaluation of estrogenic endocrine disruptor compounds (EDCs) from vegetal (formonometin, FORM; daidzein, DAI; genistein, GEN; biochanin A, BIO-A; and sitosterol, SITO), biological (estrone, E1 and estradiol, E2), pharmaceutical (ethynylestradiol, EE2) and industrial (octylphenols, OPs; nonylphenols, NPs; alkylphenols ethoxylates, APEOs; and bisphenol A, BPA) origins. For this propose, water samples were collected during one year, every two months, at 10 sampling stations covering all the area of the estuarine system. The samples were filtrated, preconcentrated in Oasis HLB cartridges, cleaned in silica cartridges, and the concentrations of the above referred pollutants were evaluated by GC-MS. Briefly, results showed the presence of FORM (ca. 2,500 ng/L), DAID (ca. 250 ng/L), GEN (ca. 7,500 ng/L), BIO-A (ca. 150 ng/L), SITO (ca. 4,800 ng/L), EE2 (ca. 14 ng/L), OPs (∑= ca. 80 ng/L), NPs (∑= ca. 350 ng/L), APEOs (∑= ca. 7,000 ng/L) and BPA (ca. 25 ng/L) in this area. Despite the efforts to protect the Sado the fact is we found all these EDCs, showing that anthropogenic estrogens are the most prevalent ones in the area, and in concentrations that do not exclude at all a toxicant potential for evoking endocrine disruption. Chemical monitoring in parallel to a biomarker approach seem particularly advisable to implement in this unique ecosystem.
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TU 040

Monitorization of pesticides reveals a high contamination load in the Douro River estuary and nearby seacoast, Porto, Portugal

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The Douro River flows along ca. 900 km, from its source in Spain to its estuary at Porto (Portugal). Due to its dimensions and huge watershed (98,000 km2), this river passes throughout many different regions that include large agricultural fields, where pesticides are widely used and its water is commonly used for local irrigation. In spite of this, the evaluation of local contamination by these compounds has been neglected. Moreover, because we believe that upper stream contamination may arrive at the estuary where they may remain for long periods of time due both to the presence of a dam inside the estuary, the Crestuma-Lever, and also the recent construction of jetties that protect houses and stores at the estuary margins from the aggressiveness of the North Atlantic Ocean, but diminishes the flow of the river into the sea. In this sense the main objective of this study was to check for the presence of 39 priority pesticides, both in surface water and sediments, collected in the Douro River estuary and in the Porto sea coast. Target compounds included several groups: organochlorines, organophosphorous, triazines, pyrethroids and others miscellaneous. Analyzes were performed using previously validated methods involving solid-phase extraction and ultrasonic extraction of pesticides from surface water and sediment samples, respectively, followed by GC-MS. Our data revealed the present of several pesticides in water (ng/L) and sediments (µg/kg dw). Those compounds found simultaneously in water and sediments were the DDTs (ca. ∑DDTs= 150 ng/L and 15 µg/kg dw), endosulfan sulfate (ca. 100 ng/L and 15 µg/kg dw), chlorfenvinphos Z (ca. 160 ng/L and 10 µg/kg dw), hexachlorocyclopentadiene (ca. 360 ng/L and 17 µg/kg dw) and atrazine-desethyl (ca. 6.5 ng/L and 14 µg/kg dw). So the obtained results not only prove that there is an ongoing significant pollution by these compounds but also agree with former findings reporting bioaccumulation of these type of compounds (mainly DDTs) in local fish, with unknown impacts for them and for the consumers health.
Acknowledgements: Research Projects CESPU 04-GCQF-CICS-09 and FCT PTDC/MAR/70436/2006 (POCI2010, FEDER).

TU 041

Occurrence of Irgarol, terbutryne, and a degradation product in German surface waters

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The structural closely related herbicides Irgarol (cybutryne) and terbutryne came up worldwide in the 1950s and are still in use outside the EU for weed control. From the 1980s Irgarol was also used as an antifoulant, mostly combined as a co-biocide. According to the EU Biocidal Products Directive Irgarol and terbutryne are also used as preservatives and in masonry.The occurrence of both s-triazines and the degradation product M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine, GS 26575) were measured in 208 water samples at 94 sites of German surface waters from 2005-2008 in cooperation with regional authorities.Sampling sites cover large streams like Elbe and Rhine as well as tributaries like Havel and Spree, backwaters, various channels, large lakes like 'lake Constance' and 'Müritz' as well as smaller lakes or lake-like enlargement of smaller rivers also addressed as lakes, and some creeks and ditches. Sampling stations were situated at hot spots like local pleasure boat marinas and landing stations, close to effluents of factories and sewage treatment plants, and at greater distance of these in the middle or at the outlet of lakes. Samples preparation and analyses were done by use of solid phase extraction (RP-ENV+) and GC-MS detection.Seasonal patterns were detected and local pollution sources identified. The origin of the metabolite M1 from Irgarol and terbutryne is discussed.

TU 042

Spatial and seasonal trends of organic pollutants in a small temporary Mediterranean river impacted by sewage effluents

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Contaminants such as alkylphenols, organotins or polycyclic aromatic hydrocarbons (PAHs) present in sewage effluent have to be monitored in surface waters and european union members are required to respect environmental quality standards (EQS). The temporary rivers, where many processes concerning contaminant behaviours remain poorly studied, provide thus a significant challenge in developing appropriate sampling strategy for their monitoring. Our study took place in the Vène River, a small intermittent coastal river located on the French Mediterranean coast. The objective of this work was to assess organic contamination of the Vène river for different hydrological conditions. Four spatial one-day sampling campaigns using key locations (i.e., sites located downstream and upstream point-pollution sources) were realized at different hydrological periods (the beginning and the end of the dry period and a period of high flow). At each station, grab water and sediment samples were collected. Alkylphenols, PAHs and polychlorobiphenyls were determined with chemical analyses in sediment and organotin in water and sediments. In addition to the organotin concentrations in water, nutrient and major ion concentrations and physico-chemical data were used for multivariate analysis such as principal component analysis (PCA) to study the spatial variations of water quality. Results of the PCA showed that sites downstream direct sewage effluents were strongly correlated with the first factor which was positively correlated with anthropic variables (nutrients and organotins) during low flow period but not for high flow period. For the temporal variations, the analyse of variance revealed significant differences for monobutyltin concentrations among the different hydrological periods ($p<0.05$) but not for dibutyltin and tributyltin (TBT) concentrations. However, large variations of TBT concentrations were observed among the different hydrological periods (from 0.11 to 0.72 ng/l during the high flow period and from 0.05 to 2.47 ng/l during the low flow period). For sediments, preliminary results showed that organic contaminant concentrations seemed more stable in time (i.e., from 0.05 to 0.23 µg/kg for TBT concentrations). The use of EQS as an annual average could be difficult in temporary rivers because of the large variations of contaminant concentrations observed among the different hydrological periods.

TU 043

Dicyclohexylamine: discovery of an environmental contaminant using in-silico screening tools

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Dicyclohexylamine has been discovered in water and sediments in the Stockholm region. Concentrations in water from the Stockholm Harbour region were in the range of 1-5 ng/L, while water from Lake Mälaren, a large lake to the west of the city, contained about 0.5 ng/L. The concentrations in a small lake with no discernable anthropogenic influence was <0.1 ng/L, which excludes long range atmospheric transport and deposition as the major source of contamination to the other water bodies. Dicyclohexylamine was also found in Stockholm drinking water at concentrations similar to those found in Lake Mälaren, which is Stockholm's major source of drinking water. Dicyclohexylamine is a high production volume chemical with a wide range of uses including in paints, as an antiseptic agent, and as a fuel additive. Which sources are mainly responsible for its emissions to the environment remains to be explored.
Dicyclohexylamine was discovered as a result of a chemical screening exercise. A model of chemical fate and bioaccumulation in the environment was used to screen a total of 12600 high- and low-production volume chemicals for their concentration in the environment and in top predators. An initial estimate of the total emissions of each chemical was made using production volume information and emission factors. Key physical chemical properties of each chemical were estimated using QSARs. For the chemicals with the highest predicted concentrations, a literature search was conducted and the models' predictions were closely scrutinized. From the chemicals which passed this test and which were not known environmental contaminants, several were selected. One of these was dicyclohexylamine.

TU 044

Persistent organic pollutants in farmed sea bass (Dicentrarchus labrax L.) in two different rearing systems in the Mediterranean Sea

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Persistent organic pollutants (POPs) are ubiquitous toxic compounds, highly persistent and bioaccumulative. Due to their chemical properties, POPs pose a risk of causing adverse effects to human health and environment. In this study we measured the concentration levels of polychlorobiphenyls (PCBs), perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in the edible part of farmed sea bass reared in two different farms (site 1 and site 2) in Liguria, a region situated in northern Italy. The analytical method was based on isotope dilution with 13C labeled internal standards, followed by instrumental analysis using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS) for the determination of PCBs, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of PFOS and PFOA. We analyzed 47 samples with the aim of establishing the contamination level and human exposure to these pollutants through fish consumption.
Concentration levels (mean ± standard deviation) of DL-PCBs were 0.39 ± 0.41 and 0.45 ± 0.40 pg ΣTEQ-PCB /g (f.w.) in site 1 and 2, respectively, whereas concentrations of the six indicators of NDL-PCBs were 8.66 ± 21.4 and 10.7 ± 8.27 ng Σ6 PCB /g (f.w.) in site 1 and 2, respectively. The concentration levels found in the farm located in site 1 were generally lower than those detected in the samples from site 2. Moreover, principal component analysis (PCA) showed differences also in the congeners profile between the two sites, since higher-chlorinated PCBs were more abundant in site 1 whereas lower-chlorinated PCBs were more abundant in site 2. Concerning PFOS and PFOA, most of the measured concentrations, especially of PFOA, were below the limit of detection (LOD = 0.05 ng/g f.w.). In particular, only about 10% of the samples analyzed showed levels slightly higher than the LOD value. A possible reason of these low levels, could be that these pollutants do not accumulate in lipids but mainly in liver and blood. Exposure assessment using these data showed that farmed fish consumption may contribute significantly to PCBs through diet, whereas for PFOS and PFOA such contribution seems to be low.

TU 045

Evaluation of the potential risk of contamination of the guarani aquifer in Santa Catarina, Brazil

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Of the total area of the Guarani Aquifer (1,195,500 km2), approximately 12.8% is represented by the zones of outcrop, i.e. 153,000 km2 (ANA, 2001), with 67.8% (104,000 km2) located in Brazil, 30.1% in Paraguay and 2.1% in Uruguay. The Guarani Aquifer, which consists of sandstones, is more vulnerable to contamination in its area of outcrop because this is an important site of direct recharge of the aquifer. In Ponte Alta , SC, Brazil, the Canoas riverbed overlaps areas of outcrop of the Guarani Aquifer. The aim of this study was to investigate the possible contamination of the aquifer Guarani and which sources. Possible changes in the degree of surface and groundwater pollution of the Guarani Aquifer and Canoas river were regularly monitored (four months) by analysis of the physicochemical and biological parameters. The results showed that the Canoas river in this area exhibited altered physicochemical characteristics, particularly regarding to total phenols concentration (0.3 ppm), sulfides (1.4 ppm) and nitrate (0.5 ppm) caused by the release of waste from a pulp and paper mill. Toxic effects were found for algae, daphnia and fish. Some samples of water from the aquifer exhibited altered concentrations of phenols and metals. It was noticed that extensive outcrop and recharge areas of the aquifer are busy with the planting of pine. The thickness of the sandstone in this area was quite variable, being the 100m thicknesses the most common.

TU 046

Status of environmental contamination indicated by multi-proxies around an important commercial port of South America (Paranaguá Harbor, Brazil)

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Paranaguá Harbor is the main South American grain shipping port, being the third most important of Brazil in loading and unloading operations with 8,540,000 ton (2010). It is located in Paranaguá Bay (25°30'S; 48°25'W) considered one of the most important estuarine complex at Brazilian coast. Its economical importance is related to fishing activities, urban and touristic exploration, besides industries that produce fertilizer compounds. Anthropogenic inputs include domestic discharges and sewage from the port, industries and population (around 126,076 people). In order to assess the contamination level of sediments in the area under human activities, the organic compounds (PAHs, aliphatic hydrocarbons and coprostanol) and metals were analyzed. Results of PAHs and metals were compared to two Sediments Quality Guidelines (SQGs), the ERL/ERM and the TEL/PEL values. The concentration of total PAHs (16 EPA) varied since 1.0 to 1630 $ng.g^{-1}$ and it did close the TEL level in one of eleven sites analyzed. Only 2 (18 %) of stations studied presented individual PAHs higher than TEL and ERL. According to the individual PAH isomer pair ratios, the main sources of these compounds are petroleum, biomass and coal combustion. Metals (As*, Cd, Cr, Cu, Ni, Pb and Zn) presented concentrations below TEL levels in all sites analyzed. Total aliphatic (including UCM) and corpostanol varied, respectively, since 0.2 to 2221 $\mu g.g^{-1}$ and < LD to 16 $\mu g.g^{-1}$, showing contamination by oil hydrocarbons and sewage in three sites analyzed (27 %). The contamination level of sediments around Paranaguá Harbor and city could be considered high in sites very close the main human activities. Levels decreased in 10-100 times 1km far from the main sources of pollution. Given that this environment may be relatively unpolluted, the monitoring and bioaccumulation of contaminants in this ecosystem should be avoided.

TU 047

Historical accumulation of polycyclic aromatic hydrocarbons (PAHs) in important economic area of a large South American tropical estuary (Paranaguá Estuarine System, Brazil)

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Polycyclic aromatic hydrocarbons are organic pollutants prevalent in the sediments of marine and freshwater environments. They are mainly derived from anthropogenic sources including the combustion of fossil fuels (oil and coal) and biomass, sewage, vehicular emissions and spillages of petroleum. The aims of this study were to describe the temporal distribution and sources of PAHs in sediment cores collected in three different sectors of Paranaguá Bay, where human activities have intensified over the last 100 years, and to assess the historical record of local anthropogenic material input as result of the influence of human occupation in this area. The Paranaguá Estuarine System is one of the most important estuarine environments of the South American coast where fishing, urban and tourist activities, industries and the main Brazilian grain shipping port are potential sources of PAHs in this area. Sediment cores (50 cm depth) were taken by scuba diver from sites P1, P2 and P3 during September, 2008. The cores were sectioned at 2 cm intervals and PAHs were determined by gas chromatograph with a mass spectrometer after Soxhlet extraction and purification by column chromatography. In general, the sources of PAHs were fossil fuels combustion, industrial and domestic residues, introduction of petroleum by boats and ships, charcoal and biomass combustion. The parameters involving different PAHs isomers indicated that the majority sources of PAHs as related to pyrolitic processes. In the site P3, the PAHs were associated to multiples sources, and the high concentration of PAHs in intermediate layers, related with the dredging activities realized in adjacent areas of this site. Natural sources of perylene for the environment were verified, associated to input by terrigenous precursors. The concentration of PAHs varied between 1.72 and 168.5 $ng.g^{-1}$, however the individual concentration did not exceed the limits TEL and PEL, established by EPA, with an exception of acenaphthylene, found with values slightly higher compared to TEL. The comparison of total PAHs values with another studies in different locations around the world, suggest that the Paranaguá Estuary Complex, even though the urban and industrial development have been increased, it is still a local area that has not yet suffered a relative significant impact related to input of organic contaminants analyzed in this study.

TU 048

Biomonitoring of polycyclic aromatic hydrocarbons (PAHs) in eastern Baltic Sea: effects on flounder (Platichthys flesus) and eelpout (Zoarces viviparus)

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Eastern Baltic Sea near Estonian coast is heavily navigated sea by numerous cargo ships and oil tankers. Hundreds of accidents and oil spills happen in this area every year. Still there is a lack of data about distribution of polycyclic aromatic hydrocarbons in enninronment. In case of major accident there is no supplementary data about area's background level. Starting from 2006 we have measured PAH metabolites, enzyme activities (EROD), parasites and other biomarkers from European flounder (Platichthys flesus) and eelpout (Zoarces viviparus). A wide range of monitoring stations in coastal areas in Gulf of Finland and Gulf of Riga were chosen. The aim of our research was to specify the distribution of PAHs in a different sea areas, as well as evaluate the physiologycal effect of pollutants in two species of fish. The results showed that fish from the Gulf of Riga had lower levels of PAH metabolites, suffered less by parasites and had better liver somatic index (LSI) and body mass index (CF) than fish close to the Baltic proper and in the Gulf of Findland. This might be due to major shipping routes close to coastal areas of North-West of Estonia. The relationships between biomarker values and study areas are discussed.

TU 049

Pesticides and polychlorinated biphenyls in bowhead whale blubber layers

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Bowhead whale blubber samples (n = 36) were collected during the 1996ð'2001 native Inuit subsistence harvests in the arctic marine environment of Barrow and Kaktovik, Alaska. Full depth blubber samples were available for 18 of the bowhead whales. Five depth layers each ~8-13 cm thick were available for most of the bowheads. Blubber layers (~ 1.5 g) were examined for historic and current-use pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). Contaminants were simultaneously extracted and isolated using pressurized liquid extraction combined with silica gel utilizing an accelerated solvent extractor. Silica gel was used to remove the more polar interferences and was present in the ASE cell during the extraction. Larger-molecular weight interferences, such as lipids, were removed using gel permeation chromatography. Contaminants were analyzed for using gas chromatography-mass spectrometry with electron capture negative ionization. The method was validated by a triplicate spike and recovery experiment. Using isotopically-labeled surrogates, the average percent recovery was 103% with a relative standard deviation of 9.4%. The precision and accuracy of the analytical method was validated in triplicate using NIST standard reference material 1945 for organics in whale blubber. The average absolute percent difference and standard deviation were 21% and 12%, respectively. Generally, pesticides and PCBs were detected in all blubber layers, and PBDEs were only detected in a single layer. Pesticide, PCBs, and PBDEs in blubber layers were analyzed to determine their trends in individual Bowhead whale blubber.

TU 050

Analysis of glyphosate and AMPA in several matrixes using SPE-LC-MS/MS and their environmental fate in two calcareous vineyard parcels of western Switzerland

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Glyphosate [N-(phosphonomethyl)glycine] is a broad spectrum herbicide largely used in Switzerland for weed and vegetation control representing 30% of all herbicides sales in 2005. As it shows strong sorption ability and a relatively low persistence in soils, it is often assumed to not reach water bodies. Two vineyard parcels of a small catchment were investigated in order to study glyphosate transfer within soils and surface waters. Based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), the analysis of glyphosate and its main degradation product aminomethylphosphonique acid (AMPA) has been set up. The method is based on derivatization with 9-fluorenymethylchloroformate (FMOC-Cl) and their concentration by solid phase extraction (SPE). Spiking tests in several matrixes revealed satisfying LOQ and recovery rates for Evian© bottled water, river water and soil solutions, as well as for calcareous soil samples. The parcels were equipped with lysimeters and runoff collectors in order to get soil solution at 4 different depths, as well as runoff water. River water was sampled together with drainage water of several vineyards. Despite its assumed low mobility, glyphosate was found to be leached and run off from the soil surface layer. The role of preferential subsurface pathways was studied, revealing the importance of textural boundaries between the more clayey deep and the silty surface layers in the transport of these compounds. Moreover, runoff water was highly loaded after rain events and glyphosate and AMPA turned out to be transported mainly by small colloids: the median of 'dissolved' fractions (<0.45µm) is located between 70 and 80% of the total concentration. The adjacent river showed concentration peaks over the 0.1µg/l threshold limit during main rain events, revealing the transfer of these compounds from the field to surface water. The highest peak had a concentration of 0.8 µg/l of glyphosate in August. Moreover, drainage systems showed much higher concentrations of up to 4 µg/l, revealing the importance of artificial channels within the urbanised landscape in the transfer of these compounds. These results give a better understanding of the environmental behaviour of glyphosate, which is largely used in viticulture and agriculture in Switzerland, due partly to the increase of simplified tillage practices.

TU 051

PAHs, PCBs and chlorinated pesticides in sediments and fish species from Milazzo (South Italy) marine area

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Marine sediments are usually regarded as the ultimate sink for persistent pollutants discharged into the environment. The aim of the present study is to determine the levels of several organic pollutants (PCBs, PAHs, DDTs, HCHs and HCB) in order to assess the contamination in Milazzo (South Italy) marine area. The distribution of pollutants of environmental interest was investigated also in fish (*Serranus scriba, Serranus cabrilla* and *Serranus hepatus*) collected from Milazzo marine area. Sediment and fish samples were lyophilized and extracted for organic contaminants with accelerated extractor (ASE) system and quantified by GC-MS and HPLC. Muddy sediments with high organic matter content dominate the study area. Total PAH concentrations ranged LOD-211.17 ng/g dry weight indicating low to moderate PAH pollution. The comparative evaluation of PAHs with low/high molecular weight and isomeric ratios suggest that the pollution source is pyrolytic and that terrigenous flows do not affect the PAH balance of the marine area. Organochlorine compounds levels indicate that sediments are not significantly polluted by HCH (max mean value of total HCHs = 8.52±8.17 ng/g d.w.), HCB (max mean value of HCB = 1.85±1.55ng/g d.w..), DDT and its metabolites (max mean value of total DDTs = 30.57±3 ng/g d.w.) and PCBs (max mean value of total PCBs = 93.60±92.78 n/g d.w.). The identification of organic contaminants sources in the Milazzo marine area ecosystem could be useful to plan the polluted sediments management activities and the restoration strategies. The organic pollutants concentrations indicate a low to moderate pollution level with an heterogeneous distribution in

the marine area. The maximum mean level of total PAH was measured in liver of *Serranus hepatus* (6.04±1.54 ng/g w.w.), while organochlorines were never detected in this species.

TU 052

Distribution and partitioning of polybrominated diphenyl ethers in East River, South China

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Distribution and Partitioning of Polybrominated Diphenyl Ethers in East River, South China
The distributions and partitionings of polybrominated diphenyl ethers (PBDEs) in water columns from the Dongjiang River were investigated in the present study. Water samples were taken in Shilong Section of Dongjiang River, South of China, during four intensive sampling campaigns in 2008 and 2009. ∑14PBDEs (BDE28, 47, 66, 100, 99, 85, 154, 153, 138, 183, 206, 207, 208, and BDE209) were analyzed . The levels of ∑14PBDEs were 0.04-2.69 ng/L, whereas those of BDE47, 99, 153 and 209 were 0.01-0.09 ng/L, 0.01-0.10ng/L, 0.001-0.11ng/L and 0.009-2.05 ng/L, respectively. BDE47, 99 153 and BDE209 were detectable in all dissolved and particle samples, which contributed higher than 80.5% of total ∑14PBDEs. The PBDEs concentrations in water samples were lower in dry season than in flood season. SPM could play certain roles in determining the distribution and partition of PBDEs between particle and dissolved phases, but their effects varied with the water properties. Calculated values of the organic carbon-water partition coefficient (KOC) were strongly correlated with literature values of the octanol-water partition coefficient (KOW). The data suggest that sorption of BDEs to colloids is important in this system, although quantifying the extent of colloid sorption is difficult.

TU 053

Evaluating daily PCB exposures to children from dietary supplements containing fish oils

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Children's dietary supplements containing fish oils have become a popular means of increasing children's intake of polyunsaturated fatty acids (PUFAs). However, there is growing concern that the levels and potential health effects of lipophilic organic contaminants such as polychlorinated biphenyls (PCBs) may diminish some of the health benefits associated with daily consumption of fish oil supplements. In this study, thirteen over-the-counter fish oil supplements available in the United States were analyzed for PCBs and daily exposures were calculated. Concentrations of ∑-PCBs ranged from 0.4 to 20.0 ng/g wet wgt and were low compared to our recent study of adult fish oil supplements (3.1 to 264 ng/g wet wgt). Based on manufacturers' recommended dosages, a child's daily intake of PCBs would range from 2.8 to 50.3 ng/day. Daily consumption of fish oil supplements expose children to PCBs, albeit at low levels. However, in comparison to ingestion of fresh fish to gain equivalent amounts of PUFAs contained in supplements, children's fish oil dietary products may decrease daily PCB exposure.

TU 054

Use of innovative technology for analysis of 54 VOCs in water: ITEX solution

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Contamination of aquifers by one or more volatile organic compounds (VOCs) is a national issue of potential concern because of the widespread and long-term use of many of these compounds. Many (VOCs) are toxic and some are considered to be carcinogenic, mutagenic, and teratogenic. Contamination also depends on the locations and types of VOC sources, the relative locations of wells, and the transport and fate of VOCs. The occurrence of low-level contamination of one or more VOCs in an aquifer also can provide managers with an early indication of the presence of VOCs that eventually might adversely affect the quality of water
Considering the high variability of VOCs concentration in groundwater especially in the case of industrial impacted groundwater, VOCs analysis is an important and sensitive point for understanding behaviour, attenuation or determining sources of the pollution.
Rapid sensitive and robust analysis of 54 VOCs including, BTEX and chlorinated solvents was developed using In Tube Extraction (ITEX) combined with GCMS for analysis in groundwater, effluent and surface waters. The study presents optimization of the method, validation on several matrices (occurrence of suspended, matter, organic matter content). Validations, considering potential trap needle saturation process and matrix effects were undertaken, highlighting robustness of the ITEX technology.
Detection limits ranged from 0.25 µg/L to 2 µg/L were validated with recoveries ranged from 85% to 103% depending on the studied compounds. Applications for the monitoring of VOCs in polluted areas will be presented showing the efficiency and accuracy of ITEX use and the obtaining of robust data concerning degradation of VOCs and natural attenuation for some of them.

TU 055

Chemo- and bioanalyses of the partitioning of radiolabelled organic chemicals in sediment-water-organism-systems

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Sediments can act as a sink as well as a source for environmental pollutants. A multitude of factors can influence this dual quality and the related partitioning between organisms as potentially affected targets, water and sediment. Those factors include sediment properties, water quality, the type of organism and substance characteristics. Direct sediment-contact tests are excellent tools to assess these complex systems. In these whole-sediment toxicity tests, the test species are introduced directly onto or into the sediment. Aim of the present project was to investigate the influence of short-term ageing on the partitioning of organic pollutants in a system comprising sediment, water and organisms.
Zebrafish (Danio rerio) embryos and the sediment dwelling worm Lumbriculus variegatus were used as model organisms. Two natural sediments (Altrip, back water of the Rhine and Höytiäinen, lake in eastern Finland) as well as an artificial sediment (OECD 218) were used, each spiked with pentachlorophenol (PCP) and/or fluoranthene (FA). Concentrations of labeled chemicals were below lethal effect levels for the bioaccumulation study (10000 dpm/g dw sediment), whereas in the ageing study with non-labeled PCP, 15 mg/kg dry weight sediment were applied. In the L. variegatus test, scintillation counting was carried out in order to quantify the radioactivity in the different test compartments after 14 days of exposure after ageing the sediment for 5 and 18-21 days, respectively. In addition to accumulated concentrations, bioaccumulation factors were determined after 14 days of exposure. The steady state of bioaccumulation was not reached within 14 days, however, one short-term ageing effect became apparent. For PCP, bioaccumulation could be shown in both sediments. A twofold ageing-related decrease in accumulation was found in the natural sediment. On the contrary, no bioaccumulation of FA was observed independent on the aging period of 5 or 18-21 days.
Furthermore, zebrafish samples were analysed at different time-points after the initial spiking to obtain data on ageing-dependent changes in observable effect. As expected, ageing natural sediments for 6 weeks and artificial sediments for 9 weeks resulted in a complete loss of effects for both chemicals.
In conclusion short-term ageing effects within only 3 to 6 weeks of ageing were uncovered in the two experiments. This clearly shows the need to account for ageing when assessing results of direct sediment contact tests.

TU 056

Trophic magnification of poly- and per-fluorinated compounds and determination of known and unknown organic fluorine content in biological samples from Mai Po Marshes Nature Reserve, Hong Kong

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Perfluorinated compounds (PFCs) have been manufactured and widely used for various applications for over 60 years. Concern about PFCs has been growing since the 1990s because of their toxicities, environmental persistence, and ubiquitous occurrence in wildlife and human populations. Several field studies from the Canadian and European Arctic and tropical regions have indicated that PFCs biomagnify in aquatic food webs, especially PFOS and some long-chain perfluorocarboxylates (PFCAs). However, no PFC bioaccumulation studies in the sub-tropical region have been reported thus far. In the present study, bioaccumulation of PFCs was investigated in a food web composed of phytoplankton (p=3), zooplankton (p=2), gastropods (p=3), worms (p=10), shrimps (p=4), fishes (n=21), and waterbirds (PFCs analyzed in the livers of grey herons (*Ardea cinerea*) and Chinese pond herons (*Ardeola bacchus*); n=6) sampled from a tidal shrimp pond in the Mai Po Marshes Nature Reserve in Hong Kong. Biota samples were generally extracted by the ion-pair method, followed by ENVI-Carb and SPE OASIS-WAX cleanup. Individual PFCs were quantified using HPLC-MS/MS. Trophic magnification was observed for PFOS, PFDA, PFUnDA, and PFDoDA and the trophic magnification factor (TMF) values ranged from 1.30-1.75. PFUnDA (TMF=1.75) was found to have the greatest TMF. In addition to known PFCs, recent studies demonstrated the presence of large amounts of unidentified organofluorines in wildlife. In the present study, extractable organic fluorine (EOF) (based on the ion-pair method) and total fluorine (TF) in the samples were analyzed using refined combustion ion chromatography for fluorine (CIC-F). A large proportion of inorganic fluorine and non-extractable organic fluorine (around 99%) were found in lower trophic organisms (i.e., gastropod, worm and shrimp) when compared to fish liver (57-71%). Relatively similar EOF contents were found in all samples. The contribution of known PFCs to EOF increased significantly with increasing trophic levels (i.e., fish liver (41-76%) > shrimp (10-12%) > worm (0.5-3.7%) > soft tissue of gastropod (0.4%)). The small PFC-to-EOF ratios found in lower trophic organisms might be due to differences in the exposure level, rate of uptake and elimination of PFCs in different species.

TU 057

Transport of particle-bound pollutants by urban runoff to the bottom of boreal Lake Vesijärvi

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Urban runoff is known to be contaminated with a range of hydrophobic organic compounds (HOCs), which are usually bound to particulate matter and therefore settle to the sediments. Polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) are among the best known compounds. They are recognized as priority pollutants, often toxic to aquatic life and known to have urban origin. The city of Lahti has approximately 100 000 inhabitants and is a growing Finnish urban area. It abuts on Lake Vesijärvi, which is an important provider of recreational and fishing activities in the region. Most of urban runoff in Lahti discharged directly to Lake Vesijärvi. However, knowledge on the amount and fate of HOCs in urban runoff is insufficient. In previous studies PAH and PCB compounds were found in Lake Vesijärvi sediments and their concentrations tended to decrease with the distance from urban shore and stormwater outlets which could be possibly caused by the impact of urban surface runoff.
Present study aims to evaluate concentrations of HOCs in sediments from stormwater traps and possibly link them to concentrations in the Lake Vesijärvi. Grab sediment samples were taken from stormwater traps at 15 locations in areas with various degrees of urbanization (urban, mid-urban and suburban). Samples were analyzed for 16 PAHs (EPA) and 28 PCBs using isotope dilution technique and gas chromatography-mass spectrometry (GC-MS) method. Concentrations of both PAHs and PCBs varied greatly with different sampling locations but were of similar level with concentrations found in Lake Vesijärvi. Local factors (bigger road or parking lot; smaller road or parking lot; side walk or green area) tended to have more significant effect on PAH than PCB concentrations, while general urbanization level seemed to impact distribution of PCBs stronger than PAHs. Pollutant profiles in different study areas were also compared.

TU 058

Distribution and seasonal variation of organic pollutants in surface marine sediments in the Mar Menor Lagoon (SE SPAIN): Flood impact

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Mar Menor is a hypersaline (42-47 psu) coastal lagoon with a mean depth between 3-4 m and the maximum depth is over 6 m. It is one of the largest lagoons of the Mediterranean Sea located in the Cartagena Field area at the South East of Spain. This semi-arid area is subject to intensive agriculture, phreatic level rise, recreational activities and sporadic torrential rainfall regime. In this study we have characterized the distribution of PAH´s, PCB´s, and pesticides, before (Spring 2009) and after (Autumn 2009) two flood events. The aims of this study were: a) to characterize the distribution of organic pollutants in sediments from Mar Menor Lagoon, b) to evaluate the seasonal variation of organic pollutants levels in sediments and c) to estimate the input and the impact of floods events on the distribution of organic pollutants in sediment.

PAHs and organochlorinated pollutants were extracted using a Soxhlet apparatus and were analyzed by HPLC for PAHs and GC-MS for PCBs and organochlorinated pesticides.The rest of pollutants (triazines and organophosphorus pesticides) were extracted using sonication extraction and were analyzed by GC-MS. Organic pollutant concentrations were also determined in water and suspended particulate matter during the flood event. These water extractions were performed using Stir Bar Sorption Extraction (SBSE) and thermal desorption coupled to GC-MS. The distribution of PAHs, PCBs and pesticides in the Mar Menor lagoon on Spring 2009 was heterogeneous. The three target groups of pollutants did not show the same pattern. The highest PAHs concentrations were detected in the northern and southern areas, particularly in the south(≈ 230 ng/g). The distribution of PCBs showed the highest concentrations in the central, and particularly in the south area. In general, the organophosphorus and organonitrogenated pesticides concentrations were lower than PAHs ones. During flood events several water and suspended solid matter samples were taken in the Albujón Watercourse mouth to estimate the organic pollutant input in these rare events. These water samples were analyzed by SBSE/GC/MS using full scan mode. As a result of the massive influx of water and suspended materials during the flood events especially through Albujón Wadi, the distribution in the sediment of all the contaminants groups studied was heterogeneous, and significant seasonally differences were observed in the organic pollutants concentrations associated to flood impact.

TU 059

Bioaccumulation of mercury in birds: a comparative study in Ciconiiformes, Charadriiformes and Pelecaniformes

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One of the most widespread pollutants threatening both wildlife and human heath, mercury (Hg) occurs in a wide range of forms, both organic and inorganic, being methylmercury (organic) the most stable and toxic form. Aquatic birds, as top predators, show high mercury levels due to biomagnification of methylmercury through the food webs and are therefore good indicators of mercury contamination in aquatic ecosystems.

The main aim of this study was to monitor mercury exposure in different species of birds from the orders Ciconiiformes, Charadriiformes and Pelecaniformes, which show different feeding preferences. To achieve that, liver samples from necropsied animals were lyophilized and then Hg was measured in triplicate by atomic absorption spectrometry (ASS) with thermal decomposition. Data obtained reported high levels of mercury burdens accumulated in almost all species. Significant differences in the burdens of mercury accumulated were also reported, being mercury levels higher in species with a fish-selective diet than in species with a generalist diet. This corroborates the hypothesis that the main source of mercury contamination in top predators such as birds (and in parallel men) is through uptake of contaminated fish. Even though some mercury levels reported seemed to be high enough to induce sub-lethal or lethal effects on the individuals, it was not possible to establish any correlations as these values are difficult to interpret without data associating tissue and exposure levels.

TU 060

Relationship between pollutant accumulation and toxic potency in deep sediments collected in the Seine estuary (Normandy, France)

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Abstract
Aquatic sediments are well known sinks for numerous persitent pollutants such as POPs and heavy metals. Pollutants accumulated in sediments can be partially bioavailable and may represent a threat for aquatic ecosystems.
In order to study time-course of pollution in the Seine estuary and also toxicity of accumulated pollutants in bottom sediments, a 110 cm-depth sediment core was sampled in the Seine estuary in a disused dock in Rouen harbor in april 2008 (Rhapsodis project). The core was splited in twenty 5 cm-thick slices which were analyzed for size grain, organic carbon, sulfurs, metals (13 elements), PAHs and persitent organic pollutants (PBDEs, OCPs, PCBs). Toxicity of sediment organic extracts was measured by using a large panel of in vitro toxicity assay : Microtox*, ER and DR-Calux*, SOS Chromotest and AChE inhibtion assay.
For all analyzed chemicals except Be, Mo and Cd a clear increase of pollutant concentrations was observed with depth. Strinkingly, peak of concentration for various polluants (PCBs, PAHs, sulfured PAHs, OCPs, Hg and Pb) was observed at 92.5cm depth. All sediment extracts exhibited high toxic potency. Acute toxicity (Microtox*) was shown to increase with depth. DR-Calux* activity (dioxine-like activity) was also increasing with depth while ER-Calux* activity related to estrogen-like compounds was globally low. AChE inhibition activity and genotoxicity (SOS-Chromotest) were mainly measured in surface and sub-surface sediments.
This study brings first evidences of global pollution decline in the Seine estuary during the last three or four decades notably for heavy metals, PCBs, OCPs and PAHs. It also highlighted the high toxic potency of accumulated pollutants and putative risk they could represent for aquatic organisms living in the Seine estuary.
This study was supported by the Seine-Aval program.

TU 061

Bioaccumulation of organochlorine pesticides and biomarker responses in Dreissena polymorpha and Dreissena bugensis after exposure to native suspended particulate matter

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The zebra mussel, Dreissena polymorpha, is widely used as sentinel organism for the assessment of environmental contamination in freshwater environments. In Germany, it is collected e.g. for regular chemical monitoring and for long-term storage of samples for retrospective research in the frame of the German environmental specimen bank (ESB). However, in the river Rhine the D. polymorpha population is declining while the closely related quagga mussel Dreissena bugensis is found in high numbers at some sampling locations. In the present laboratory study, D. polymorpha and D. bugensis were exposed to natural sediments for up to two weeks. Wet sediment (< 63 µm, 100 mg L-1 dry weight) was simulated as suspended particulate matter to mimic the mussels' main uptake route for chemicals. The sediments were sampled in a) the river Elbe in Dessau, a site known to be highly polluted with e.g. organochlorine pesticides, and b) in Havelberg in the river Havel, one of the Elbe's tributaries and a relatively unpolluted site.
Chemical analysis of organochlorine pesticides (7 PCBs, DDT and metabolites, HCHs, HCB) in soft tissue of mussels showed significantly higher values of PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, the sum of seven PCBs as well as p,p'-DDD in D. bugensis compared to D. polymorpha. 14 days of exposure increased the concentration of p,p'-DDE and p,p'-DDD in both species. Furthermore, exposure to the Dessau sediment resulted in elevated levels of p,p'-DDD in mussel tissue compared to Havelberg sediment. Interspecific differences were less pronounced when regarding chemical concentrations to lipid content instead of dry weight of tissue since D. bugensis had higher levels of total lipid than D. polymorpha.
DNA damage in gills, measured with the COMET assay, was higher in D. bugensis compared to D. polymorpha. Simultaneously, the content of heat shock protein (Hsp70) in gills was higher in D. polymorpha than in D. bugensis. DNA damage and Hsp70 were not induced by neither exposure time nor sediment type. Protein carbonylation in gills and soft tissue, however, showed no difference between species and exposure time, but in soft tissue it was slightly elevated in mussels exposed to the Havelberg sediment.

TU 062

Dioxin-like activity of sediments of the Elbe River and associated flood areas

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The aim of the present study was the determination of cytotoxic hazard potential and dioxin-like activity of sediments from the riverbed of the Elbe River and of associated flood areas. Of peculiar interest were industrial locations with high contamination. Soil samples from the flood areas of the Elbe River were taken in the year 2003, one year after the flood disaster. The riverbed samples originate from the river head down to the estuary and were sampled in the year 2008, after the flood. Freeze-dried sediments were extracted with n-hexane:acetone (1:1, v/v) by means of pressurized liquid extraction (PLE). The Neutral red assay on cytotoxicity and the EROD (7-ethoxy-resorufin-o-deethylase) assay on dioxin-like activity were then applied with the resulting extracts using the RTL-W1 permanent cell line. Within this study the main focus was set on polychlorinated biphenyles (PCBs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/Fs), which interact with the aryl hydrocarbon receptor (AhR) and cause EROD induction. Moreover, in order to identify whether PAHs, PCDDs/Fs or PCBs cause the strongest effects in the EROD assay, a multilayer fractionation was performed. Finally, results of the bioassays were compared with available analytical data to evaluate the significance of the used in-vitro bioassays.

TU 063

Monitoring of glyphosate residues in environmental groundwater samples by enzyme-linked immunosorbent assay (ELISA) and LC-MS/MS

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TU 064

Accessibility and chemical activities of PAHs and PCBs in anthropogenic impacted sediment cores of the main sedimentation basins of the Baltic Sea

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Many polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been classified as "Priority Pollutants"by the Stockholm Convention on Persistant Organic Pollutants (POPs), due to their ubiquity, persistence, bioaccumulation carcinogenic or mutagenetic potentials. The distribution of POPs between pore water and sediment affect their fate, transport and ecotoxicological risks in the marine environment. To date, there are only a few limited studies that have used field data to give information about the direction of the diffusive mass transfer, which always takes place from areas of high to low chemical activity. A very important question remains. Does the sediment act as a diffusive source or as a diffusive sink for the POPs in the water column? This question calls for the determination of the chemical activity of POPs in the pore water, which can be measured by equilibrium sampling techniques. To calculate chemical activity, freely dissolved concentrations of PAHs and PCBs were measured in the pore water of sediment samples using solid-phase microextraction, a cost- and time-efficient method with detection limits in the lower nanogram per liter range. Additionally, total sediment concentrations were measured in sediments to calculate the distribution of contaminants between the two compartments. Sediment-pore water partitioning of POPs was studied in sediment cores of the main sedimentation basins of the Baltic Sea. Chemical activities of the POPs in the sediment cores were used to predict the regional baseline toxic potential of the contaminant mixture. Finally, gradients in Cfree and chemical activity were used to determine the direction of diffusion within the sediment and to obtain a spatial characterization of pollutant exposure. Cfree and chemical activity are important exposure parameters for the prediction of bioconcentration and toxicity in sediment organisms, and their measurement should be included in risk-assessment and pollution-management strategies.

TU 065

Photo-transformation of AhR-active hydrophobic organic compounds in presence of humic substances

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Hydrophobic organic compounds (HOCs), frequent anthropogenic environmental pollutants, can cause many adverse effects in organisms. Activation of arylhydrocarbon receptor (AhR) can be very important mechanism of toxicity for so-called AhR-active HOCs, such as benzo[a] pyrene (B[a]P), dibenz[a,h]anthracene (DB[a,h]A), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Humic substances (HS), being naturally occurring compounds (up to 50 mg/L in waters), can occur together with HOCs in contaminated water. Beside other important ecological properties, HS serves as a natural source of reactive oxygen species that are formed after HS irradiation. Direct photolysis of HOCs is a very important way of their degradation in the aquatic environment. Nevertheless, oxygen species, formed after HS irradiation, can enhance the photochemical degradation of HOCs. In present study, we have assessed the ability of various HS to enhance photo-degradation of upper mentioned HOCs, and its mixture. Aqueous HOCs+HS solutions were irradiated by low/pressure mercury lamp in Pyrex tubes (retaining UV radiation <300 nm). Photo-degradation of HOCs, studied by chemical analysis using GC/MS(MS), was observed, where HOCs in mixtures with HS were photo-degraded more rapidly. To evaluate whether any new AhR-active compounds has been formed as a products of HOCs photo-transformation, AhR-mediated toxicity of complex samples was assessed using *in vitro* assay based on the H4IIE-*luc* transgenic cell line. Obtained results have been in good accordance with results from chemical analysis. Supported by projects GACR 525/08/P464 and CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 066

Equilibrium sampling of environmental pollutants in fish - Comparison with lipid-normalized concentrations and homogenization effects on chemical activity

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Equilibrium sampling of hydrophobic organic pollutants into the silicone polydimethylsiloxane (PDMS) has been used in numerous environmental matrices and has recently been applied in lipid-rich biological tissues [1, 2] including fish. The concentrations of chemicals in the polymer (C_{PDMS}) can then be multiplied with lipid/PDMS distribution ratios ($D_{Lipid,PDMS}$ [3]) according to eq. 1 to obtain concentrations in fish lipids ($C_{Fish,lipid,equilibrium}$):

$C_{Fish,lipid,equilibrium} = C_{PDMS} * D_{Lipid,PDMS}$ (1)

In this study, PDMS thin-films [4] were placed in intact tissue of two eels and one salmon for equilibrium sampling of polychlorinated biphenyls (PCBs). A 'classical' exhaustive extraction technique to determine lipid-normalized PCB concentrations, which assigns the body burden of the chemical to the lipid fraction of the fish, was additionally applied. Lipid-based PCB concentrations obtained by equilibrium sampling were in good agreement with those determined using total extraction. These results support the validity of the equilibrium sampling technique, while at the same time confirming that the fugacity capacity of these lipid-rich tissues for PCBs was dominated by the lipid fraction.

Further, we provide equilibrium sampling data obtained in homogenates of the same fish tissues. The PCB concentrations were 1.2-2.0 times higher in the PDMS immersed in homogenized tissues as compared to PDMS immersed in intact tissues, indicating that homogenization increased the chemical activity of the PCBs and decreased the fugacity capacity of the tissue.

On the basis of the presented data, we describe a novel approach to confirm that (a) the equilibrium sampling technique and (b) the widely used environmental monitoring approach of assigning the body burden of pollutants to the organism's lipid fraction are valid in lipid-rich biota. Further, we provide experimental evidence of reduced fugacity capacity of homogenized tissue as compared to intact tissue. This observation may have consequences for (1) equilibrium sampling, (2) physiologically based pharmacokinetic models since these are often based on tissue/air and tissue/water partition coefficients determined using tissue homogenates and (3) the mechanisms of dietary absorption of organic contaminants.

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TU 067

Bioaccumulation of micropollutants in an estuarine food chain

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Due to anthropogenic activities trace metals and organic micropollutants have been introduced in the aquatic ecosystem where they can accumulate and persist for many years. Those pollutants can become available for uptake by organisms and cause toxic effects. Persistent pollutants have the ability to be transferred through food chains and even become a threat for human health. Bioaccumulation of chemical substances depends on two main factors. First, pollutants must be available for uptake by organisms. This bioavailability is strongly influenced by the chemical properties of the pollutant, by several physical and chemical processes and by environmental characteristics of the surrounding water and sediment, which can be highly variable in certain environmental systems like estuaries. These characteristics determine the chemical speciation and distribution of pollutants over the environmental compartments.

Secondly, bioaccumulation is influenced by the exposure route. Which exposure route is important for an aquatic organism is highly dependent on its physiology, habitat preference and feeding strategy.

This project aims to determine the exposure route most responsible for bioaccumulation in organisms with different feeding strategies and trophic level, and this for various pollutants. Therefore we conducted passive as well as active biomonitoring studies in the Scheldt estuary. We related accumulated levels of micropollutants (trace metals, organic pollutants) in caged and free living animals to levels in environmental compartments (water, SPM, pore water, sediment).

TU 068

Bioaccumulation and trophodynamics of emerging contaminants in tropical mangrove food webs

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Tropical mangroves are important ecosystems in Southeast Asia, as well as other tropical and subtropical regions of the world. These unique ecosystems provide essential habitat for numerous species of aquatic and terrestrial organisms. In 2004, field measurements in Singapore have demonstrated legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethanes (DDTs) and chlordanes (CHL) exhibit a high degree of biomagnification in local mangrove food webs. The objectives of the present study were conduct a field study to assess the occurrence, levels and bioaccumulation behavior of various emerging contaminants of concern in Singapore mangrove food webs. Target analytes included a comprehensive list of pharmaceuticals and personal care products (PPCPs), current-use pesticides (CUPs) and brominated flame retardants (BFRs). A comprehensive field survey of surface waters, sediments and mangrove organisms, including various species of algae, plankton, mollusks, crabs and fish, was conducted at several mangrove sites around Singapore. Water samples were filtered/extracted using a filtration apparatus containing glass fibre filters and solid-phase extraction (SPE) disks in order to determine freely dissolved and particulate bound chemical concentrations. Sediments and tissue samples were extracted using accelerated solvent extraction (ASE) and further cleaned-up using gel permeation chromatography (GPC), SPE and/or Florisil chromatography. Identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) or gas chromatography mass spectrometry (GC-MS). 13C or deuterated mass-labeled standards were used as internal standards to correct for matrix effects. Measured concentrations and observed trophic magnification factors (TMFs) of the various emerging contaminants of concern are compared to observations of legacy POPs. The bioaccumulation behavior and overall biomagnification potential of these contaminants in the mangrove food webs are evaluated and discussed.

TU 069

Accumulation of butyltin compounds in minke whales and long-beaked common dolphins from the Korean coast

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Butyltins (BTs) including mono- to tri-butyltins (MBT, DBT and TBT) were determined in the liver of minke whales (Balaenoptera acutorostrata) and long-baeked common dolphins (Delphinus capensis) caught off the Korean coast. Total concentrations of BTs ranged from 15.7 to 297 ng/g on a wet weight basis (wet wt) in minke whales and from 59.0 to 412 ng/g wet wt in long-baeked common dolphins. DBT was the predominant BT in the liver, contributing to 63% of total BTs. Significant species-specific difference in concentrations and composition of BTs was found between minke whales and common dolphins. A gender difference in BT residues between male and female common dolphins was not observed. However, concentrations of BTs in minke whales increased with body length. The overall BT levels in cetaceans from Korean coastal waters were lower than those reported worldwide, and the levels were consistent with BT contamination in the Korean coast.

EC07 - Integrated chemical and biological approaches for toxicant identification

TU 074

Estimating metal bioavailability in sediment by DGT for avoiding overdosing of chelatants in TIE protocols

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The protocol for Sediment Toxicity Identification Evaluation ("sediment TIE") recommended by the US-EPA proposes generic quantities of resin to be added to sediments for adsorption of different compound classes and then toxicity reduction. However, the overdosing of a given chelating resin aiming at specifically binding divalent metals may lead to misinterpretation of observable toxicity reduction as its organic polymer matrix is also able to interact with organic toxicants. The aim of the current work is to better understand the effect of chelatants on the reduction of the metal bioavailable fraction and thus to optimize the quantity of chelating resin to be added according to sediment physico-chemical properties.

For this purpose, artificial sediments were prepared as a mixture of silica sand, clay minerals (kaolinite), calcite and additional components (iron oxide like goethite or ferrihydrite, Humic acids) and spiked with copper (Nia et al, 2011). Bioavailable fractions of Cu (before and after chelatant addition) and kinetic effects of the chelatant were estimated by the technique of diffusive gradients in thin-film (DGT): DGT devices were inserted into each sediment and removed at different contact times. A multi-compartmental model simulating transport in diffusion layer (DGT gel) and sediment porewater, as well as sorption/desorption kinetics (Ciffroy et al, 2011) and allowing to estimate Cu-adsorbent capacity of the sediment (total Kd and labile Kd) and adsorption/desorption kinetics rates (kads;kdes) was used for estimating the effect of chelatant concentration on copper lability.

In parallel, toxicity of this set of spiked sediments (with or without resin addition) was assessed by the Danio rerio embryo-larval biotest. Comparative analysis aimed at showing whether the reduction of lability (estimated from DGT) and of toxicity follow similar patterns and thus to demonstrate if DGT can be a good surrogate for predicting chelatant effects. As a final result, our study provides a tool to optimize resin quantities with respect to the sediment characteristics and to optimize TIE protocols.

TU 075

Optimization of accelerated solvant extraction (ASE) of wide range of endocrine disrupting chemicals (EDC) in sediment

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Aquatic sediments are the sink for thousands of chemical pollutants. Among them, EDCs encompass a wide diversity of chemical classes from natural and anthropogenic origins, acting on various signaling pathways, and the use of in vitro bioassays is now widely accepted as an integrative method for their detection in complex samples. However, the diversity of EDC chemical properties makes difficult their common extraction and validation of extraction method for bioanalysis is still weakly documented. Hence, the aim of this study was to optimize and validate a method allowing a rapid and efficient extraction of a broad range of active chemicals.

A mixture of 12 EDCs (i.e. estrone, 17β-estradiol, bisphenol A, o,pDDT, 4-tert-octylphenol, fenofibrate, triphenyl phosphate, clotrimazole, PCB-126, 2,3,7,8 TCDD, Benzo[k]fluoranthene and dibenzo[a,h]anthracene) with wide range of polarity (2<logKow<8) at known active equipotent concentrations was used to spike a blank sediment. These chemicals are ligands of estrogen receptor (ER), Pregnane X receptor (PXR) or Aryl hydrocarbon Receptor (AhR). Several extraction solvents (acetone, methanol, dichloromethane, heptane, dichloromethane/acetone (50:50, v/v), dichloromethane/methanol (50:50, v/v), heptane/acetone (50:50, v/v), heptane/methanol (50:50, v/v)) were compared and chemical recoveries were determined on the basis of

both biological and chemical analyses. The same approach was applied to a natural sediment for native EDCs including polycyclic aromatic hydrocarbons, polychlorobiphenyls, organochlorides pesticides, alkylphenols, pharmaceuticals, steroids and more polar pesticides.
In spiked blank sediment, bioassay-based recoveries showed that mixtures such as DCM/MeOH or Heptane/MeOH yielded the best rates (> 80 %) whereas the heptane was the less efficient (50-60 %). First results obtained with chemical analyses were in accordance with bioassay data. Finally, similar results were obtained in natural sediment.
In conclusion, this study reports a comprehensive assessment of extraction conditions before application in different in vitro bioassays, which has been rarely studied. Although there was no marked differences between extraction solvant, mixture of polar and non-polar solvent were shown to offer the best extraction of a wide range of active EDCs.

TU 076

Relationship between pollutant accumulation and toxic potency in deep sediments collected in the Seine estuary (Normandy, France)

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Aquatic sediments are well known sinks for numerous persitent pollutants such as POPs and heavy
metals. Pollutants accumulated in sediments can be partially bioavailable and may represent a threat
for aquatic ecosystems.
In order to study time-course of pollution in the Seine estuary and also toxicity of accumulated pollutants in bottom sediments, a 110 cm-depth sediment core was sampled in the Seine estuary in a
disused dock in Rouen harbor in april 2008 (Rhapsodis project). The core was splited in twenty 5 cmthick
slices which were analyzed for size grain, organic carbon, sulfurs, metals (13 elements), PAHs and persitent organic pollutants (PBDEs, OCPs, PCBs). Toxicity of sediment organic extracts was
measured by using a large panel of in vitro toxicity assay: Microtox®, ER and DR-Calux®, SOS Chromotest and AChE inhibtion assay.
For all analyzed chemicals except Be, Mo and Cd a clear increase of pollutant concentrations was observed with depth. Strinkingly, peak of concentration for various polluants (PCBs, PAHs, sulfured
PAHs, OCPs, Hg and Pb) was observed at 92.5cm depth. All sediment extracts exhibited high toxic
potency. Acute toxicity (Microtox®) was shown to increase with depth. DR-Calux® activity (dioxine-like
activity) was also increasing with depth while ER-Calux® activity related to estrogen-like compounds
was globally low. AChE inhibition activity and genotoxicity (SOS-Chromotest) were mainly measured
in surface and sub-surface sediments.
This study brings first evidences of global pollution decline in the Seine estuary during the last three or
four decades notably for heavy metals, PCBs, OCPs and PAHs. It also highlighted the high toxic potency of accumulated pollutants and putative risk they could represent for aquatic organisms living
in the Seine estuary.
This study was supported by the Seine-Aval program.

TU 077

Genotoxicity of sediments from river Bosna

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Among others toxic effects that can posses persistent organic pollutants, for the certain PAHs is well documented their mutagenicity and genotoxicity. In this study we have investigated genotoxic potential of river sediment as risk indicator, primarly for aquatic ecosystem. Having this in mind according to previously performed analysis, repeated sampling of sediment in River Bosna from heavily contaminated and reference site was performed. Genotoxicity was determined by use of the Cytokinesis blocked micronucleus assay (CBMN), method developed by Fenech and co-workers, with and without metabolic activation with S9 microsomal fraction. This technique consists in adding to cell cultures cytochalasin-B (Cyt-B), an inhibitor of the mitotic spindle that prevents cytokinesis. As a consequence, cells that have completed one nuclear division are identified by their binucleated appearance. It is a very sensitive and simple indicator of chromosome damage, which also provides information on cell cycle progression, it is less time-consuming in respect to chromosomal aberrations and slides scoring is relatively easy.
Genotoxic evaluation with in vitro CBMN assay of investigated river sediments shows presence of substances with clastogenic/aneugenic potential. Sediment from the reference site shows direct clastogenic activity, while heavily polluted sediment have dose-dependent clastogenic activity after metabolic activation. According to clastogenic profile of sediment from heavily polluted site it can be concluded that this activity is caused by PAHs present in sample. For the reference site it can be concluded that clastogenic activity is only partly due the PAHs present in the sample, and that there are obviously other substances with direct genotoxic potential. Minimal clastogenic concentration (MCC) of sediment from the polluted site was 10 fold higher than the MCC for the reference site, which correlates with concentrations of PAHs previously determined.
Our results pointed on significant clastogenic/aneugenic activity of sediments from River Bosna, which indicates chronic hazard for aquatic organisms. Risk for human health by consumption of contaminated fish can be assumed, but for the risk assessment additional separate studies should be performed.

TU 078

Effect-directed analysis of potentially genotoxic compounds in sediments collected from Laguna Lake (The Philippines) using the Ames Fluctuation assay

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Waters from lakes and rivers, which are being used in irrigation activities and as potential sources for drinking water, are repositories for many hazardous chemicals. Even more alarming are the sediments beneath the waters which possess high potential to accumulate contaminants that have damaging impacts on human health and ecosystems. Previous studies also reported that Laguna Lake, one of the largest aquatic resources in the Philippines, is under stress as a result of excessive pollution. The presence of high levels of perylene and other polycyclic aromatic hydrocarbons (PAHs) could be identified as potential carcinogens, inducing frameshift mutations. Addition of liver homogenates (S9) can induce mutagenicity by metabolic activation of several PAH´s. The objectives of the study were (1) to assess the mutagenic potential of sediment extracts from Laguna Lake using the Ames fluctuation assay and (2) to identify the bioactive fractions and compound groups that are responsible for the observed genotoxic responses using the strategy of Effect directed analysis (EDA). Samples from Central Bay and East Bay were investigated. Central Bay may contain effluents from highly industrialized metro Manila, whereas East Bay is mainly surrounded by agricultural areas. For an efficient evaluation of sediment toxicity, a fractionation of the complex sediment extracts of both sampling sites was accomplished using a recently developed multicolumn HPLC technique and each fraction was tested in three replicates.
Results of all fractions measured from Central Bay with TA 98 and activation by S9 detected the highest induction in fraction 14 with an induction factor (IF) of 2.0. Without S9 supplementation, the highest induction factor was found in fraction 1 with an IF of 4.6. Results obtained with the tester strain TA 100 and supplementation of S9 revealed an IFmax of 2.08 in fraction 13. In the absence of S9, inductions were found in fractions 1, 11 and 15 with induction factors between 1.64 and 2.23. The sample from East Bay showed the highest induction in fraction 12 with an induction factor of 2.78 when using TA 100 and S9 supplementation. Without activation by S9, inductions were found in fractions 4, 11 and 12 with a range of induction factors between 1.97 and 2.38. Investigations with TA 98 and metabolic activation revealed maximal induction in fraction 3 with an IF of 2.78. Without S9 supplementation, the highest induction factor was detected in fraction 8.

TU 079

Application of the transthyretin binding assay in effect-directed analysis of sediments: identification of thyroid hormone disrupting compounds

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In Effect-Directed Analysis (EDA) studies, various bioassays have been implemented that have an endpoint related to endocrine disruption, with the emphasis on estrogenic and androgenic effects. To extend the scope of EDA, we have used the radioligand T4*-TTR binding assay (shortly, TTR assay) to determine the thyroid hormone like (TH-like) activity to direct our fractionation for the identification of unknown thyroid hormone disrupting environmental contaminants.
From a human and animal health perspective, the thyroid function is vital for the normal development of the central nervous system of the fetus.
To assess what classes of environmental toxicants are capable of interfering with the thyroid hormone system, an inventory of the different classes of compounds with reported TH-like activities was made. Compounds that are known to influence the thyroid system are, among others, hydroxylated polybrominated diphenyl ethers, hydroxylated polychlorinated biphenyls and triclosan. More recently, also polyfluorinated compounds were shown to have moderate TH-like activities.
In the framework of the EU funded Modelkey project we have carried out EDA studies at several locations connected with European river systems using a variety of bioassays. Sediment extracts were fractionated using sequential reversed and normal phase liquid chromatography.
In the course of this project, we identified several different classes of environmental toxicants in fractions of the responsive sediment extracts, by conventional gas chromatography coupled with mass spectrometric detection as well as liquid chromatography coupled with high resolution mass spectrometry. A selection of these compounds was tested in the TTR assay in order to assess whether they were responsible for the observed TH-like activity in the fractions of the sediment extracts.

TU 080

Biochemical biomarkers applied on benthic species with different feeding strategies: Improvements in a weight-of-evidence approach for sediment quality assessment

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Weight of evidence approach for sediment and dredged material quality assessment provides more complete knowledge, which facilitates decision making. Suitability of biochemical biomarkers measured in species with different feeding strategies as potential line of evidence was studied. Sediment pollution of Cádiz Bay (SW Spain) and Algeciras Bay (S Spain) was assessed. Lipid peroxidation, DNA strand breaks and activities of biotransformation and antioxidant enzymes were analyzed as health status indicators in Arenicola marina (deposit feeder), Ruditapes decussatus (filter feeder) and Carcinus maenas (predator) after a field assay based on organism caging and transplantation. Sediment metals and PAHs significantly ($p < 0.05$) induced biomakers compared with day 0 mostly in organisms caged at Saladillo dock (Algeciras Bay), but chemicals only provoked significant ($p < 0.05$) lipid peroxidation increase in crab tissues. Sediment metals, especially Ni, caused antioxidant enzyme significant ($p < 0.05$) induction regarding day 0 in lugworms and clams from rivers Guadarranque and Palmones (Algeciras Bay) and lipid peroxidation significant ($p < 0.05$) increase only in clams from these sites. Most biomarkers measured in Cádiz Bay and Algeciras Bay caged organisms, including lipid peroxidation and DNA damage, were not associated with contaminants analyzed in this research, what suggested the presence of other xenobiotics in the environment, presumably lipophilic, which provoked severe alterations in the exposed animals. A WOE approach which includes multi-specie biochemical biomarker measures allows a more comprehensive sediment quality assessment covering a wider range of biota sensitivities and offers more thorough information for ecological risk evaluations

TU 081

Optimization of the SPE step in the analysis of β-blockers and β-andenomimetics in natural water samples by SPE-GC technique

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Environmental water sample matrices, especially sewage and marine-water samples are complex and often contain interfering elements that can mask or interfere with the analysed pharmaceuticals. In this situation direct analysis these samples may not be possible. Additionally, the low concentrations in which the pharmaceuticals are generally found cause that an initial stage of concentration and purification of the analytes prior to their analysis is necessary. The solid phase extraction (SPE) is the most common sample preparation technique used in environmental areas. Choice of sorbent is a crucial in SPE because it can control such parameters as selectivity, affinity and capacity. This choice depends strongly not only on the target analytes and the interactions of the chosen sorbent through the functional groups of the analytes, but also on the kind of sample matrix and its interactions with both the sorbent and the analytes. This work describes the application of the different kinds of SPE sorbents: C18 bonded silica gel (Strata C18), copolymers (Oasis HLB, Strata X, and Lichrolut EN), functionalized copolymers (Isolute ENV+), mixed-mode ion-exchange (Strata C-X, Oasis MCX) and a three-function sorbent (Strata Screen C) for extraction of six β-blockers (acebutolol, atenolol, metoprolol, nadolol, propranalol, pindolol,) and two β-adrenomimetics (terbutaline, salbutamol) from natural water samples. Parameters such as pH of the loading samples, the amount and the kind of solvents used in conditioning, washing and eluting steps, were selected and optimized. The obtained extracts were evaporated to dryness, subjected to silylation using BSTFA, and finally analysed by GC-FID technique. The recovery of the analytes form natural water samples in the mentioned above SPE conditions will be discussed.

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TU 082

Mutistep fractionation based on normal phase SPE and reverse phase HPLC (RP-HPLC) for isolation of endocrine disrupting chemicals in environmental extracts

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Effected Directed Analysis (EDA) approach aims to identify adverse pollutants by reducing the complexcity of environmental matrices. Single hyperfractionation combined to bioassays is usefull to isolate known active chemicals and to direct chemical analyses to these "classical"pollutants. However, although the emergence of promising chemical tools (e.g. Orbitrap®), identification of unknown active chemicals is still time and cost consuming due to the complexity of each active fraction (e.g. mixture effect). Hence, further fractionation steps are often needed. The aims of this study was to develop and to test the use of a first pre-fractionation step on SPE that will be followed by a RP-HPLC fractionation. First the separation of 12 EDCs have been evaluated with several elution conditions. Silica cartridges with 4 step elution - heptane, heptane/dichloromethane (50/50, v/v), ethyl-acetate and methanol/water (50/50, v/v) - allowing the best and reproductible isolation of chemicals, have been chosen for further investigations. For these conditions, recoveries were assessed for the mixture alone and for a blank sediment extract spiked with this mixture. Finally, a natural sediment known to exert estrogenic, PXR-like, anti-androgenic and dioxin-like activity was fractionated following these conditions. Good mixture recoveries (74-110 %), were obtained. The fractionation F1 contained only the PCBs and the PAHs, while 4-tert-octylphenol, triphenyl phosphate and fenofibrate were detected only in F2. Finally, steroids, bisphenol A and clotrimazole were found in F3 while F4 contained more polar chemicals.

Fractionation on natural sediment allows isolation of TCDD-like activity in F1 and F2 while PAH like activity was detected in F1, F2 and in F3. Then estrogenic compounds were only detected in F2 and F3. Interestingly, the sum of the estrogenic activity found in these 2 fractions is higher than the activity found in the crude extract, sugggesting the occurrence of anti-estrogenic chemicals. Finally, PXR-like activity was mainly detected in F3.

this pre-fractionation protocol allows, in the present case study, the isolation of several biological activities. Based on this first isolation directed hyperfractionation has then been undergone, RP-HPLC hyperfractionation on C18 has been calibrated for the separation of 35 EDCs with broad range of chemical properties and will be readly used for the isolation of active chemicals in the polar and semi-polar pre-fractions.

TU 083

Towards a common mass spectra database for the identification of unknowns in environmental samples

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The identification of unknown compounds in environmental samples isolated during non-target screening or effect-directed analysis (EDA) is often a challenge on the way to the successful outcome of theses studies. Gas chromatography - electron ionisation mass spectrometry (GC-EI-MS) is frequently used to generate mass spectra due to its potential to produce many fragments and therefore unique and/or easily identifiable spectra. This technique is commonly used and a lot of commercial and a few free mass spectra libraries are available to support identification. The advancement of database search strategies and publishing of online databases has improved tentative identification of many compounds in recent years, but many chemicals and their transformation products are still not included in such databases.

Improvements in the analytical technology and tools such as accurate and multidimensional mass spectrometry (e.g. QToF-MS, ToF-MS, FTICR, Orbitrap) in combination with liquid chromatography and soft ionisation techniques such as electro spray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) allow the analysis of a broad range of compounds including polar one (or polar substances) and the restriction of the elemental composition in many cases to one or few formulae. However databases containing accurate mass spectra generally contain relatively few spectra and are not yet widely used, as many compounds relevant in environmental samples are still absent from these databases. One obstacle is the comparability of mass spectra generated with different settings, ionisation and spectrometric techniques due to increased instrument specificity, compared with the relatively reproducible EI-MS spectra.

Our aim is to improve the identification of unknowns in environmental samples using a common and open access mass spectra database including MS data from all instrument types and with sophisticated data evaluation tools. The web-based database MassBank [1] was developed within a metabolomics consortium [2] and is a possible tool to achieve this target The database is free and allows the storage of a wide variety of spectra including EI-MS, ESI-QToF-MSMS and ESI-FTI-CR-MS. Different tools are available to process the raw data and upload the data to MassBank including a spreadsheet based record editor for the addition of metadata.

References:

[1] http://www.massbank.jp
[2] Horai H et al. (2010), J. Mass. Spec. 45, 703-714

TU 084

Construction of a water toxicity sensor based on luminescent bacteria

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To ensure safe drinking water it is critical to have a reliable toxicity monitoring system. Although there are several chemical and biological detection methods, there is no suitable system yet for the real-time monitoring of toxicants in water, taking endpoints into account with human relevance. This gap may be partly bridged by a sensor that applies genetically modified bacteria that respond to specific groups of toxicants by emitting luminescence. These bacteria carry a plasmid with a promoter-gene that is known to be activated in case of exposure to certain types of toxicants, for example DNA damaging agents or heavy metals. This promoter gene is coupled to a luminescence gene-set, so that luciferase is formed when the promoter is activated. The resulting production of light can then be detected and used as a measure of the toxic stress the bacteria were exposed to.

A new prototype of a flow-through sensor for on-line water monitoring based on these modified bacteria is being developed at KWR. The bacteria are fixed on an optic fiber or a glass slide and placed in a continuous water flow. The light generated by the bacteria is then measured by photomultiplier tubes. The current prototype is highly adjustable and allows control of pH, temperature, flow, and pressure. Additionally, it is possible to add nutrients as well as test compounds to the water. This sensor prototype is being tested in both the laboratory and at monitoring stations along Dutch rivers. The ultimate aim is to develop a sensor that measures several types of toxicity and that can be applied continuously in the field, both at surface water inlets and in the distribution network.

TU 085

Toxicity of coastal waters: use of a quick algal bioassay

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Primary production by microalgae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain industrial chemicals may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuarine and marine ecosystems. At the same time the frequency and intensity of toxic algal blooms in the coastal zone are increasing globally, resulting in increased levels of toxins prospected to affect coastal ecosystems. These different chemical stressors are hypothesized to disturb regulatory mechanisms within algal communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavourable algal species that destabilize the food chain. It remains however difficult to quantify the toxic effects of these chemicals: the relative contribution of anthropogenic and natural chemicals on the total chemical pressure is unknown. Also insight in the potential synergistic action of toxicants and toxins is lacking, while in the field many confounding factors (e.g. changing nutrient and light regimes) may mask effects.

The first step to unravel the complex interaction between algae and toxic pressure is to provide knowledge on chemical compounds causing phytotoxic effects. In this study we use passive samplers which extract the freely dissolved concentration in the water during a period of 6 weeks to take episodic events into account. The concentrated extracts are tested in an algal bioassay with different marine algal species (e.g. Dunaliella tertiolecta, Phaeodactylum tricornutum) to include differences in algal sensitivity. Use of Pulse Amplified Modulation (PAM) fluorometry provides a quick (4.5h) method to determine toxicity to algae based on changes in photosynthetic efficiency. An Effect Directed Analysis (EDA) will be performed to unravel which chemical compounds are responsible for the toxic effect on the algae. In 2010-2011 passive samplers are exposed at Hansweert (Westerscheldt, The Netherlands) and collected every 6 weeks to include the seasonal dynamics of both anthropogenic as well as natural compounds. Here, first results of this sampling campaign are presented and discussed. The results of the EDA analysis will be used in experiments where mixture toxicity, multi stress and community effects are taken into account to describe the overall toxic effect under relevant field conditions.

TU 086

Dissolved and intracellular microcystins in lake waters during a Planktothrix rubescens algal bloom: HPLC quantification and crustacean acute toxicity test

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Microcystins, highly toxic cyclic peptides, are a group of hepatotoxins produced by a number of aquatic species of cyanobacteria, such as Microcystis, Anabaena and Planktothrix. Worldwide contamination in water has prompted the development of detection methods for their identification and quantification. A massive seasonal development of Planktothrix rubescens in a reservoir destined for crop irrigation located in Southern Italy has lead to quantify algal toxin content in the lake water to verify the possible health risk. Microcystins dissolved into the water were separated from intracellular ones by filtering raw samples. Filters were extracted by methanol/water solutions after frozen/defrozen treatment over night. Water samples were concentrated and extracted by SPE-C18 cartridges. Toxin content was detected and quantified using high performance liquid chromatography (HPLC-DAD). The only microcystin detected was [d-Asp3] microcystin-RR. It was identified by retention time and spectrum comparing with a certified standard. Quantification was made by means of a calibration curve obtained at 238 nm. Microcystin extracellular concentration was never above the WHO limits for drinking waters (1 µg/L). Maximum level as dissolved microcystin was 0.7 µg/L on April 2009 sample. In the same sample the highest endocellular concentration (30.8 µg/L) of [d-Asp3] microcystin-RR was measured. As predictable, endocellular toxin was 90-95% of the total microcystin content; the endocellular

content ranged from 10 to 40 times greater that the dissolved content. Probably it depends on the physiological state of the alga; for example the extracellular content of microcystin is different, if the samples are collected from a fresh or aged population of P. rubescens; the lysis of dead cells can release endocellular toxins into water environment. Acute toxicity tests (24 h exposure) with Thamnocephalus platyurus were carried on using endocellular and extracellular water extracts. Only the endocellular extract, 10 times concentrated, obtained from April 2009 sample, showed a measurable inhibition (12.5%); it means that the acute toxicity effects on T. platyurus appeared starting from a concentration of about 300 µg/L of [d-Asp3] microcystin-RR. The 24 h dose-response curve showed that the EC50 of desmetyl-microcystin-RR to T. platyurus was 845 µg/L. Comparing with literature results, this microcystin is less toxic than microcystin-LR, but 3 times more toxic than microcystin-RR.

TU 087

Integrating biological indicators and geochemical markers to evaluate contamination in an organic matter enriched area on South Brazilian coastline

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Paranaguá Bay (S Brazil) is one of the largest and best preserved estuarine complexes along the western South Atlantic. It houses approximately 19% of the Brazilian Atlantic forest remnants and was considered a biosphere reserve by UNESCO in 1995. The city of Paranaguá has approximately 140,000 inhabitants and one of the largest harbours of the South Atlantic. More than two thirds of local domestic sewage is discharged into the estuary without any treatment. The contamination associated with the input of heavy metals, hydrocarbons, sewage and the development of anoxic conditions may lead to changes in macrofaunal associations. In order to find reliable biological proxies of contamination levels, we evaluated the composition and abundance of local polychaete assemblages. Sediment samples were collected by SCUBA diving at 11 stations around Paranagua in a grid spaced up to 2 km from the main contamination sources. Nutrients, fecal sterols, hydrocarbons and metals were analyzed in the surface sediment layer. A PCA analysis showed that the stations adjacent to the city and the Port of Paranaguá are in fact characterized by high concentrations of nutrients (P, N, TOC), metals, fecal sterols and PAHs. The total density and species number of polychaete assemblages were significantly lower ($p < 0.001$) at stations closer to the harbour and sewage discharge points, Most of the differences reflected the low polychaete abundance and diversity in two hypoxic stations, with high concentrations of total PAHs (3786 $ng.g^{-1}$), metals, nutrients and coprostanol (16 $ug.g^{-1}$). Occurrence patterns of the species *Magelona papilicornis*, *Glycinde multidens*, *Chaetozone sp.*, *Capitella sp.*, *Aricidea taylori* and *Poecilochaetus australis* differed significantly among stations. This set of species can be used as early indicators of organically enriched areas.

TU 088

Assement of contamination of watershed for waste produced by the rice fields

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The southern region of Brazil is dominated by wetlands, which are attractive ecosystems for irrigated rice production. This type of farming is generally practiced in the vicinity of water sources, and requires intensive use of chemical inputs resulting in serious risk of water contamination. For these studies three sites were chosen along the Cachoeira do Sul river (nº 2, 3 and 4), from which water is pumped for the management of the rice fields. In order to define a possible site to be used as a reference, without contamination, another sampling site(7) was chosen on the basis of its clear water, without the turbidity found at the other sites. The samples were collected in November 2008, during the rice cultivation period and therefore during the application of pesticides to crops. The water samples were examined for toxicity using Scenedesmus subspicatus (microalga) and Daphnia magna (Microcrustacean) as bioindicators. The tests with the microalgae showed that the sample of water coming from the tables of the rice field (site 1) and the sample from the Rio Cachoeira do Sul (site 4) in direct contact with rice cultivation, exhibited the highest growth inhibition rates, 44% and 37% respectively. Typically, samples 2 and 3 also presented a toxic effect as indicated by the reduced algal growth rate of 31%. The control sample from the Cachoeira River showed a growth inhibition rate of 4%. Therefore, comparing the various values of the rate of inhibition for the samples, the inhibitory effect appears to be related to exposure to or contact with rice crops and the application of pesticides in connection with rice cultivation. The toxicity tests using Daphnia magna also showed that samples from sites 1, 2, 3 and 4 were associated with lower numbers of neonates and therefore the highest rates of reduction of the reproductive capacity of organisms tested.

TU 089

The integration of chemical, ecotoxicological, and ecological data obtained from an extended study carried out in an industrialized area

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The aim of the project was to study urban soil by chemical, ecotoxicological and ecological methods and to assess the environmental risk index for each soil samples. Four sites have been selected on the basis of the predicted patterns of chemicals deposition.
Soil samples were took from depths of 5-20 cm at five locations along the main industrial region of the Kirov, Russia (Latitude: 45° 5' 20 N, Longitude: 40° 16' 11 E) in May 2010. The soil samples were air-dried and sieved into coarse and fine fractions. The labile fraction of heavy metal to determine the concentration by Atomic Absorption Spectrophotometry SpectrAA-220FS (Varian, USA) by flame Atomization.
The six bioassays evaluated were 1) Crustacean Daphnia magna- acute toxicity 96-hour, 2) Higher plant Sinapis alba - seed germination, growth rough 4-day, 3) Algae (Scenedesmus quadricauda acute toxicity 72-hour, 4) Protozoan Paramecium caudatum - acute toxicity 24-hour, 5) E. coli (with gene-modified luciferase) - bioluminescence, 30-min and 6) Micromycetes Fusarium oxysporum - the rate of radial growth of colonies, 5-day.
Ecological monitoring includes measures of function and structure of different populations and communities in the soil. The composition of the microbial community was determined by molecular techniques of gas chromatography - mass spectrometry (GC-MS). Analysis was performed on GC-MS system HP-5973 Agilent Technologies (USA).
The present study show that chemical data was input principal investment to assessed EnvRI. The battery of toxicity tests and ecological monitoring data point out the important information about the resistance soils community to heavy metal pollution. The greatest sensitivity of structure microbial community to pollution was shown by the ecological monitoring. Bioluminescent bacteria E. coli and micromycete F.oxysporum were found as the most sensitive test-species to HM polluted.
Estimation of ecological risk have been proposed follows the Triad approach and integrates data by computing different indexes which contribute to the final estimation of the EnvRI (Environmental Risk Index). The highest weight is assigned to parameters describing the effects at population-community level. An intermediate weight is applied to bioassays, measuring ecotoxicological effects at organism level and to data which describe bioavailability of pollutants. The lowest weight in EnvRI computation is assigned to ChemRI.

TU 090

Multi-biomarker approach to investigate the state of contamination of the R. Lambro / R. Po Confluence (Italy)

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The response of Zebra mussel (Dreissena polymorpha) to chemical pollution derived from the R.Lambro/R. Po confluence, one of the most polluted European aquatic environments, was assessed by using a wide battery composed by eight different biomarkers: the single cell gel electrophoresis (SCGE) assay, the apoptotic cell determination, the micronucleus test and the Neutral Red retention assay (NRRT). Moreover, we measured the activity of three antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as the activity of the detoxifying enzyme glutathione transferase (GST). We exposed mussels at laboratory conditions to water sampled in spring and fall at three sampling sites located both upstream and downstream the confluence and directly on River Lambro. We measured each end-point every 5 days for a total time exposure of 15 days, when mussel soft tissues were also taken to evaluate the bioccumulation of several organic pollutants (PAHs, PCBs, DDTs, HCHs and HCB). Biomarker responses revealed a heavy genotoxicity of the pollutant mixture at all the sampling stations with significant increase of DNA strand breaks, apoptosis and micronuclei with no significant seasonal differences. We obtained a clear induction of the enzyme activities measured in spring that showed the increase of oxidative stress, but also a very complex enzymatic activity trend in fall with several end-points that showed a parabolic slope that bring back the activities to baseline levels, suggesting a possible seasonal change in chemical mixture characteristics. This study confirmed the utility of a wide biomarker battery in biomonitoring studies and the suitability of Zebra mussel as bioindicator organism also for river basins.

TU 091

Stage-dependent β-naphtoflavone-induced cytochrome P450 1A (CYP1A) activity in medaka (Oryzias latipes) embryos using an in vivo quantitative approach

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Worldwide continuous increase in chemical production has prompted the development of chemical management programs for a correct assessment of their risk to human health and to the environment. Examples are in the US with the US EPA HPV Challenge Program and here in the EU with the recent REACH Program. Within the environmental assessment, there is a strong current towards the development of alternatives to existing ecotoxicity tests without reducing the validity of the risk assessment. In this context the use of fish embryos and eleutheroembryos are promising alternatives. These stages also provide the application of in vivo techniques for the evaluation of biomarkers indicative of exposure/toxicity. Here, we propose original methods for a non-invasive quantification of the CYP1A activity and its stage dependent β-naphtoflavone (BNF) induction (up to 5 developmental stages were selected) over medaka (Oryzias latipes) embryonic/larval development. CYP1A activity was measured from fluorescent images from metabolite resorufin using intact embryos and at two observational levels: specific induction in gallbladder (vesica biliaris), and non-specific induction in the whole embryo. A battery of analytical procedures is also proposed to quantify BNF in small volume of aqueous samples from biological assays reaching limits of detection up to 30 ppt. This work was made possible by Spanish Government Grants RTA2010-00004-C02 and CTM2010 19779-C02

TU 092

Hormonal control of HSP70 and apoptosis in sea bream cells

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Evidence as to whether heat shock protein 70 (HSP70) chaperones life (stress-tolerance) or death (apoptosis) is equivocal, as HSP70 can either inhibit or promote apoptosis, depending on cell type. Underlying these observations is the tripartite relationship between hormones, HSP70 expression and apoptosis and using a range of in vitro models we have been investigating this in silver sea bream (*Sparus sarba*). Using whole blood preparations it was found that camptothecin-induced apoptosis, as determined by elevated DNA fragmentation. However, exposure of whole blood preparations to growth hormone was found to attenuate the occurrence of DNA fragmentation in parallel with increased HSP70 family gene expression. In a sea bream macrophage primary culture and a fibroblast cell line it was found that chemically induced HSP70 expression (via azetidine treatment) protected against camptothecin-mediated apoptosis, as assessed by lowered caspase 3 activity and reduced DNA fragmentation. When these cells were treated with cortisol different responses were found as macrophages were more prone to apoptosis and had decreased HSP70 amounts whereas fibroblasts were protected against camptothecin- mediated apoptosis with a concomitant increase in HSP70 levels. Also in sea bream gill cell preparations, prolactin was found to protect gill cells against camptothecin-induced apoptosis, an effect that occurred alongside increased HSP70 amounts. It appears from these data that HSP70 may play a central role in mediating apoptosis under the influence of key hormones such growth hormone, prolactin or cortisol. (This research was supported by a General Research Fund (CUHK 4783/09M) from the Research Grants Council, Hong Kong).

TU 093

Comparison of bioassays for screening dioxin-like activities in food samples

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In our environment we encounter a large number of hazardous substances almost on a daily basis. Knowledge about the toxicity of these substances is important to ensure human health as well as integrity of the ecosystems we live in. Polychlorinated dibenzo-para-dioxins (PCDDs or dioxins), polychlorinated dibenzofurans (PCDFs or furans) and polychlorinated biphenyls (PCBs) are highly ubiquitous toxic substances. They enter the food chain and become health hazards. This has caused the European Commission in 2001 to limit the presence of dioxins in food and feed. Strict but feasible maximum limits had been implemented at the beginning of 2002. Any food or feed exceeding these maximum limits is considered unsuitable for human consumption. Hence,

monitoring of dioxin levels is of high importance and should be of primary relevance. Bioassays are a valuable pre-screening tool to investigate dioxin like activity. A newly introduced method to test food and feed is the Micro Ethoxyresorufin-O-deethylase (EROD) bioassay with H4IIe cells. The Micro EROD bioassay is an aryl hydrocarbon receptor based assay to detect the receptors interaction with dioxin-like compounds. In this study the feasibility and application characteristics of this bioassay for food testing were evaluated. A comparison with the H4IIE-Luc and RTL-W1 bioassays was conducted, and acidic column chromatography as sample clean-up methodology to gain fat free samples was characterized. Additionally, a review of alternative methods was performed. The acidic column chromatography in combination with the Micro EROD bioassay revealed to be a promising tool to pre-screen food and feed samples. It can be integrated as a pre screening technique to determine dioxin-like activity. High levels detected should then be further analyzed by instrumental analysis. Nevertheless, further investigation and modification of the Micro EROD bioassay is recommended for streamlining and improvement of the protocol to achieve a sophisticated, integrated pre-screening tool for dioxin-like activity in food and feed samples.

TU 094

Changes of AhR-mediated toxicity of hydrophobic organic compounds after UV-VIS irradiation

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Hydrophobic organic compounds (HOCs) were shown to cause many adverse effects in organisms. Arylhydrocarbon receptor (AhR) activation is among the frequent mechanisms of toxicity of these pollutants. They are present in all environmental matrices, thus, it is almost impossible to avoid the exposure of the organisms to this type of chemicals. HOCs can be degraded and removed from the environment by many processes, such as volatilization, adsorption to solid-phase particles, leaching, bioaccumulation, biodegradation, chemical oxidation or photooxidation. Direct photolysis of HOCs evoked by solar irradiation is a very important way of degradation in the aquatic environment. The aim of this study was to assess (i) changes of AhR-mediated toxicity of benzo[a]pyrene (B[a]P), dibenz[a,h]anthracene (DB[a,h]A), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a mixture of the three chemicals in the aquatic solution, and (ii) changes of concentration of each compound after UV-VIS irradiation. The chemicals were dissolved in ethanol, ethanol was evaporated under a gentle stream of nitrogen and distilled water was added. The water solutions were irradiated by a low pressure mercury lamp. AhR-mediated toxicity was assessed using *in vitro* assays based on the H4IIE-*luc* transgenic cell line. Chemical analysis of B[a]P, DB[a,h]A and TCDD was performed using GC/MS(MS). We observed a significant decrease of HOCs concentrations in samples after irradiation which corresponded well with the decrease of AhR-mediated activities of irradiated samples. Therefore, we suppose that there had not been created any AhR-active photooxidation products of HOCs. Supported by projects GACR 525/08/P464 and CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 095

Application of EDA to identify hydrophobicity causing compounds in olive oil mill waste waters

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In the Mediterranean region waste waters as olive oil mill waste waters (OMWW) are widely applied at agriculturally used soils due to positive properties as high nutrient content. This application is often accompanied with negative effects as increasing phytotoxicity and hydrophobicity of the soils and thus, decreasing field capacity. Although several studies investigated the composition of OMWW, the compounds or class of compounds responsible for this process are still unknown. To facilitate the identification of the responsible chemicals a fractionation procedure was validated for typical OMWW substances as model compounds. The different classes of compounds as e.g. polyphenols, aliphatic hydrocarbons and carboxylic acids are separated by extraction at different pH values and the use of selective solvents. The optimised method was used to fractionate OMWW samples from Palestine. For prioritization of fractions the phytotoxicity in germination tests and hydrophobization potential using the sessile drop method was evaluated.

TU 096

Eco design of environmental benign ionic lubricants: assessment of toxicity and biodegradability

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The philosophy of green or sustainable chemistry is the development of high efficient technical processes and applications using chemicals with a reduced or eliminated hazard potential for man and the environment. Therefore the development of chemicals with optimised technical properties should run in parallel with the minimisation of (eco)toxicological hazard potentials. In the field of designing chemicals, ionic liquids represent an excellent model substance class. Ionic liquids are salts with melting points below 100°C or even room temperature. Their physicochemical properties like high thermal stability or low vapour pressure raised the interest of this substance class for their application as lubricants in resent years. Additional the high structural variety is an advantage of these compounds. By changing the anion or modifying the fine structure of the cation it is possible to define attributes like viscosity or density, which allows for an optimized application-related design of ionic liquids. Beside the optimization for application also the environmental impact should be considered. Therefore studies on toxicity and biodegradability have been performed for selected ionic liquids. The compounds, mainly ammonium based cations, were chosen according to structure-activity-relationships and their potential applicability as lubricants. Toxicity was tested with several model organisms varying from isolated enzymes, mammalian cells, marine bacteria and algae to higher organisms like crustacean. Data about biodegradation are discussed as well. The present study shows that the design of environmental benign ionic lubricants is- with limitations- feasible.

TU 097

Prospective hazard assessment of new industrial chemicals: the case of ionic liquids as high performance lubricants

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The interest in ionic liquids for their potential in different chemical processes is increasing, as they are claimed to be environmentally benign and very good non-volatile solvents for a wide range of applications. Responsible product design however should always take into consideration not only technological demands but also the risks arising out of possible (eco)toxicity. This contribution presents our strategy where technical properties of ionic liquids, potentially used as lubricants, are investigated in parallel with the examinations of (eco)toxicological hazard potentials. Toxicological studies of imidazolium, ammonium and choline based ionic liquids presented here using test systems at different level of biological complexity, such as isolated enzymes, mammalian cell lines or algae. In addition data about biodegradation and sorption of selected ionic liquids to different types of soil and marine sediment are also discussed. A set of instrumental methods for analyzing ionic liquids in environmental and biological samples is presented as well.

EP05 - Plastic pollution - polluted plastics: Fate, effects and life cycle assessment

TU 100

Distributions of marine microplastics in the Puget Sound estuary

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Marine microplastic debris are particles composed of plastic polymers with dimensions between 0.33 and 5 mm. Early reports of pre-production resin pellets in Northwest Atlantic waters suggested losses during manufacturing or shipping may supply plastics to the oceans. Since those initial studies in the 1970's there have been very few reports of microplastics in the oceans. Recently, we have developed standarized protocols to sample and analyze microplastic particles in surface waters, sediments, and beach sands. The goal of our study is to make the first systematic characterization of microplastics in the Puget Sound estuary. Nets with 0.33 mm mesh size were towed horizontally through surface waters using standard plankton sampling conditions and tow durations in several locations throughout the Puget Sound, ranging from highly urbanized, stormwater-dominated embayments to relatively unimpacted open waters. Samples were oxidized with hydrogen peroxide to remove marine carbon. Samples from nearshore that contained more recalitrant terrestrial carbon were oxidized with a sulfuric acid/potassium dichromate solution. After oxidation, inorganic solids were separated from the plastic by density gradient and the remaining solids were isolated and weighed to determine the total quantity of microplastic mass. Concentrations of solid material collected from Puget Sound surface waters in the 0.33 to 5 mm size range vary widely from 1.5 x 10-5 to 0.11 g/m3, and the geometric mean plastic content of this material is 1.8% (range 0.008 to 27% plastic). Strong temporal and spatial gradients indicate a significant source from urban stormwater.

TU 101

Analysis, occurrence and aerobic biodegradation of polyethylene glycols of different molecular weights in seawater, wastewater and sludge

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The biodegradation of polymers plays an important role because polymers are used in high amounts in our daily life. Biodegradation is a major topic today in risk assessment strategies. Mostly, the focus is set on freshwater systems because in generally the direct entry of chemical substances is through these systems.
There are major differences between freshwater (WWTP activated sludge, OECD 301) tests and those in marine (synthetic and native marine water) tests. The differences span from the time of biodegradation of the same substances to differences in the biodegradation graphs of reference substances and also to differences in the metabolic pathway. The potential of biodegradation in freshwater and marine tests will be presented for the group of poly(ethylene glycols) ranging from 200 to almost 60'000 g-mol-1. Specific parameters such as molecular or structural properties of the polymers do have influence on the biodegradation in different environments such as an influence by molecular weight, number of hetero atoms in the chain, specific behavior of groups that hydrolize but do not biodegrade. Possible pathways of biodegradation have been confirmed applying different mass spectrometric (MS) methods, such as matrix assisted laser desorption ionization MS as well as electrospray MS following liquid chromatographic and size exclusion separation techniques. Beside these water soluble polymers examples of unsoluble as well as well as per se biodegradable polymers will be given. Additional methods for the determination in the aquatic environment will be presented.

TU 102

Environmental and health hazards of chemicals in plastic polymers and products

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Many of the chemicals used to produce plastic polymers and products are hazardous to human health and the environment. Considering the extensive use of plastics in society and the rapidly increasing accumulation in the environment (both the marine and terrestrial), there is a need for assessing toxicity hazards and risks. In this project the environmental and health hazards of chemicals in plastic products and polymers were investigated. One part includes a survey of the chemical composition of more than 50 different plastic polymers. A hazard ranking and hazard assessment was made based on EU (CLP) hazard classifications of the chemicals that are needed to produce the different polymers. This shows which polymers that are made of the most hazardous chemicals, and the need for substitution of polymers/and or chemicals. The other part deals with the toxicity of water leachates from plastic products to Daphnia magna. In four separate studies leachates from various plastic products, synthetic (plastic polymer) textiles, and discarded electronic products were tested. Toxicity Identification Evaluations were also made to identify which group of toxicants that was causing toxicity. Some overall conclusions are that carcinogenic, mutagenic and/or reproductive toxins (category 1) substances are used to make several of these plastic polymers. Acute toxic substances were found to be leached from several products to water even during the short term leaching period of 1-3 days. Hazardous ranking systems and toxicity testing of product leachate can be useful tools in the assessment of products.

TU 103

Organochlorine pesticides in plastic pellets from Santos, southeastern coast of Brazil

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Hydrophobic pollutants such as organochlorine pesticides (OCPs) are adsorbed to the plastic pellets from seawater due to the hydrophobic nature of the plastic surfaces. In this study, the occurrence of OCPs was evaluated in plastic pellets sampled in 30 points on the sand surface in Santos Bay (23°58'06"S and 46°20'33"W), located close to an important industrial complex (Cubatão) and the biggest port of Latin America (Porto de Santos). Pellets (approximately 1 g) were extracted by soxhlet apparatus and the extract was cleaned up by chromatographic adsorption column with 5% deactivated alumina. Twenty-five OCPs were analyzed by gas chromatograph with electron capture detector (GC-ECD). The concentrations of OCPs found in plastic pellets were in ng g^{-1}: Dieldrin (<0.61 to 5.75), HCHs (<0.24 to 87.1), DDTs (12.6 to 3545), heptachlor (<0.33 to 39.6), ɤ-chlordane (0.47 to 121), endosulfan (<0.46 to 6.20), HCB (1.92 to 557) and mirex (0.73 to 692). OCPs concentrations in plastic pellets varied considerably (more than two orders of magnitude for some compounds) in a single site. Variability can be associated with several factors such as the residence time of the pellet in the environment. The proximity of an industrial area can contribute to the presence of contaminants (e.g. HCB) in this region. The occurrence of HCB, one of the predominant chemicals in the samples, can be associated either with improper disposal occurred in the past and/or with waste from chemicals production or with other sources far from the sampling sites. DDT showed higher concentrations than DDD and DDE in 47% of the samples, which can be attributed to a recent contamination. However, studies about DDTs contamination in several compartments of Santos ecosystem showed low concentrations of DDT and the predominance of the metabolites DDE and DDD. Therefore, plastic pellets can be mainly an important source carrying hydrophobic pollutants coming from different places and OCs concentrations found in plastic pellets may reflect not only local sources of contamination, once the pellets float on the ocean and are in contact with the pollutants along the entire transport pathway before reaching the beach.

TU 104

Sorption of Bisphenol A to poly(methyl methacrylate) in aquatic environment measured by MALDI-TOF-MS

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Plastic debris is one of the most significant pollutants of the marine environment. Depending on their hydrophobic character plastics are acting as carriers for organic pollutants [1]. Thus, ingested plastic particles can play an important role in the uptake of organic pollutants by organisms [2].
For better understanding of contaminant sorption to polymers poly(methyl methacrylate) (PMMA) samples of different molecular weight distributions were exposed to synthetic fresh and salt water contaminated with Bisphenol A. All samples were measured by solvent-free matrix assisted laser desorption ionization/time of flight/mass spectrometry (MALDI-TOF-MS) to observe Bisphenol A sorption according to molecular weight of PMMA. The solvent-free sample preparation was compared to solvent based preparation with regard to the observation of Bisphenol A sorption to PMMA. With this information it is planned to investigate sorption and desorption of other relevant aquatic pollutants.
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TU 105

Sorption of two antifouling compounds (Irgarol 1051 and Diuron) into plastic pellets

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Plastic pellets, the raw material for plastic objects, are commonly lost to the aquatic environment and abundantly found worldwide. In the environment, the pellets are able to retain organic contaminants through sorption process. This process is defined as the interaction between the solute and the sorbent, occurring in the sorbent's surface (adsorption) or in the sorbent's matrix (absorption). Here, the sorption process of two antifouling compounds, Irgarol 1051 and Diuron, were chosen to be analyzed, because these compounds were the main substitutes of Tributyl Tin (TBT) in antifouling paint for ships, and there are no studies concerning the sorption of these compounds into plastic pellets. Thus, the aim of this work was to assess the concomitant sorption of Irgarol 1051 and Diuron in polypropylene plastic pellets, evaluating the effects of solute concentration and water salinity. For the solute concentration test, solutions containing 10, 100 and 1000 ng.mL-1 of each compound were tested. For the salinity test, ultrapure (salinity 0) and salt water solutions (salinity 15 and 30) containing 100 ng.mL-1 of each compound were tested. The solutions were introduced to beakers and 1g of plastic pellets was added. All tests were conducted in triplicates along with a triplicate blank. After 7 days, the pellets were removed and extracted with methanol (ultrasonification). The extract was analyzed by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The increase of solute concentration affected significantly the sorption of both Irgarol (Kruskal-Wallis, H= 11,35, p=0,00) and Diuron (Kruskal-Wallis, H= 7,94, p=0,01). Also, the sorption of Irgarol was relatively lower than Diuron. The salinity variation affected the Irgarol sorption (ANOVA, F=40,75, p=0,00), but did not affect the Diuron sorption (ANOVA, F=1,87, p=0,24). It is known that salinity only affects superficial processes (adsorption). Thus, the present results may indicate that adsorption was the main process occurring for Irgarol 1051, while absorption was the main process occurring for Diuron. Moreover, the greater sorption of Diuron also indicates the occurrence of absorption. The sorption of Irgarol and Diuron into plastic pellets can pose an environmental risk, since the pellets are ingested by aquatic organisms and easily dispersed in aqueous media.

TU 106

Using three-dimensional solubility parameters as a screening tool for prioritizing types of plastics and pollutants most likely to lead to elevated exposure

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The presence of plastics in the environment has been linked with increased exposure of organisms to certain pollutants through ingestion of contaminated plastics. Here we present the use of a screening tool based on calculations of Hansen solubility parameters for a wide range of common plastics and pollutants of environmental relevance. Results suggest that non-polar organic chemicals with log K_{OW} > 5.5 have the potential to have significant sorption capacity to plastic polymers, such as polyethylene and polypropylene. These observations are supported by the use of Hansen solubility parameters, which indicate that non-polar organic chemicals have a greater potential for dissolution in polyethylene, than do polar organic chemicals. Surface interactions for polyethylene and polypropylene are characterised by van der Waals interactions, which help explain the strong dissolution capacity of these polymers for non-polar organic chemicals. Plastic polymers with hydrogen-bonding capacity and polar functional groups, however, have lower sorption capacity for non-polar organics, but may represent sources/sinks of polar organic chemicals. Understanding the interactions between PBT substances and different types of plastic material in the environment, and their relative abundance, is thus an important component towards quantifying potential exposure pathways.

TU 107

Inventory of the presence of plastics in the digestive track of North Sea fishes

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Plastic debris can be found in seas and oceans all over the world and ingestion of smaller plastic parts by marine birds, turtles and mammals has been reported by various researchers. As far as we are aware of, an inventory of the presence of plastics in the digestive track of North Sea fishes has never been made. In 2010, IMARES started such an inventory using fish samples from various positions in the North Sea.
After being measured and weighted, the complete content of the digestive system of each individual fish was collected. The organic matter was chemically digested and the remaining material was searched for any plastics by stereo microscope. This method allows the detection of even very small plastic particles. When drafting this abstract small plastic fragments have been found in 1% of 500 individual Herrings from the Northern North Sea that were processed. Other species like Mackerel, Grey gurnard and Hake were sampled in smaller numbers (45-170 individuals) without plastics being found. Samples of Horse-mackerel and Whiting have been collected and other species and locations are planned to be sampled the coming months. Plastics found in these species (if in significant amounts), plastics retrieved from stomach content from Northern fulmars and plastics collected during surveys with bottom trawling in the North Sea will be chemically analysed by GC-GC-MS. Comparing contaminant levels and profiles also with sediment and fish data, gives a first insight in the importance of plastic debris as carrier of organic contaminants in the North Sea.

TU 108

Influence of ingested micro-plastics on the bioaccumulation of organic substances in fish - a model approach

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Marine waters are polluted with plastic litter, which can cause severe adverse health problems for marine organisms when they are taken up through ingestion or filter feeding. They can contain organic substances ad-/absorbed from surrounding water body or as additives, which might pose an additional chemical risk due to bioaccumulation in the marine food chain [1]. Of particular concern are small particles of less than 1 mm in diameter (micro-plastics), which were recently found in mussels and other organisms [2]. A simple equilibrium model predicts lower bioaccumulation for lipophilic compounds if plastics material is added as separate compartment. To enable a more realistic consideration of the effect of ingested plastics on bioaccumulation in fish, we developed a dynamic bioaccumulation model to investigate the influence of ingested micro-plastics on the bioaccumulation in fish.
We modified the dynamic Gobas fugacity model [3] taken from the well-acknowledged bioaccumulation model ACC-Human [4] which describes the bioaccumulation of pollutants by baltic herring growing from birth to the age of 5 years. To model the influence of plastic ingestion on bioaccumulation of organic substances additional assumptions were made:
i) In addition to plankton herring ingests micro-plastics. Ingested plastics material is completely excreted such that there is no plastic accumulation.
ii) Micro-plastics and the fish at birth are in equilibrium with the surrounding water. Within the gastrointestinal tract plankton and micro-plastics are homogeneously mixed.
iii) It is assumed that ingested micro-plastics consist of low-density polyethylene. The fugacity capacity of micro-plastics is determined by the content of organic carbon.
The additional body burden due to ingestion of organic pollutants with micro-plastics depends on the substance properties. For compounds with fast metabolic transformation ingestion of plastics increases pollutant accumulation, while for persistent compounds it decreases the mean body burden. This study provides first insights into how ingested micro-plastics may influence the bioaccumulation of aquatic organisms.
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TU 109

Polyethylene microplastics select for 'plastiphilic' bacterial assemblages in coastal marine sediments

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Synthetic plastics constitute the majority of anthropogenic debris in the Earth's oceans. Recently, microplastics (fragments of ≤ 5 mm) have been highlighted as emerging pollutants in coastal sediments. Despite the potential for this debris to impact upon macrobiota, our understanding of the interactions between microplastics and microorganisms is negligible. This study initiates characterisation of the ecological interactions between bacteria and microplastics within coastal sediments. Specifically, this research aims to determine whether and how rapidly sediment bacteria colonise microplastic pollutants and to investigate the structure, diversity and successional dynamics of plastic-associated bacterial assemblages over time and across different types of sediment.
Fourteen-day laboratory microcosms were established using sandy and silty sediments from three sites within the Humber Estuary (United Kingdom). The microcosms were spiked with polyethylene as a model plastic. Changes in the structure and diversity of bacterial assemblages at the plastic-sediment interphase and within sediments were investigated by terminal restriction

fragment length polymorphism and sequencing analyses of 16S ribosomal RNA genes. Plastic-associated bacterial assemblages formed in all sediment types with the onset of colonisation varying from six to 48 hours. The plastic-associated bacterial communities were distinct from those in sediments (ANOSIM R = 0.5, P < 0.001). Successional changes occurred in the structure of the plastic-associated bacterial communities, with increasing convergence in the structure of these communities across different types of sediment. Epsilon- and gamma-proteobacteria were identified as dominant classes within the plastic-associated bacterial communities. Whilst temporal variability in the structure of the sediment bacterial communities was found, convergence of these communities between sediment types was not observed.
This study provides the first evidence for the potential for rapid bacterial colonisation of microplastics in marine sediments and for the formation of microplastic-associated bacterial assemblages whose composition differs from those in bulk sediments. The results also provide insight into the existence of 'plastiphilic' bacterial taxa that may become selected for in the presence of plastic across several types of sediment. These findings have opened an avenue for microbial research into the ecological impacts of microplastics.

TU 110

Environmental impact of treatment processes for plastics and composites at the end-of-life vehicles in Spain

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From an environmental perspective, the automotive sector is currently faced with two major challenges:

- Reduce the energy demand.
- Reduce the amount of waste generated.

Reducing the vehicles' weight -among other factors- will reduce the energy demand. In this sense and in a similar way as has happened in other transport sectors, the increased use of lighter materials such as plastics, composites and light alloys is postulated as part of the solution.
In order to meet the second challenge -reducing the amount of waste- Directive 2000/53/EC on end-of life vehicles states that no later than 1 January 2015, the reuse and recovery shall be increased to a minimum of 95% by an average weight per vehicle and year, for all the end-of-life vehicles. Directive 2000/53/EC focuses on the reduction of waste dumped into landfills, but expresses no limits on the emission levels of the treatments applied to achieve this objective.
In this work, the Life Cycle Assessment methodology has been used in order to assess the environmental impact of all the emissions of the different treatments applied to the plastics of the end-of-life vehicles in Spain. This assessment will be useful as a reference document to analyze the improvements in the environmental impacts that the future compliance of Directive 2000/53/EC will mean and it will also show the influence of the use of plastics and composites. In addition, the emission levels obtained through this assessment will be useful to check which end-of-life treatment is the most environmentally sustainable.

TU 112

Stepwise D-Optimal design based on latent variables

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In the course of REACH, each chemical compound produced in or imported into the EU in amount of more than 1 ton has to be registered according to a number of environmental endpoints, including bioaccumulation and toxicity. Experimental determination of these properties requires a high number of animal tests. Apart from ethical reasons, animal experiments are expensive and time consuming. Therefore, the number of these tests should be kept as small as possible. This can be achieved by testing only a small representative subset of compounds, using them to build QSAR models and predict the remaining compounds.
There are several standard approaches for the selection of diverse sets of compounds for model purposes, such as factorial or D-Optimal design. The later method is frequently considered to be a better choice. The D-optimal design selects compounds using principal component analysis (PCA) of molecular descriptors. The analysis is done in one step and does not take into account the target property. Therefore, the selected compounds may not be optimal for modelling of the given property. Moreover, most labs, e.g. because of restricted capacities, test compounds not in parallel but in a stepwise procedure. The question is whether there is a better strategy that could provide better selection of compounds by taking into consideration the target property and available data.
We introduce a stepwise solution for experimental design, that utilizes the D-Optimal approach and combines it with partial least squares techniques to iteratively refine the descriptor space for the compound selection. This refinement is realized by the usage of the PLS latent variables, that are correlated with the target property, instead of principal components to extend the initially selected set of compounds. We show, that especially for global datasets, this approach can significantly increase the quality of a model, built on compounds suggested for testing, compared to a model, based on the compounds selected by the traditional D-Optimal design approach. The developed methodology was applied to predict several important endpoint required for REACH. We show that the new approach significantly decreased ($p < 0.01$, binomial test) the average RMSE about 0.05 log units and increased R2 and Q2 of the models compared to the traditional D-Optimal design.

TU 113

Hormetic effect of textile wastewaters to sea urchin early development

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The relevance of hormesis has been recognized leading to increasing concern in ecotoxicology. We applied an experimental design (optimal and sub-optimal control quality) for evaluating the occurrence of hormesis in wastewaters of textile industry, physico-chemical parameters were analyzed. We used sea urchin (Paracentrotus lividus) bioassays to assess toxic vs. hormetic effects. Sea urchin embryo cultures were exposed to untreated and treated (chemical and biological) textile wastewater at dilutions ranging from 0.1 to 10% in seawater. A total of eight culture repeats showed "optimal"cultures with normal larvae in controls >70%, and "suboptimal"cultures with <70% larvae in controls. The results showed that both untreated and treated wastewaters of textile industry at all dilutions, up to 10%, failed to induce any increase in developmental defects in sea urchin larvae in the cultures with optimal control quality (94.1% normal larvae). In contrast, developmental defects were significantly decreased in larvae reared in either untreated or treated wastewaters at levels ranging 0.1 to 0.3% when tested against control cultures displayed suboptimal quality (66.1% normal larvae). Increasing concentration-related toxicity was detected in cultures reared in either untreated or treated wastewater at concentrations ranging from 1 to 10%. These findings provide evidence that suboptimal control quality is needed for detecting hormetic effect of environmental agents in an ecosystem. Moreover, the data cast doubt about effective removal of toxic components along with wastewater treatment at the facilities investigated in this study.

TU 114

Optimization of fertilization and larval development toxicity tests using two marine sea urchin species. Study of salinity influence.

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Embryo larval development (ELD) and fertilization tests with sea urchin have been widely used in ecotoxicity studies to assess pollution of marine ecosystems, and included in a regulatory framework due to their sensitivity and reproducibility. Salinity could act as a confounding factor when determining the mentioned toxicity responses, since these biological processes properly occur in a salinity range which depends on the species. In an attempt to determine the optimum salinity range, ELD and fertilization bioassays were performed at different salinities (15 to 40.5 "ð) using two species of Atlantic sea urchin: Arbacia lixula and Paracentrotus lividus. A. lixula embryo larval development assay showed wider optimum salinity range (from 29 to 35 "ð) compared with P. lividus (from 29 to 31 "ð). Regarding fertilization assay using P. lividus as bioindicator species, the highest percentages of fertilization (90 %) were measured when the salinity ranged from 29 to 31 "ð. More research is needed concerning A. lixula, since the fertilization success did not show higher values than 50 %. In the present study it has been demonstrated that salinity could be a confounding factor when interpreting ecotoxicological results, consequently this parameter should be controlled in order to optimize the described toxicity tests.

TU 115

Comparing the response of neonates, juvenile and adults of Daphnia exilis (Herrick, 1895) exposed a toxic metals

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In this study an sensibility evaluation in neonates, juvenile and adults (5 days) of *Daphnia exilis* exposed to the metals Cd, Cr, Cu, Mn, Ni, Pb, Va and Zn was carried out in order to determine their response and propose the use of *D. exilis* like alternating method for to evaluate the toxicity of water and sediments samples of aquatic systems. Static bioessays, were done with neonates and juvenile exposed during 48h to five concentrations of each metal (0.625, 0.156, 0.078, 0.039, 0.018 mg/ L) and control without toxic. With the gathered data the lethal concentration 50 (LC_{50}) were determined for probit method. Lethal tests showed that neonates and juvenile were more sensitive to copper. LC_{50} obtained were 0.013 mg/L, and 0.010 mg/l respectively . No significant differences ($p > 0.05$) were observed between LC_{50} of Cd, Cr, Cu, Mn, Ni and Zn obtained in the neonates and juvenile assays. This data suggest that the response observed in the Daphnia exilis neonates is similar to juvenile.

TU 116

Evaluation of sensitive of veliger and pediveliger larvae of Catarina scallop Argopecten ventricosus (Sowerby II, 1842) exposed a toxic metals

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In this study an evaluation of the effects of Cd, Cr, Pb and mixtures in veliger and pediveliger Catarine scallop larvae and their settlement was carried out. Bioessays, lasting 48 hours, were conducted with 5 concentrations of each metal and of their mixture (1:1). Lethal Concentration 50 (CL_{50}), and larvae settlement percentage were determined. Significant differences between control and exposed organisms were observed at 24 and 48 hours ($p < 0.05$). Cadmium was the more toxic metal for veliger and pediveliger larvae, The Cd+Cr + Pb mixture interaction was boosted, with a magnification value of 1X. A reduction in the larvae settlement was observed 80% in Lead tests, 71% with Cadmium, 83% with Chromium and 88% with the mixture. *A. ventricosus* larvae are more sensitive to metals in comparison with *Artemia franciscana* nauplii organisms utilized to evaluate the residual waters toxicity that are spilled in the coastal zone, it is important to continue carrying out research to detect the possible damage in the scallops populations.

TU 117

Sub-lethal toxicity of zinc to Mysidopsis juniae (Crustacea: Mysidae)

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In aquatic environments, due to the great dilution factor, organisms are exposed to sub-lethal concentration of pollutants, such as metals resultant from industrial activity. Long-term exposure to this waste may cause physiological or behavioral disturbances. These effects are not detectable in acute toxicity tests, and so, the use of chronic assays poses as such a relevant approach. In this sense, the present study investigated the toxicity of zinc in a short-term chronic bioassay using a microcrustacean, *Mysidopsis juniae*, as the experimental model. Since the Brazilian legislation has yet to regulate bioassays such as this, the study aims to validate this species and this method as a viable ecotoxicological routine. The methodology used herein was modified from the norm proposed by the United States Environmental Protection Agency for *Mysidopsis bahia*. Concentrations of the test compound varied from 0.0375 to 0.60 mg/L, in 200 mL quadruplicates on 5 organisms younger than 24h per group. Two static 7-day experiments were conducted at 24 ± 1°C and 35 ppm salinity. Mortality, dry weight and length of organisms were evaluated. Mean LC50 was 0.22 ± 0.11 mg/L. Variation of length was significant reduced at the highest tested concentration, however, for the dry weight, no statistical differences were observed between exposed and control organisms. As there is only little information concerning sub-lethal effects in *M. juniae*, it is adequate to state that this method still requires adjustments for its effective standardization. However, based on this data, is reasonable to conclude that length, rather than weight, is a sensitive parameter to evaluate sub-lethal effects of zinc toxicity.

TU 118

Antioxidant action of two polyphenols, resveratrol and piceid, on a biological model in vitro.

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Among natural antioxidants found in many natural foods and beverages, the flavonoid resveratrol and piceid have been proven to be effective against a broad range of diseases whose origin can be

attributed to oxidative damage. Resveratrol is a phytoalexin, a secondary metabolite synthesized by Spermatophytes in response to stress conditions caused, for example, by exposure to UV radiation or fungal infection. Piceid, called also polidatina, is the glucosylated form of resveratrol, and, as its parental compound, also shows two isomers *cis* and *trans*; it is more resistant to enzymatic oxidation of resveratrol, enters the cell through an active transport mechanism that uses the glucose transporters, and, because of its higher solubility in water, is more efficiently absorbed from the mammal's intestine. This work was planning to evaluate the antioxidant action of these polyphenols on a biological model *in vitro* and to assess, then, any protective action of these stilbenes when the same organelle are exposed to toxic environmental substances known to give rise to a state of oxidative stress. The choice of using a mitochondria-based bioassay was determined by the need to clarify the biochemical mechanisms underlying the antioxidant effect of resveratrol and piceid on natural membranes. In particular, starting from some studies conducted to date on model membranes (<*ie*. synthetic organelles like micelles or liposomes), we tried to investigate resveratrol and piceid antioxidant action performed at mitochondrial membranes, using frozen beef heart mitochondria. Initially, they has been utilized with the classical mitochondrial endpoint, <*ie*. respiratory chain inhibition. Then, because it is reported that the antioxidant activity of polyphenols is linked to their ability to block or slow down the lipid peroxidation, reacting with peroxy radicals formed in the propagation phase, we investigated the ability of resveratrol and piceid to interact with initiator radicals, since the possibility exists that oxidation is inhibited at the stage of initiation. Indeed, the action of resveratrol and piceid as a scavenger against radical initiators and propagators of membrane oxidation, and its effectiveness in preventing the lipid peroxidation in different types of substrates, is well known.

TU 119

Genotoxicity induced by amorphous silica powders in murine alveolar macrophages (RAW 264.7) and human epitelial lung cells (A549). Effect of dimension and superficial morphology

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No sufficient evidence exists about the health hazards caused by nanosized amorphous silica, nor as regard its superficial conformity. The aim of the study is to investigate the potential genotoxic effects of silica particles with different superficial morphology (mesoporous and dense spheres) and dimension (250,500 nm), by in vitro experimental models. Pure quartz, vitreous silica and imogolite (1 nm diameter) were also investigated. Murine alveolar machrophages (Raw 264.7) and human epitelial lung (A 549) cell lines have been used as representative of occupational and environmental exposure. Genotoxicity was evaluated by Comet Assay (4 and 24 hrs exposure) and Micronucleus Test. Cytotoxicity was tested using Trypan Blue method. Cell lines have been treated with 5-10-20-40-80 µg/cm2 of different silica-based compounds. The same doses of exposure were tested by Micronucleus test. MMS was used as positive control in Comet assay, Mytomicin C for micronucleated cells.
Cell suspensions exposed to the lowest dimension of porous particles show a particular distribution of these particles inside the cell, with respect to the one across the slide. When observed with TEM, cells treated with smaller porous powders showed enlarged vacuoles filled with the particles, indicating an active endocytosis process.
Data analyses confirmed crystalline particles to possess genotoxic activity. Interestingly, amorphous silica particles were also found to possess genotoxic activity.
In particular, physical characteristics like superficial porousity and dimension seem to play a role in modulating the genotoxic potential.
Even in absence of clear dose-effect trends, an effect of superficial morphology was found to modulate genotoxic and cytotoxic potential of amorphous particles in both cell lines: increased MN frequencies, increased degree of DNA fragmentation and decresed viability were induced by particles with porous surface in comparison with dense ones. On the other hand an effect of particle dimension was found as well: smaller dense particles induced higher frequencies of MN, higher amount of DNA migration and loss of viability.
Vitreous silica and imogolite were not found to possess genotoxic activity at the tested doses.
Future research directions are going to extend the study to pure amorphous silica particles at nano-metric scale and to investigate the mechanisms of cell damage induced by amorphous SiO2.

TU 120

Possibility to use porcine cumulus oocyte complexes (COCs) as in vitro toxicology assessment of heavy metal environmental pollutant bioavailability

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This work aimed to study the possible method to culture female porcine cumulus complexes (COCs) for use as in vitro toxicology assessment of heavy metal contamination. The oocytes isolated from ovaries were round in shape and surrounded by zona pellucida with layers of cumulus cells ranging between 90-400 micrometer in diameter. COCs were classified into intact-, multi-, partial-, completely denuded oocyte, and expanded cumulus cell layer at the percentage composition of 53.86%, 14.32%, 4.32%, 19.20%, and 8.30%, respectively. COCs classified as Type I and II were further investigated for morphological changes by culturing at 37°C with 5% CO2, 95% air atmosphere and high humidity for 48 h. in culture medium with and without hormones induction. On the early stage of culture, the oocytes were still in a round shape, and surrounded with zona pellucida and several cumuli cell layers. Expansion of the cumulus cell layers was observed when the cells were cultured for longer periods. Compared to the COCs induced with hormones, the groups that were cultured without hormone appeared in a spherical shape with process of sticking granular end point into the zona pellucida of oocyte. Meanwhile, at the cumulus cell surface, they were many microvili communicating with the surrounding cumulus cells. Interestingly, morphological changes of the complexes were observed after culturing for 24-48 h. In the groups supplemented with hormones (FSH, LH, and estradiol), the COCs showed the expanded cumulus layers, where the cumulus cells were peel off from the oocyte surface. For the round-shape cumulus cells, they adapted themselves into either an elongate shape or a columnar shape, and no communication between microvilli of cumulus cells. As a result, peel off, expansion and elongation of the cumulus cells from the oocytes occurred to allow oocyte maturation after culture with hormones. The further study is to use COCs culture cells to assess cadmium and copper toxicity.
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TU 121

Soil health assessment through primary coelomocyte culture of Eisenia fetida: In vitro exposures to elutriates of artificially contaminated soils and real soils

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Exposures to contaminated soils of individuals *Eisenia fetida* are widely used to assess soil health, being some of the tests applied with such a purpose well stablished and standarized by the OECD. However, recently a protocol for coelomocyte primary culture of *E. fetida* have been designed, and it was validated through the exposure of cells in vitro to different metals and performing the Neutral Red Retention (NRR) assay to assess cell viability. Although the *in vitro* exposures of coelomocytes to a single metal compound was tested, the soils are usually polluted with mixtures of different contaminants, and therefore the coelomocyte primary cultures should be also tested with real soils to validate them as a tool for soil health assessment. Elutriates of OECD soils artificially contaminated with Cd as well as elutriates of mine soils were extracted and primary coelomocyte cultures were exposed to elutriates for 24 h. The cell viability was assessed by the NRR assay and riboflavin content and the activity of the MXR were also measured to detect the effect of the elutriate exposure. The results showed that elutriates of the contaminated OECD soils decreased the NR retention of coelomocytes in a dose-dependent manner, and the riboflavin content was also reduced. This response seemed to be a general stress signal since the decrease was not dose-dependent. In addition, the activity of the MXR decreased, indicating a possible inhibition of the transport of toxic compounds outside the cell (i.e.damaged detoxification). The exposures to elutriates of mine soils showed also a decrease of the NRR capacity, and a similar decrease of the riboflavin content was also detected. The results of these experiments indicate that the *in vitro* exposures of coelomocyte primary cultures could be a useful tool for assessment of soil health.
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TU 122

Application of the modified Comet assay (Fpg) to three fish cell lines for genotoxicity assessment of pollutants

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In a context of growing regulatory pressure, the need for in vitro model in genotoxicity assessment is currently increasing. This apply to the need for genotoxicity testing of new and existing chemicals (REACH), to the monitoring programs of water quality recommended in the Water Framework Directive and responds to the societal demand to avoid testing on whole organism. Amongst the known genotoxicity biomarkers frequently used to assess genotoxocity potential of pollutants, the modified Comet assay using restriction enzyme allows an increased sensibility of the Comet Assay. Thus, in this work, it has been chosen to detect genotoxicity at environmentally relevant concentrations, since most of the aquatic genotoxic contaminations are characterized by chronic exposure at low levels of concentration. The Fpg restriction enzyme has been chosen, amongst other, for its capacity to detect various lesions such as base oxidation or alkylation, therefore broadening the type of DNA damage detected by the Comet assay.
First a Fpg modified comet assay has been applied to three piscine cell lines (RTL-W1, RTG-W1, PLHC-1) exposed to various model genotoxicants exhibiting known mode of actions (MMS, H2O2[3DOTS]).This allowed to assess and compare the feasibility of the modified comet assay with cell lines currently used in ecotoxicological studies (DNA damage basal level, cell aggregation, response to genotoxicants). Second, the genotoxicity potential of different classes of environmental chemicals (phytosanitary products, heavy metals, pharmaceuticals[3DOTS]) was tested on some of those cell lines.

TU 123

Results of an international round robin study with the Ames fluctuation test

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The German mirror committee of ISO TC147 SC 5 WG 9 'Genotoxicity' organised an international round robin study on the Ames fluctuation test (ISO DIS 11350). Nineteen laboratories from seven countries participated in the study. The laboratories had to test four coded water samples. Sample one, a river water sample, was used as a negative control water sample and for spiking with strain-specific mixtures of mutagens (samples two and three). Sample four was an effluent from an industrial waste water plant. Validity criteria were fulfilled in 86 to 100 % of all test conditions (bacterial strain / ± S9-mix). The overall test sensitivity was 100 %. The test specificity ranged from 80 to 100 % depending on the test condition. For statistical evaluation an arcsin-square-root transformation was applied to generate a metric data set. An ANOVA was performed to identify samples with a significantly higher numbers of revertants. A threshold value derived from a large set of negative controls was used in a final step to exclude results of individual tests

with unexpected low numbers of spontaneous revertants. The lowest ineffective dilution values (LIDs) were then determined by this new approach. The variability of the LID values among the laboratories amounted in most cases to no more than two dilution steps around the median. The data produced in this round robin study proved a sufficient reproducibility of the test procedure and provide validity data for the finalization of the ISO standardization process.

TU 124

Improvement on frozen mitochondria bioassay: a methodological remark.

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The protocol for the bioassay with the mitochondria of beef heart frozen at -22 ° C, developed by Iero et al. (2003) requires that the reaction cell is thermostatically controlled at 25 ° C: this value was chosen because it is used as a reference for the state environmental standard (Standard Ambient Temperature and Pressure, SATP). The choice is not, therefore, been supported by assessments on the effectiveness of the test at this temperature, but was dictated by the practice of reporting results to a standard temperature value. Finally, it was decided to make a comparison between the working temperature of 25 °C and 37 °C, chosen as close to the body temperature of cattle (estimated to be 38.6 °C) then working temperature 'normal' mitochondria extracted from ox heart. In the first phase, a series of tests only for the basal respiration were carried out in order to compare respiration rates and the quality of the regressions obtained, and subsequently mitochondria have been tested for toxicity using some known mitochondrial toxic substancesthe (e.g. Pb (NO3)2) to compare the trend of effect to the two temperatures. Running the tests at 37 °C, it can therefore calculate the toxic effect of the compound at concentrations lower than those attainable with the test at 25 ° C: it can be concluded that the bioassay with frozen beef heart mitochondria is more sensitive and accurate at 37 °C. This reason, together with considerations concerning the rate of respiration higher (due to higher enzyme activity) and increased signal stability, lead to the conclusion that in order to optimize the performance of the test is preferable to control the temperature of the cell reaction at 37 °C.

TU 125

A novel contact bacterial bioluminescence assay with Photorhabdus luminescens for testing of freshwater samples including sediments

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Testing of the bioluminescence inhibition with *Vibrio fisheri* has become a world standard in rapid screening of ecotoxicity. However, ecological relevance of using this marine bacteria for assessment of freshwater samples has been disputed. Further, use of the bioluminescence assay for colored or turbid samples is limited with respect to possible false positive results (attenuation of the luminescence by the colour). Here we present development of a novel assay with the luminescent bacteria *Photorhabdus luminescens* originally isolated from soil, which aims to overcome both above mentioned limitations. Low-salt media are used for the culture of *P. luminescens*, and the rapid kinetic design (also known as 'Flash-test' at *V. fisheri*) allows contact toxicity testing of the turbid and coloured samples such as sediment suspensions. Experimental protocol and the sensitivity has been tested with a number of various samples including model chemicals and complex sediments and compared with *V. fisheri* test. The results demonstrate good applicability of the novel test for direct toxicity assessment in complex field samples. Supported by the projects INCHEMBIOL (Ministry of Education C.R. - MSM0021622412) and CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 126

Effects on cholinesterase activity in erythrocytes of wild birds exposed in vitro to lead, cadmium and their binary mixtures

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Lead and cadmium effects on erythrocytes have been widely reported, including calcium and potassium homeostasis alteration, changes in cell membrane, osmotic alterations and mechanical fragility of the membrane, synthesis inhibition of haem group, interferences in oxygen exchange, alterations in carbohydrates and energy metabolism, cholinesterase inhibition, and oxidative stress induction due to reactive oxygen species (ROS), as well as alterations in antioxidant mechanisms. Although cholinesterase inhibition by metals is still a controversial topic in scientific literature. The aim of this study was to evaluate the lead and cadmium-induced effects in mallard (Anas platyrhynchos), Eagle owl (Bubo bubo) and Eurasian buzzard (Buteo buteo) erythrocytes exposed in vitro to cadmium, lead and cadmium:lead combination (1:10) in propotiortions equal to those previously found in wild birds. After determination of cell viability with Propidium Iodide, different concentrations were selected in order to evaluate cholinesterase activity in haemolysates. Cholinesterase activity was measured according to Ellman's method. Variations in this enzyme system after exposure to these metals depended on the species used. In general trends, mallard erythrocytes showed a decrease in this activity. In Eagle owl erythrocytes, a great increase in this activity was observed. In buzzard erythrocytes no significant changes were observed following lead exposure, whereas cadmium provoked an increase in this activity in all doses, and metal mixture produced an increase at EC25 and EC50. Little is known about the effects of these metals on cholinesterase activity in erythrocytes, although a decrease in rodents exposed in vivo to lead was observed. On the other hand, no variations were found in workers exposed to lead. For cadmium, it has been observed an enzyme inhibition in human and rodent erythrocytes exposed in vitro. However, activation on cholinesterase activity has been related with membrane alterations in human erythrocytes. In conclusion, the increase in cholinesterase activity in raptor erythrocytes exposed to cadmium and/or lead, especially in Eagle owl, could be a sign of structural membrane damage. Acknowledgments: Work supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS, NOVEDAR-CSD00C-07-22204) and Seneca Foundation (08758/PI/08). Special thanks to P. María-Mojica for his help in sampling.

TU 127

Acute and chronic toxicity testing of pharmaceutical compounds on primary cultures from the zebra mussel (Dreissena polymorpha)

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There is growing concern over the potential toxic effects of emerging contaminants, particularly pharmaceuticals, being released into the aquatic environment. There is much renewed interest in alternative toxicity test methods, and the use of alternative (invertebrate) animal species owing to the recent REACH legislation. *In vitro* techniques are one such alternative method and are an important tool in investigating the mechanisms of action of toxins on individual cells or tissues removed from the process of the entire organism. A recently developed method for the *in vitro* culture and maintenance of cells and tissues from the zebra mussel (*Dreissena polymorpha*) was used in toxicity tests to investigate the acute (cytotoxicity) and chronic (glutathione S-transferase (GST) induction) effects of the non-steroidal anti-inflammatory drug diclofenac and the lipid regulator gemfibrozil. This technique offers a screening evaluation method providing information on both the cytotoxicity and mode of action of these contaminants on cells in suspension and tissue explants (respectively) on the three most common targets of environmental pollutants in the zebra mussel: hemocytes, gill and digestive gland. Both compounds showed similar levels of cytotoxicity with gill and hemocyte cells show a significant decrease in viability at 0.001 mg/L. The trypan blue exclusion test was found to be more sensitive than the mitochondrial MTT reduction assay. An increase in GST expression was seen in both gill and digestive gland explants after a preliminary 24h exposure to 1 and 10 mg/L gemfibrozil indicating their potential biotransformation capacity. Toxicity results from these acute and chronic *in vitro* exposures shall be presented along with their comparison to in vivo whole animal tests to assess their sensitivity.

TU 128

Novel approach for ecotoxicological assessment of pesticides: Nnn-invasive neural and vascular responses in aquatic oligochaete, Lumbriculus variegatus, exposed to pyrethrins

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Oligochaete worms are key non-target macroinvertebrates in terrestrial and freshwater ecosystems. Aquatic and terrestrial pollution has attracted much public interest in the past two decades. Yet despite a major research thrust in this field, a non-invasive sensitive biomarker that would allow repeat measurements to monitor both onset and recovery from toxic insults has not emerged. Sub-lethal effects of agricultural pyrethrin pesticides [commercial formulations and pure permethrin (PMT) and fenvalerate (FEN)] on nervous and vascular functions of the freshwater oligochaete Lumbriculus variegatus were studied non-invasively following immersion (upto 6 h) in aqueous concentrations ranging from 0.001 to 10 ppm. Neurotoxicity effects evaluated included conduction velocity (CV) of the medial giant fibers (MGF), helical swimming, and body reversal behavior. Vascular system effects included blood pulsation rate in tail segments. Both PMT and FEN induced a significant ($P < 0.05$) dose-dependent decrease in the CV of the MGF, loss of swimming and reversal reflexes, and increased frequency of blood pulsations. Results indicated that FEN was more toxic than PMT and that commercial preparations of both pyrethrins were more toxic than the respective pure compounds. Effects of commercially prepared FEN occurred at doses as low as 0.001 mg/L and usually within 3 h of chemical exposure. Thus pyrethrins caused marked nervous, behavioral and vascular effects even at very low concentrations. The CV technique reported here is sensitive, gives accurate and reproducible results, does not require anaesthesia or immobilization, and allows quantification of neurotoxic effects with non-invasive repeat measurements in the same oligochaete. This novel technique could be used as an early-warning biomarker to monitor toxicity of pesticides and other chemicals in non-target aquatic and also terrestrial (earthworms; not reported here) organisms.

TU 129

EROD as a biomarker in zebrafish embryo

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The present study is part of the joint research project "DanTox" funded by the German Federal Minister of education and Research (BMBF). The overall aim of this project is to develop a eukaryotic test system which can be used to investigate the ecotoxicological effects of contaminated sediments. In addition to native sediments and sediment extracts, selected model chemicals from the list of priority substances of the European Water Framework Directive (EWFD) will be tested. These substances are methylmercury(II)chloride (heavy metal), chlorpyrifos (organophosphate insecticide) , aroclor 1254 (polychlorinated biphenyl, PCB) and Bisphenol a (diphenylmethane derivative). The visualization of specific activation of the aryl hydrocarbon receptor (AhR) via live cell imaging is one of the bioassays that will be developed. The measurement of ethoxyresorufin-O-deethlyase (EROD) activity in fish is a well-established in vivo biomarker of AhR-mediated induction of cytochrome P450-dependent monooxygenases (CYP1) and is a highly sensitive indicator of specific planar polycyclic aromatic hydrocarbons (PAH) as well as of structurally related compounds. The CYP-induced fluorescence signal results from the ability of CYP1A to convert the substrate 7-ethoxyresorufin to the fluorescent product resorufin. The transparency of the zebrafish embryo allows a direct detection of an EROD-induction via epi-fluorescence microscopy and confocal laser scanning microscopy (CLSM) according to Otte 2010. The aim of this study is to develop a stable and easy-to-handle EROD-assay by elucidating the spatio-temporal pattern of basal and induced CYP1 activities in zebrafish embryos. β-Naphtoflavone (10 µg/L) is used as a positive control and shows CYP1 induction at any time of inspection after 24, 48, 72, 96 and 120 hours post-fertilization. Due to their molecular structure, methylmercury(II)chloride and chlorpyrifos showed no EROD-induction. Aroclor 1254, on the other hand, is a well-known inducer of CYP1A and interesting spatio-temporal patterns can be expected. The role of Bisphenol a as an inhibitor of CYPs, however, has still to be clarified. In order to ensure that there is no toxic effect related to the substances that influences EROD activity, fish embryo toxicity tests (FET) are carried out. Semi-static exposure systems turn out to be well-suited to ensure a constant distribution and uptake of heavy metals as well as of hydrophobic PAHs.

TU 130

In vivo and in vitro hepatic transcriptional and proteomic response in Oncorhynchus mykiss exposed to ethinylestradiol and benzo(a)pyrene

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The European Union (EU) established in 2007 the chemical regulatory legislation Registration, Evaluation, Authorisation and Restriction of Chemical Substances (REACH) with the objective to improve the human and environmental health status. Through performing assessments, early identification of potentially hazardous chemical compounds could be made, thus prohibiting or regulating their production and use. It is estimated that approximately 30,000 chemicals are in need of risk assessment due to their production volume, persistent and/or bioaccumulation potential (vPvB). REACH rely on using aquatic organisms, foremost fish as a test organism in these assessments resulting in the potential use of millions of fish for regulatory testing. Besides the high costs, the implementation of the 3Rs (reduction, refinement and replacement) are of con-

siderable ethical importance. Therefore, search for and evaluation of alternative or non-animal testing methods to serve as surrogates for whole fish tests are of high priority.
This study has evaluated the hepatic transcriptional responses in *Oncorhynchus mykiss* exposed *in vivo* and *in vitro* to benzo[a]pyrene (BaP) and ethinylestradiol (EE_2), as to determine whether primary hepatocytes may serve as an (alternative) non-animal test method. Analyses focused on characterising the global transcriptional changes and determining whether the cellular responses where manifested at the protein level in both *in vivo* and *in vitro* experimental models. In addition, bioassays involving cytotoxicity (metabolic activity and membrane integrity) were measured on *in vitro* samples. Results presented will determine whether primary hepatocytes exhibit comparative responses to that of native liver cells in rainbow trout.

TU 131
AlterREACH - an evaluation of non-animal testing methods as environmental endpoints in REACH
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The Registration, Evaluation, Authorisation and Restriction of Chemical Substances (REACH) is the European Union's chemical regulatory legislation for new and existing chemicals. The REACH legislation requires that chemicals, considered to be persistent, bioaccumulative and/or toxic (PBT), have to undergo regulatory testing using aquatic vertebrates. Estimates are that about 30,000 single chemicals may require testing with up to three million fish using currently available and validated methods. With the strong drive towards implementing the 3Rs (reduction, refinement and replacement) into ecotoxicological testing, the need for developing and evaluating alternative (non-animal) experimental methods is clearly warranted. This multi-disciplinary project, involving various international research groups, intends to meet this challenge through the development and evaluation of alternative test methods to carry out rapid, reproducible screening for the bioaccumulative and toxic properties of chemicals. Ecotoxicological testing using zebrafish (*Danio rerio*) embryos, complimented by *in silico* quantitative structure-activity relationship (QSAR) models, *in vitro* methods (cell cultures) and toxicogenomics, will be assessed. Results will then be compared to findings from *in vivo* experiments to evaluate whether these approaches may be applied as part of the regulatory testing within REACH.

TU 132
Alterations in gene expression after exposure of zebrafish (Danio rerio)embryos to sediment extracts
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The present study is part of the joint research project DanTox, which has the objective of developing a combined vertebrate-based sediment contact assay to investigate mechanism-specific endpoints indicative of the exposure to sediment bound pollutants. Specifically, this study focused on the analysis of changes in gene expression patterns in embryos of zebrafish (*Danio rerio*) after exposure to contaminated sediments. Together, these goals will contribute to the development of a targeted cDNA-microarray which will be useful for environmental screening.
Three sediment samples, one from a contaminated site located in the outer Vering Canal in Hamburg-Wilhelmsburg and two from the Rhine River, each representing a different level of contamination, were used. After freeze-drying, the sediments were extracted with acetone in a Soxhlet apparatus. Fertilized zebrafish eggs were exposed to the extracts for 48 hours according to a standardized fish embryo toxicity test guideline (DIN 38415-6). Subsequently, mRNA was extracted from the embryos and subjected to quantitative real-time polymerase chain reaction (Q-RT-PCR) to analyze the transcript abundance of selected genes. Specifically, a battery of phase I and II metabolism genes (CYP1A1, AHR2, GST and UGT1A1) as molecular biomarkers of dioxin-like exposure as well as MT1 and MT2 as markers for metal exposure were measured. Clear increases in the transcript abundance of CYP1A1, GST and UGT1A1 were observed. The greatest fold changes, up to 600-fold, were observed in embryos exposed to the extract from the Vering Canal. Dose-response relationships for CYP1A1, GST and UGT1A1 were observed in embryos exposed to extract from the least contaminated Rhine site, Ehrenbreitstein. No comparable dose-response relationships were observed in embryos exposed to extracts from either the Vering Canal or the second Rhine site, Altrip. This was likely due to already maximal induction of transcript abundance in embryos exposed to the least concentration of both sediment extracts. Transcript abundance of MT1 and MT2 was not significantly altered in embryos exposed to extracts from any of the sites. Based on the results it appears that dioxin-like pollution may be more prevalent than metal pollution in these areas.
A goal for the future is to correlate results from gene expression analysis to results obtained with in vitro biotests such as the EROD assay and the H4IIE assay for the same samples.

TU 133
Embryotoxic and Ah-receptor-mediated effects of sediment extracts from the Vering Canal of Hamburg and the Rhine River in Danio rerio embryos
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The European Water Framework Directive (EWFD) aims to achieve a good ecological and chemical status in the surface water of European rivers until 2015. However, there is still need for basic research in order to fulfill this legal obligation. In particular, since sediments and particulate matters are well known for being sinks and secondary sources for pollutants, applied sediment toxicology is of major relevance in achieving the objectives set by the EWFD.
The present study is part of the joint research project DanTox, which - among other specific endpoints - investigates embryotoxic and AhR-mediated effects of sediment extracts from the Rhine River and the outer Vering Canal of Hamburg-Wilhelmsburg using Danio rerio embryos.
Sediment samples were collected from three sites, two from the Rhine River (Altrip (A) and Ehrenbreitstein (EB)) and one from the outer Vering Canal of Hamburg (VC). Freeze-dried sediments were extracted with acetone in a Soxhlet apparatus. In order to investigate dioxin-like activity in embryos of Danio rerio a combination of the fish embryo toxicity test (FET) and the cell culture based EROD-assay was applied.
Exposure to the sediment extracts led to different malformations in zebrafish embryos. The LC50 values ranged from 1.3 mg SEQ/ml (VC) to 25.8 mg SEQ/ml (EB) Moreover, the results showed a clear increase of the acute embryotoxicity over time (24, 48, 72 and 96 h) for all three samples. Exposure to TCDD led to a decrease in EROD activity below basal level of the negative control. This was unexpected, since TCDD is one of the strongest EROD inducers and used as a positive control in the cell culture based EROD assay. These results indicate that the barrier function of the chorion prevents TCDD from entering and harming the embryo. In contrast to TCDD, the exposure to sediment extracts showed an EROD induction above the basal level of the negative control.
To achieve the goals of the EWFD it is of particular interest to establish a combined vertebrate-based sediment contact assay with different biological endpoints (e.g. embryotoxicity, teratogenicity, and AhR-mediated toxicity). Therefore, the present study is a first step for establishing such specific biomarkers to determine the ecotoxicological effects of sediment-bound pollutants. However, there is still need for research to improve and standardize the measurement of dioxin-like activity and embryotoxicity in sediments using zebrafish as a vertebrate-based test model.

TU 134
Evaluation of epigenetic DNA modifications in a zebrafish (Danio renio) cell line model
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Influences of environmental factors, subsequent epigenetic DNA modifications and potential development of diseases emphasize the importance of epigenetics and its application as a potential environmental biomarker. However, the study of multigenerational epigenetic DNA modifications is a novel approach in ecotoxicology which can be both time and labour consuming. Therefore the potential of a high throughput in vitro screening method were assessed by exposing a zebrafish cell line to model substances 5-azacitidine, ethinylestradiol, diethylstilbestrol and arsenite. Methylation of CpG islands of selected genes were monitored by bisulphite conversion and high resolution melt analysis after short term exposure to sub-lethal concentrations. The genes selected for targeted CpG methylation analyses were DNA-methyltransferase 1, vitellogenin 1 and c-Myc. Results will be compared to existing in vivo data for validation of a high throughput in vitro alternative zebrafish model.

TU 135
Combining the acute Danio rerio embryo toxicity test with the assessment of endocrine disruption endpoints
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Effluents from the wastewater treatment plants (WWTP) are important sources of pollution in the natural water bodies. Whole effluent toxicity tests evaluate the overall toxicity of chemical mixtures present in these effluents. One of these tests is the fish embryo toxicity (FET) test with zebrafish (*Danio rerio*) which is a modern test recently standardized by ISO. Nonetheless, the embryo mortality, which is evaluated in the standard test, may miss more subtle endpoints such as endocrine disruption, which is a known environmental problem for fish populations and other ecosystem components. In the present work, we combine the standard FET test with determination of the estrogen-controlled genes by PCR method. WWTP effluent samples have been collected across the European Union (the FATE-SEES project coordinated by the JRC, Ispra, Italy), and tested (i) by the in vitro reporter gene test for estrogenicity and (ii) by the modified FET test. We evaluated the applicability of the PCR within the standard test, and compared the results of both in vitro and in vivo methods. Outcome of the currently running work will be presented.
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TU 136
Tweaking the fish embryo test with zebrafish (ZFET) for a refined alternative toxicity evaluation of pesticides
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Great efforts have been made to develop the zebrafish embryo toxicity test (ZFET) as an alternative to ethically questionable and costly animal experiments. The recently revised European directive on the protection of animals used for scientific purposes (DIRECTIVE 2010/63/EU) now specifically tolerates the use of not yet independently feeding embryonic and larval stages of non-human vertebrates for testing, what will further promote the development of methods to broaden the scope of the ZFET within the regulatory framework. A large number of chemicals have already been tested in the ZFET, but pesticides were found to be underrepresented and a lack of mode-of-action (MoA) specific testing is apparent. As for many other substances, pesticide effects on the zebrafish embryos often occur only after 48hpf, thus outside the time frame of the standard ZFET to date. In line with current validation activities towards a OECD guideline for the ZFET, we tested several pesticides in the 48h-ZFET and in an extended 120h-ZFET to investigate the consequences of a prolonged test period on the toxicity and the sensitivity compared to the acute fish toxicity. Additionally, embryos exposed for 48h to a selection of pesticides at morphological effect concentrations below the EC20 were subjected to microarray analyses to study potential MoA specific responses of the transcriptome. As anticipated, the first 48hpf were found not to be the most sensitive exposure period for the majority of pesticides tested and morphological effects were often confined to minor, non-lethal effects. Exposure beyond 48h led to either delayed or failed hatching or to severe and often lethal effects. However, differentially expressed genes at 48h relate to molecular functions, which can be linked to the morphological effects observed and to the given MoA of the pesticide, even at the very low concentrations investigated. These findings provide strong evidence, that the application of transcriptomics to a mode-of-action specific testing of pesticides in the ZFET can increase the sensitivity and improve the mechanistic understanding without prolonging the test period.

TU 137
Acute and chronic zebrafish embryotoxicity toxicity for two narcotic chemicals using a passive dosing exposure system
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Recent computational models, such as the target lipid model, have been used to derive envi-

ronmental quality objectives that are intended to be protective of chronic toxicity endpoints. However, reliable experimental chronic/early life stage (ELS) effects data for fish are limited and further test data are needed to confirm this prediction. An effort has been underway for the past several years to develop a zebrafish embryotoxicity (zFET) OECD Test Guideline, currently in draft form, to meet various drivers to Reduce, Refine and Replace the use of vertebrates in animal testing. Here we present an adaptation of this method using a closed system to allow for longer-term embryo toxicity testing of sparingly soluble or volatile substances. A passive dosing system using test substance-saturated silicone oil in silicone tubing was employed to generate constant test concentrations without carrier solvents. We present acute and ELS endpoint data for 1,2,3-trichlorobenzene as well as phenanthrene, two narcotic compounds of differing aqueous solubilities. The observed acute and chronic effect concentrations and the empirical acute to chronic ratios are compared to predictions derived using the target lipid model. Implications of this comparison and need for further research are discussed.

TU 138

Update on the OECD validation study on the transferability and intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test

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The OECD Acute Fish Toxicity Test Guideline (TG 203) is an integral component in the environmental safety assessment of industrial chemicals, agrochemicals, pharmaceuticals, feed stuffs, and biocides. One of the most promising alternative approaches to the acute fish toxicity test is based on the use of zebrafish embryos. In 2005, the German Federal Environment Agency submitted the draft TG on 'Fish embryo toxicity (FET) test' to the OECD Test Guideline Program and a supportive Background Paper. Subsequently, OECD established the ad hoc Expert Group on the Fish Embryo Toxicity Test. Based on the outcome of expert meetings, OECD decided to perform a validation study (coordinated by ECVAM and steered by a validation management group). The validation study aims to evaluate the transferability, and the intra/interlaboratory reproducibility of the Zebrafish FET (ZFET). Newly fertilised zebrafish eggs (20/concentration and control) are exposed for up to 96h to chemicals. 4 apical endpoints are recorded daily as indicators of acute lethality in fish: coagulation of embryo, lack of somite formation, non-detachment of tail bud from the yolk sac and lack of heart-beat. LC50 values are calculated for 48h and 96h exposure. 20 chemicals are tested at 5 different concentrations in 3 independent runs in at least 4 laboratories with appropriate controls. The presentation will give an overview on 1) the validation study design, 2) preliminary results 3) positive and negative controls and 4) the correlation of the ZFET with acute fish LC50 data.
"Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries'"

TU 139

Development of a long term strategy for chronic ecotoxicity and animal alternatives

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The ILSI Health and Environmental Sciences Institute (HESI) held a workshop June 2010 on the "Development of alternatives to chronic ecotoxicity tests: predicting early-life stage and endocrine-mediated toxicity in aquatic vertebrate species."The meeting was attended by over 50 scientists representing industry, academia, government, and NGOs from North America, Europe, and Japan and was aimed at examining critical science needs related to the development of alternatives to chronic fish toxicity and endocrine disruption tests. It is estimated that demands for long-term (chronic) fish toxicity testing represents approximately 6% of the total animal testing needs for REACH, and there is a great need to develop scientifically-sound alternative methodologies that can accurately predict chronic toxicity and endocrine disruption while maximizing the use of limited resources. Various approaches such as the use of fish cell lines, modeling, and new technologies (such as 'omics) are promising strategies to improve testing and should ultimately result in reducing animal use. However, their application to chronic toxicity and endocrine disruption tests hinges on a fundamental understanding of chemical's adverse outcome pathway. By focusing on the concept of adverse outcome pathways in reviewing new and existing data and methodologies, the workshop participants began the design of a long-term platform to address animal alternative testing strategies for chronic toxicity and endocrine discuption.
Discussions at the workshop led to eight major recommendations that will aid to focus and refine the development of alternative methodologies for ecotoxicology: 1) Focus on and further develop adverse outcome pathways; 2) Perform critical evaluations of existing tests; 3) Increase global coordination of test development across disciplines & sectors; 4) Create centralized data repositories for fish & amphibian data; 5) Develop standard reference chemical lists for chronic ecotoxicity & EDC testing; 6) Promote increased use of fish and amphibian embryo-based toxicity tests to predict chronic toxicity; 7) Modify current EDC tests for use in quantitative risk assessment & promote the development of robust alternatives; and 8) Consider mixture toxicity and effluent discharges when designing alternative strategies. This poster will highlight these conclusions and provide an update on the ongoing HESI committee activities.

TU 140

Molecular assays enabling 'animal-free' toxicology tests

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The exploitation of genetic endpoints provides the potential to develop in vitro screening procedures. The resulting high throughput toxicology screens would have utility for rapid compound assessment within pharmaceutical pipelines and in tiered frameworks for the assessment of industrial and agro-chemicals.
We have developed a molecular assay for gene expression analysis based on chemiluminescence which utilises the high signal output from acridinium-ester (AE) and has the potential to measure changes in gene expression without amplification technologies. AE-labelled probes bind in a sequence-specific manner to RNA and light output is directly proportional to the number of transcripts present. A homogenous assay format can be achieved by exploiting the way the AE is protected by the nucleic acid duplex. Unbound probe can be removed by a chemical means which leads to very low background levels hence simplifying the assay format.
This technology is capable of achieving 0.1fmol sensitivity with a dynamic range of 4 orders of magnitude and has previously been exploited to measure endocrine disrupting chemicals in aquatic toxicology applications1,2,3. We are currently optimising assays for genotoxicity (RAD51C, Cystatin A, p53) and steroidogenesis (CYP21A2, CYP19A1 and HSD2) endpoints in order to demonstrate broader application to toxicology testing.
The assay requires only a tube luminometer and waterbath and has the potential to provide a cheap and precise alternative to qPCR. Development of novel AE molecules will allow dual measurement of control and marker genes in a single measurement and use of target capture using magnetic beads covalently modified with streptavidin will also further improve sensitivity. When combined, these technologies will provide a powerful toolkit to measure steriodogenesis and genotoxicology markers.
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TU 141

A review of freshwater fish sublethal tests over the past 60 years

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Pressure on animal testing has traditionally been the purview of mammalian toxicological science, but in the past few years, more attention is given also to environmental safety. As with higher vertebrate animal alternatives, balance between reduce animal use without impairing or increasing uncertainty in risk assessment is needed. Testing demands for long-term (chronic) fish toxicity is anticipated to be the third largest pool of needs following 2-generation mammalian developmental toxicity test (OECD TG 416) for REACh. In 1984, in a review of sublethal tests, Woltering suggested that that the growth endpoint could be deleted from the sublethal fish tests, without affecting the predictive nature of the tests. As this endpoint has not been excluded and other endpoints have been added (e.g., biomass), we evaluated toxicity data from years of fish life cycle chronic, partial chronic and early life stage tests to assess the utility of the standard fish chronic toxicity endpoints, in particular the sublethal response. Approximately 500 studies involving >100 fish species were summarized using several databases, literature and unpublished industry sponsored reports. We found the most sublethal tests have been performed with the fathead minnow, rainbow trout, zebrafish, Medaka, and bluegill. The fathead minnow has the greatest frequency of use over a wide variety of research applications and use of bluegill has become uncommon since the 1990's. With the additional development of shorter tests, i.e., the 7-d growth and survival test and 21-day reproduction tests, a review of the tests and their endpoints is due. The choice of species and method is often based on what makes it a strong model for addressing new challenges in aquatic toxicology, including the identification of sensitive life-stages/endpoints or chemicals with differing modes/mechanisms of action, predicting population-level effects based on data collected from lower levels of biological organization, and exploring/understanding the emerging role of genomics in research and regulation. Much of the ability to application to chronic toxicity hinges on an understanding of chemical mode of action. A summary of the most common methods, species, and duration and will be presented, and endpoints for the tests will be explored.
KEYWORDS: Aquatic toxicity, freshwater, early life stage, fish, sublethal toxicity

TU 142

Evaluation of the efficiency of the industrial effluent treatment using the luminescent bacteria toxicity bioassay

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Introduction: The toxicity assay with the luminescent bacterium Vibrio fischeri is recommended in the assessment of environmental quality, being the only one that can be used to evaluate the toxicity of effluents before and after the treatment, due to the bacterium's ability to tolerate low concentrations of oxygen; it can also be used to evaluate the efficiency of effluent treatment applied by the industries. Considering that the release of industrial effluents containing toxic substances is a major responsible for the contamination of water bodies, control of release of these wastewater is extremely important for the supply of quality water for the population. Objective: To determine the acute toxicity of industrial effluent samples collected in the region of Ribeirão Preto, using the test with luminescent bacteria. Methods: Samples of effluent from two industries (one food and a tannery) were collected before and after treatment of the effluents, following the CETESB collection protocol. The analyses were performed using luminometer coupled to the computer with specific software Microtoxð' and culture of lyophilised Vibrio fischeri, following

the protocol recommended by the manufacturer. The assay was performed after 5 and 15 minutes of contact. A sample was considered positive when it caused an inhibition of luminescence of 20% compared to negative control. Results: In both industries studied, the raw effluent was considered toxic. However, at the output, after treatment of these industries, no toxicity was detected under the tested conditions. Conclusion: The study showed that the treatment evaluated by both industries has been effective in removing of the toxicity in relation to toxicity for Vibrio fischeri, thus contributing to preserve the environment. Information on the type of treatment used by these industries was not provided by them.
Financial support: CNPq

TU 143
Application of Hg specific biosensors to soils: a laboratory to field study
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The development of assays using a range of environmentally relevant bacterial-based biosensors for the assessment of metal bioavailability in soils and water has advanced over the past decade. In this study, two Hg specific sensors were calibrated using aqueous Hg doses for application to Hg amended and historically contaminated soils. A constitutively-marked biosensor was used to assess the presence and effect of other soil attributes. The response of the Hg specific sensors to Hg amended soil water and soil enabled a dose response to be developed allowing a prediction of Hg dose. However, when the sensors were applied to soils historically contaminated with Hg, the predictive capacity was poor, regardless of the soil solution extraction used. The presence of co-contaminants greatly constrained the applicability of the Hg specific sensors and this was validated both by the response of the constitutively-marked sensor and chemical analysis. Bacterial biosensors may well perform effectively in solutions but this application to historic soil will require further development at the interface between the test medium and the sensor.

TU 144
In vitro biological responses to 1D and 2D nanomaterials: carbon nanotubes and graphene
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Carbon-based nanomaterials are one of the most important and variable families available. They have geometries that range from spherical (as particles), to fibrous (as one-dimensional nanotubes), and sheet-like (as two-dimensional graphene) forms. Evaluation of potential adverse health effects of these diversely engineered materials requires expensive and long-term in vivo toxicological testing. We have developed an alternative 3D in vitro culture using non-adherent macrophages in order to assess the potential of these materials to induce inflammatory responses and granulomas.
Macrophages are phagocytic cells specialized in recognition, internalization and destruction of a wide variety of stimuli including foreign bodies. They play a central role in the development and maintenance of chronic inflammation, granulomas and fibrosis. Although carbon nanomaterials are non-antigenic and may persist in biological systems, it is suggested that their different geometric structures determine the induction of pathological outcomes. In this model, THP-1 cells were transferred to cell culture wells previously coated with 3% agarose and exposed for up to 14 days to sub-lethal doses of the different carbon nanomaterials. Behaviour in biological systems, cellular interaction and internalization were evaluated.
The CB, MWCNT and FLG were characterized by high-resolution transmission electron microscopy. The capacities of these ultrahigh surface area materials to adsorb biological molecules and alter cell culture media were characterized by adsorption studies and spent media analysis. Vitamins such as pyridoxine HCl and nicotinic acid present in cell culture media were adsorbed in a dose-dependent manner; depletion of other vitamins was material specific.
Macrophages internalize CB particles and MWCNT as well as FLG up to 5 μm in lateral dimension; though they do not internalize the larger FLG, they do interact and tightly adhere to their surfaces. Both the carbon nanotubes and the graphene samples appear to induce the formation of stable cell-material aggregates in the 3D microwells. In the case of nanotubes it will be shown that the cell-material aggregates show the characteristic morphological features and biomarkers of granulomas, such as those observed in recent in vivo studies. The 3D cell culture model could become an attractive alternative to in vivo testing for the prediction of granuloma formation and inflammatory responses to 1D and 2D carbon nanomaterials.

ET02 - Assessing the exposure, effects and risks of Pharmaceuticals and Personal Care Products (PPCPs) in the environment

TU 147
Environmental risk assessment of 14 major pharmaceuticals: are the current environment concentrations in Korea safe?
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Abstract
As a part of efforts to address contamination of pharmaceutical residues in Korean waters, we evaluated potential risks of fourteen major pharmaceuticals in Korea. The study pharmaceuticals, i.e., acetaminophen, acetylsalicylic acid, chlortetracycline, cimetidine, ciprofloxacin, diclofenac, erythromycin, ibuprofen, lincomycin, mefenamic acid, oxytetracycline, sulfamethazine, sulfathiazole, and tylosin were chosen based on their occurrences and potential risks in Korea. We reviewed literatures to identify data gap in aquatic toxicity, and conducted appropriate toxicity tests to derive predicted no effect concentrations (PNECs). Occurrence levels of the study pharmaceuticals were also gleaned from available literatures. As test organisms, freshwater invertebrates (Daphnia magna and Moina macrocopa) and fish (Oryzias latipes and Danio rerio) were employed. The PNECs of acetaminophen, acetylsalicylic acid, chlortetracycline, cimetidine, ciprofloxacin, diclofenac, erythromycin, ibuprofen, lincomycin, mefenamic acid, oxytetracycline, sulfamethazine, sulfathiazole, and tylosin were determined 100, 100, 10, 110, 10.6, 100, 1, 10, 7, 0.2, 18.3, 66, 44, and 20.6 μg/L, respectively. The 95% upper confidence limits of the average of measured environmental concentrations (MECs) for acetaminophen, acetylsalicylic acid, chlortetracycline, cimetidine, ciprofloxacin, diclofenac, erythromycin, ibuprofen, lincomycin, mefenamic acid, oxytetracycline, sulfamethazine, sulfathiazole, and tylosin in Korea were determined 0.023, 0.037, 0.050, 0.217, 0.006, 0.013, 0.008, 0.067, 0.059, 0.036, 0.009, 0.024, 0.103, and 0.011 μg/L, respectively. Hazard quotients derived from MECs and PNECs for 14 pharmaceuticals were less than 1, suggesting no potential environmental concerns in Korea. Potential impact of mixture exposure, however, may not be ruled out.

TU 148
Risk assessment and management of active pharmaceutical ingredient emissions from manufacturing facilities
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Active Pharmaceutical Ingredient (API) residues have been found to be widespread in the aquatic environment, albeit in most cases at trace levels, with the route to the environment predominantly being via therapeutic use and subsequent excretion to sewer. Whilst manufacturing discharges may be a low overall contributor to environmental concentrations, they need to be managed effectively so that they do not adversely affect the local receiving environment. In order to achieve this, AstraZeneca has developed a risk based approach that identifies the long and short-term concentrations, referred to as the Environmental Reference Concentration (ERC) and Maximum Tolerable Concentration (MTC) respectively, of an API which should not be exceeded in the local aquatic environment receiving the effluent from a pharmaceutical manufacturing site. Performance against these standards has been measured across AstraZeneca's global manufacturing sites through evaluation of process operations and batch scheduling, targeted determination of effluent API concentrations using LC-MS/MS and an understanding of the off-site dilution and fate of effluents.
This poster presentation provides an overview of the ERC and MTC concept, the techniques and challenges in the application of the analytical chemistry and assessment methodologies applied and summary results of the assessments of the effluent quality discharged from AstraZeneca's manufacturing sites.

TU 149
Environmental risk assessment of ionisable organic chemicals
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The environmental release of ionisable organic compounds (where behaviour depends on pH, ionic strength, etc.) represents a number of challenges for risk assessors who must select relevant and appropriate test endpoints using methods that have historically been used with more neutral lipophilic compounds. ECETOC have thus established a Task Force to review the current understanding and available literature on partitioning property data of ionisable organic compounds at environmentally relevant pHs, including estimation methods for these properties. It is anticipated that the output of the task force will lead to suggestions and recommendations for improved methods for predicting the environmental concentration of ionisable organic compounds in aquatic environments. To date, the work of the Task Force has emphasised the need for better generic environmental fate models, with appropriate soil and water properties, and other parameters required for effective environmental risk assessment of ionisable compounds. Preliminary findings include identification of the key parameters needed to better predict the bioavailability of ionisable organic compounds, which are shown to be strongly influenced by the physical-chemical properties of the substance, for instance, pKa and log Kow, as well environmental properties such as pH. Modelling activity is supported from data assembled for a physical-chemical property database for ionisable organic compounds used as pharmaceuticals. Using the best available property data a sensitivity analysis has been performed using a number of environmental fate models to determine how variability in environmental input parameters influence the predicted environmental concentration and ultimately the bioavailabilty of a substance.

TU 150
Sediment toxicity assessment and PNEC derivation for cyclic volatile methylsiloxanes
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Cyclic volatile methyl siloxanes (cVMS) are important consumer materials that are used in personal care products as well as industrial applications. These compounds have gained increased attention in recent years following the implementation of chemical legislation programs worldwide (e.g., REACH). Industry-wide research programs are being conducted to characterize the persistence, bioaccumulation and (eco)toxicity properties (e.g., PBT) for cVMS compounds. As part of this larger effort, sediment effects data (acute and chronic) were compared to effects data for other non-polar organic chemicals using the target lipid model (TLM) framework and equilibrium partitioning (EqP) theory. The TLM provided a data-rich framework that was well suited for evaluating potential effects from CVMS compounds based on structures and probable toxic mode of action (e.g., narcosis). Many of the reported acute and chronic effects data for CVMS compounds (D4, D5, D6) were above the measured aqueous solubility when evaluated on a pore-water basis using EqP with measured organic carbon partition coefficients (Koc). This suggests that effects observed in laboratory toxicity tests could be due to physical effects (e.g., oiling) in addition to chemical narcosis and have limited relevance since total sediment concentrations of CVMS compounds are relatively very low. Those chronic effects that are below the estimated solubility limit are consistent with the PNEC derived with TLM using bioavailability adjustments for high logKow chemicals (e.g.,logKow>6), which often have reduced bioavailability due to sorption and/or kinetic limitations. The suitability of this adjustment for CVMS compounds was demonstrated by comparing measured biota-sediment accumulation factors (BSAF) to predicted BSAFs using EqP with measured Koc and the bioavailability-adjusted Kow. The TLM-derived PNEC is based on a large dataset of chronic effects data for several organisms from various taxa and is a robust benchmark for evaluating potential risks due to CVMS compounds. This work supports an on-going tissue residue-based risk assessment by evaluating the application of the TLM to CVMS compounds in sediments.

TU 151
An approach to search for potential persistent pharmaceuticals using a multivariate chemical similarity approach

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A multidimensional chemical map of drugs was constructed, using principal component analysis, covering 899 drugs described by 67 calculated chemical descriptors. The map of drugs was applied to search for potential persistent drugs using a novel structural similarity tool. The basis for the tool is a selection of anchor molecules (diclofenac, trimethoprim, and carbamazepine) representing drugs of known environmental concern. In addition 12 chemicals listed by the Stockholm Convention on persistent organic pollutants (POPs) were used representing typical environmental pollutants. Chemical similarity was quantified by measuring relative Euclidean distances in the five dimensional chemical map. More than 100 drugs were found within a relative distance less than 10% from each drug anchor, whereas the region of the map closest to the POPs was much less populated. The 15 nearest neighbours (kNNs) of each anchor are presented, showing minor overlaps between the diclofenac, carbamazepine and POP clusters. A literature survey showed that in principal no data is available on the environmental fate characteristics of the identified kNNs except for the non-steroidal anti-inflammatory drug ketoprofen, which was among the diclofenac kNNs. In total 52 individual compounds are identified using the novel read-across tool which could provide a basis for future studies on environmental fate characteristics of drugs.

TU 152

Environmental risk assessment for ancillary substances in biotechnologcal production

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More and more pharmaceutical active substances are produced through biotechnological processes. For sustained, safe growth and high yield the organisms need an optimised environment, which is attained through the use of buffers, chelators and antibiotics, beside actual nutrients. These ancillary substances are drained with the wastewater to a wastewater treatment plant (WWTP) and are eventually released to receiving waters. Potential risks of such substances to WWTPs and surface waters were investigated.
Three buffers (MES, MOPS, PIPES), one very common chelator (EDTA) and one antibiotic (Gentamycin) were literature-searched or tested for biodegradability and inhibition of activated sludge as well as toxicity to algae, daphnids and fish. Based on documented use amounts of the ancillary substances in the biotechnological production plants of F.Hoffmann-La Roche Ltd in Basel (CH) and Penzberg (D), actual wastewater fluxes through the WWTP as well as realistic dilution factors for the surface water, site-specific predicted environmental concentrations (PECs) for the respective WWTP and receiving water in Basel and Penzberg were extrapolated. These were compared with predicted no effects concentrations (PNECs) for the wastewater treatment and surface waters, derived from the toxicity results.
PEC/PNEC risk characterisation ratios for the five ancillary substances are presented.

TU 153

The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal) - Part I: Evaluation of liver impacts in zebrafish after sub-acute exposures

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The liver is crucial in detoxification and, consequently, the study of functional and structural liver impacts serve as indicators of toxic exposure and effects. Still, toxicopathological effects in liver after exposure to pharmaceuticals are not yet characterized in fish. So, our purpose was to analyze qualitatively and quantitatively the liver structure of adult zebrafish after exposures of pharmaceuticals, while looking at their estrogenicity. For the latter, vitellogenin (Vtg) induction was assessed by immunohistochemistry. In parallel, a histological and stereological approach was made at light microscopy, in the last case to estimate the so-called volume-weighted mean volume of the hepatocyte nucleus, a potential liver biomarker of environmental stress. Thus, a sub-acute exposure of 21 days was carried out, at a breeding optimal temperature using a selection of five pharmaceuticals (carbamazepine, fenofibric acid, propranolol, trimethoprim and sulfamethoxazole), previously quantified in the Douro River estuary. These compounds, with different modes of action and belonging to distinct therapeutic groups, were tested individually and in mixture. The mixtures corresponded to the maximum levels found in the estuary and to 10,000 times higher.
The immunohistochemistry evidenced that individual exposures to fenofibric acid, propranolol, and both mixtures had potential to induce Vtg expression. This is in line with recent data uncovering surprising estrogenic effects of certain pharmaceuticals. Our results disclosed sex specific reactions and distinct responses depending on the type of exposure. For instance, males had the need to increase the hepatic mass (most likely) to face the higher metabolic demand, while females overcome it quite well (using the baseline hepatic mass and organelle machinery they already had for ovary maturation). Also, the nuclear volume of hepatocytes increased in males after both mixture exposures, which is logically related to a higher metabolic demand and activity. This pattern was not seen after individual exposures.
The study confirmed the usefulness of nuclear volume increases as a general marker of toxicant exposure and highlighted the importance of studying the effects caused by distinct (non-steroidal) pharmaceuticals in mixtures, including realistic environmental levels and considering both sexes.
Acknowledgements: FCT Grant (SFRH/BD/31382/2006); FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).

TU 154

The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal) - Part II: Assessment of impacts in the maturation of zebrafish gonads after sub-acute exposures

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The inherent biological activity of pharmaceuticals, nowadays commonly detect in aquatic compartments, together with their potential to interact with similar targets in aquatic species, may induce changes in biological key functions, such as reproduction. Some effects may negatively affect individual and even also the sustainability of a wild fish population. As in aquatic environments the fish are exposed to a great variety of compounds, and there is a currently lack of data concerning the toxic effects caused by non-steroidal pharmaceuticals, this study included carbamazepine, fenofibric acid, propranolol, sulfamethoxazole and trimethoprim as selected compounds. We hypothesized that pharmaceuticals other than those classically associated with reproductive physiology disruption may also affect the gonads, directly or indirectly. Thus, the main goal herein was to assess, using histopathological and stereological analyses, the impacts of these man-made chemicals on the maturation stages of female and male gonads of zebrafish exposed during 21 days. Individual and mixtures of the above referred pharmaceutics were tested, including a low environmental mixture of the five compounds at ng/L levels, as previously quantified in the Douro River estuary (Portugal).
Overall, the stereological analyses of volumes showed a tendency for a diminishing of the most mature cell compartments, namely spermatozoids and late/mature oocytes after individual and mixture exposures and when comparing with controls. Moreover, an increase in spermatocytes and primary oocytes was obtained suggesting, at least, a slow-down in maturation. Nevertheless, a distinct pattern was observed between sexes. The females seemed to be more sensitive to pharmaceutical mixtures, while in males the pronounced differences were registered in the individual exposures. The histological analyses were clearly in line with the quantitative results and evidenced a follicular atresia phenomenon that occurred in female gonads after both mixture exposures.
The present study demonstrates the potential of pharmaceuticals from various therapeutic classes to disrupt the normal kinetic of ovary and testis maturation compartments in zebrafish and, consequently, alerts for the importance of studying the reproductive effects caused by these compounds which are not connoted as endocrine disruptors.
Acknowledgements: FCT Grant (SFRH/BD/31382/2006); FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).

TU 155

The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal) - Part III: Experimental assessment of gross developmental defects using the zebrafish embryo test

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The fish embryos are now pointed out as one of the most promising and viable animal alternative tests in environmental toxicology and the zebrafish embryos are particularly advantageous as they allow an easy identification of potential teratogenic and embryotoxic effects. Thus, this study aimed to assess the toxicological effects caused by two distinct mixtures of pharmaceuticals using the zebrafish embryos as experimental model. One mixture corresponds to an environmental realistic scenario found in the Douro River estuary (Portugal) and the other mimics an overestimated mixture of pharmaceuticals. The studied mixtures included five pharmaceutical compounds which were the following: carbamazepine, fenofibric acid, propranolol, sulfamethoxazole and trimethoprim. The assay targeted developmental toxicity.
The mixtures of pharmaceutical compounds proved to induce several physiological and morphological alterations during the embryonic stages. The anatomical changes were mainly spine deformities and yolk-sac edemas. Moreover, a significant diminishing of heart rates and of tail lengths was noted even at ng/L levels. The results showed that the toxic effects are concentration dependent. Other parameters evaluated as mortality, hatching time and spontaneous movements were not affected by mixture exposures.
Even dealing with environmental low concentrations of pharmaceuticals relevant embryotoxic effects were observed. If we face the effects on the zebrafish embryos as proxies of fish embryotoxicity or even of general toxicity potential, the data suggests that risks seem to exist locally for the biota at the current levels of pollution by those pharmaceuticals. According to other data presented at this meeting, the concentrations of compounds tested seem to be able to disturb the gonads and the progeny, indicating that these low levels are potentially more harmful than we could predict.
Acknowledgements: FCT Grant (SFRH/BD/31382/2006); FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).

TU 156

Evaluation of whole toxicity of the river waters sampled in urbanized area of Japan, Mainly contaminated by treated and/or untreated sewage.

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In European and North American countries, bio-assays have been used for the management of industrial and municipal wastewater discharged into the natural aquatic environment. Following the introduction of whole effluent toxicity (WET) regulation using acute Daphnia magna test by South Korea projected to be started in 2011, Japan also started their research on possible introduction of the system similar to USEPA's WET to Japan but information had been limited about the toxicity of the industrial and municipal wastewater in Japan. Thus, our research group has examined the aquatic toxicity of some industrial and municipal wastewater in Tokushima, Japan using the short-term chronic test in USEPA's WET Test Methods with slight modification and found significant adverse effects on the growth of green algae (*Pseudokirchneriella subcapitata*), on the reproduction of *Ceriodaphnia dubia* and/or germination/survival of eggs of Japanese medaka (*Oryzias latipes*) or zebrafish (*Danio rerio*) for several samples. On the other hand, we have been investigated on ecological risk of pharmaceuticals and personal care products and found some PPCPs including some antimicrobial agents and UV filters might be a possible threat for the aquatic organisms.
In present study, the short-term chronic toxicity tests were used to evaluate the distribution of toxicity of river waters sampled in approximately 10 urbanized sites of Tokushima, Kyoto, and Saitama, Japan, where the untreated or treated sewage water is the major source of the contaminants. The reproduction of *Ceriodaphnia dubia* was significantly inhibited in the river waters sampled in at least seven sites while the growth of green algae was significantly inhibited in those sampled in at least three sites. Some potential toxicants, mainly PPCPs, were measured for the water sampled using GC-MS or LC/MS/MS for the water samples in addition to the toxicity tests for twenty individual PPCPs. Toxicity unit (TU) for each individual PPCP was calculated based on NOEC values and compared with the TU of the whole river waters. Relatively higher concentrations of most of PPCPs were found in Tokushima compared to Kyoto and Saitama. Triclosan and other antifungal agents slightly or moderately contribute the whole toxicity of the water samples for algae but the factors other than PPCPs may play important role for daphnid.

TU 157

Biologicaly treated Latvian municipal and industrial waste water toxicity to crustaceans D.magna and A.salina

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One of the main reasons of the Baltic Sea pollution is leaching waste water from the waste water treatment plants. With HELCOM Baltic Sea Action Plan the Baltic Sea countries have commit-

ted themselves to achieve Baltic Sea with life undisturbed by hazardous substances hence reaching good environmental quality status.
The aim of this study was to evaluate Latvian municipal and industrial waste water toxicity after their treatment in the waste water biological treatment plants using freshwater (*D. magna*) and saltwater *(A. salina*) crustacean tests.
Tested biologically treated waste water samples were collected in the time period from May 2009 until August 2010 from two industrial and two municipal waste water treatment plants. Toxicity was determined by using two different acute toxicity standard methods: freshwater - ISO *Daphnia magna* immobilization test (EN ISO 6341:1996) and saltwater - *Artemia salina* test (ArtoxKit M standard method).
The acquired results showed that *A. salina* is more sensitive against biologically treated municipal and industrial waste water than *D. magna*. Both test results indicates that Latvian industrial waste water toxicity has a seasonal character showing higher toxicity in autumn and winter while municipal waste water is none or slightly toxic during all seasons. According to test results success of treatment process of Latvian municipal waste waters depend from inhabitant amount in the area the waste water is collected. Study revealed that biological waste water treatment is not sufficient for Latvian industrial waste waters.
This study was performed in the frame of project INTERREG COHIBA (Control of hazardous substances in the Baltic Sea region).

TU 158
Global hepatic gene expression in fish exposed to sewage effluents: a comparison of different treatment technologies
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Effluents from sewage treatment plants (STPs) contain complex mixtures of pollutants. The addition of more advanced treatment steps to existing plants with conventional treatment has been suggested to enhance the reduction of chemicals, including pharmaceuticals. To assess how effluents treated with different technologies affect biological responses in fish, we have performed in vivo studies with rainbow trout over two weeks. The treatments evaluated include conventional activated sludge alone or in combination with activated carbon, ozone, ozone plus a moving bed biofilm reactor or UV/H2O2 treatment. The study was performed at a semi-large scale pilot plant with parallel treatment lines at Henriksdal STP (Stockholm, Sweden). Fish were also exposed in cages up- and down-stream from another STP with conventional activated sludge treatment (Stadskvarn STP, Skövde, Sweden) to allow a comparison between rigorously controlled exposure conditions and the more realistic field exposure. Previously, we have shown that the conventionally treated effluent induced an increase in liver and heart size. These effects were normalized by several of the advanced treatments. However, we have also shown changes in the blood plasma metabolome upon exposure to e.g. ozone-treated effluents, indicating that advanced treatment steps could potentially produce effluents affecting exposed organisms by unknown modes of action. Here, effects on mRNA expression were studied in order to more specifically identify affected physiological pathways and biomarkers. Quantitative PCR data showed that exposure to conventionally treated effluent induced hepatic zona pellucida glycoprotein 3 (zp3), a marker of exposure to estrogens, and cytochrome P450 A1 (cyp1a), a marker of exposure to dioxin-like chemicals. All advanced treatments reduced the induction of both genes with the exception of cyp1a which was not reduced by UV/H2O2 treatment. Effects on global hepatic gene expression, assessed by microarray, will be reported and related to observed metabolic changes.

TU 159
Assessing fish responses to municipal wastewater in Canadian receiving environments
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Municipal Wastewater Effluent (MWWE) is the largest point-source for contaminants to the Canadian aquatic environment. This effluent is a mixture of domestic and industrial wastes, Pharmaceuticals and Personal Care Products (PPCPs). The purpose of this study was to evaluate the potential impacts of these discharges at various levels of biological organization (fish communities and populations and individuals) in the Canadian aquatic receiving environment. In 2005 and 2007, field studies upstream and downstream of two municipal discharges assessed fish communities (diversity and abundance), populations and individual responses in terms of growth (condition factor) and reproduction (in vitro sex steroid production, gonadosomatic indices, and gonad, kidney and gill histopathology). Fish community assessments in 2007 and 2008 demonstrated significant alterations in fish abundance, diversity, and changes in the key species of the river fauna downstream of the MMWE discharges. Fish [Greenside Darter (Etheostoma blennioides) and Rainbow Darter (Etheostoma caeruleum)] collected downstream of the Kitchener and Waterloo municipal wastewater plants had greater condition when compared to reference fish collections. Although fish populations did not display effects to MMWE exposure, individual exposed fish demonstrated physiological alterations in sex steroid productive capacity and male fish demonstrated intersex. Other alterations in histopathology observed included inflammation of tubules in the kidney and stunted gill lamellae.

TU 160
Relationship between Soil Sorption Coefficient (Koc) and Octanol/Water Partition Coefficient (Kow) for Methylsiloxanes
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In environmental modeling, organic carbon normalized soil/water sorption coefficients (K_{OC}) of nonionic organic compounds by natural organic matter are often estimated from their octanol/water partition coefficients (K_{OW}) using an empirical single-parameter linear relationship. When this approach was used for cyclic methylsiloxanes (cVMS) such as octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), the measured log K_{OC} values for cVMS were found to be much smaller than those estimated based on the empirical model optimized using data for non-siloxane organic compounds as the training set. In order to better understand the relationship between K_{OC} and K_{OW}, the measured room temperature values of log K_{OC} and log K_{OW} from literature were analyzed in this study for nonionic (neutral) organic compounds, including volatile methylsiloxanes (VMS). It was found that the aforementioned discrepancy for siloxanes arose from two factors. First, relative to wet n-octanol, wet organic carbon is a more cohesive matrix, stronger H-bonding donor, and more effective at inducing dipoles in polarizable molecules. Secondly, relative to the benchmark non-organosilicon compounds for which the correlation between K_{OC} and K_{OW} was derived, VMS have larger size, strong H-bonding basicity and low polarizability that signify the difference in solvation properties between organic carbon and octanol. Consequently, VMS follow the same scientific principles as other nonionic organic compounds in environmental partitioning, but may not obey the simplified single-parameter relationships.

TU 161
The effects of different synthetic Progestins in fish: potencies vary
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Synthetic progestins are widely used for contraception and hormone replacement therapy. These progestins can be prescribed alone or in combination with synthetic estrogens (for example EE2). In humans, progestins act as contraceptives by inhibiting ovulation. They have been found to be present in the aquatic environment, which is not unexpected given their widespread use. Given the pivotal role that progestins play in human reproduction, it is important to understand the hazard these compounds pose once discharged into the aquatic environment.
Our research has investigated the effects of several synthetic progestins on reproductive performance in the fathead minnow (Pimephales promelas). Using our 45 day 'pair-breeding' assay, we found dramatic effects on reproduction, specifically a complete cessation of egg-laying. Where as some progestogens were active in the low ng/l range, others prevented egg production only when concentrations were above 1ug/l. Other effects seen included induction of secondary sexual characteristics in female fish (tubercles, fin spots and fat pads) and effects on plasma steroid levels. We propose that progestins are potentially a serious threat to aquatic wildlife, perhaps more so than the much-studied estrogens!

TU 162
Dose-response effects of chronic exposure of Fathead Minnows to low concentrations of a synthetic glucocorticosteroid
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Human pharmaceuticals present in the aquatic environment could adversely affect aquatic organisms, particularly fish. Synthetic glucocorticosteroids (GC) are used in large quantities as immunosuppressive and anti- inflammatory drugs. Many GCs are halogenated to increase the persistence in the human body, so they might be expected to be relatively resistant to degradation and hence present in the aquatic environments. Some reports have quantified the environmental concentrations of GCs in the hundreds of nanograms per litre range. In order to assess the impact of environmental concentrations of GC, an in-vivo exposure experiment was conducted with adult fathead minnows. Fish were exposed (ten male and ten female in each tank in duplicate) to 100ng, 1µg or 10µg Beclomethasone dipropionate /L via a flow through system. Similar duplicate tanks served as control, with no chemical added. After 21days of exposure, all fish were terminally sampled to determine various endpoints. There was a dose-response increase in plasma glucose levels and a decrease in blood lymphocyte counts and plasma cortisol levels. Secondary sexual characters in females suggest a dose-related masculinisation of fathead minnows which is not predicted from the mechanism of GC action. Expression profiles of selected genes (PEPCK, GR and Vtg) in liver via real-time PCR studies also resulted in dose-related effects at all three tested concentrations. Hence, we are not be able to define a no effect concentration for this chemical. The results suggest that GCs could cause effects in very low (as low as 100ng/L) concentrations that could be environmentally-relevant. Therefore, studies to determine the environmental concentration of GCs and to determine no effect concentration are needed to assess if GCs pose a risk to aquatic environment.

TU 163
Effect of azole fungicides on testicular steroidogenesis in vivo and ex vivo in zebrafish (Danio rerio)
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The occurrence of several azole fungicides in aquatic environment has been recently reported but their endocrine effects in fish are poorly studied. The aim of the present study was to assess the effect of clotrimazole, a pharmaceutical fungicide, on expression of key target genes (StaR, cyp17a1, cyp11b2) involved in testicular steroidogenesis as well as on 11- ketotestosterone (11KT) biosynthesis.
For that purpose, we first exposed adult male zebrafish to a wide range of clotrimazole concentrations (from 0.01 µM to 0.3 µM) for 7 days. In vivo exposure to clotrimazole resulted in an increase of cyp17a1 and cyp11b2 gene expressions in testis. To gain further information about the mechanism of action of clotrimazole, zebrafish testicular explants were exposed to similar concentrations of clotrimazole, with or without forskolin (FSK), an activator of the cAMP pathway that stimulates steroidogenesis. FSK (1 µM) strongly up-regulated cyp17a1 and cyp11b2 gene expressions and increased 11KT release in the culture medium. Clotrimazole (0.2, 1, and 5 µM), alone or in combination with FSK (1 µM), did not change basal or FSK-induced expression of cyp17a1 and cyp11b2. However, it inhibited both basal and FSK-induced 11KT release ex vivo, suggesting it acted as an inhibitor of steroidogenic enzymes. In order to determine whether other compounds belonging to the azole family are able to elicit similar effect on testicular steroidogenesis as clotrimazole, two other azole fungicides known to contaminate aquatic environment were tested, fenbuconazole and propiconazole. We showed that exposure of male zebrafish for 7 days to fenbuconazole and propiconazole had no significant effects on steroidogenic gene expressions while ex vivo both azoles was able to inhibit the 11KT release. Overall, our results show for the first time that the pharmaceutical fungicide clotrimazole is able to affect key steroidogenic genes expressions in a fish testis. However, the marked differences observed between in vivo and ex vivo experiments suggest that clotrimazole does not act directly on testes to regulate cyp17a1and cyp11b2 transcription. Whatever, using the testis tissue explants model, we demonstrate a direct action of clotrimazole, and to a lesser extent propiconazole and fenbuconazole, on the gonad which results in inhibition of 11KT synthesis. These original data deserve further studies on the effect of these compounds on fish reproduction

TU 164
Potential endocrine disrupting effects of six pharmaceuticals in H295R cell and freshwater fish Oryzias latipes
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Pharmaceuticals in the environment are of growing concern for their potential endocrine disruptions in ecosystem. Using a human adrenal H295R cell line and in vivo systems, we investigated the potential endocrine disrupting effects of six pharmaceuticals (diclofenac, erythromycin, ibuprofen, sulfathiazole, chlortetracycline, and oxytetracycline). After exposure to each target pharmaceutical for 48 hr, production of 17β-estradiol (E2) and testosterone (T), aromatase (CYP19) enzyme activity, or expression of steroidogenic genes were measured. Among target pharmaceuticals, ibuprofen, sulfathiazole, oxytetracycline and chlortetracycline enhanced E2 production. This observation was accompanied by increased aromatase activity and up-regulation of CYP17, CYP19, CYP11β2 or 3βHSD2 mRNAs, which are important components of steroidogenic pathways. After the ~120 d chronic exposure ibuprofen, sulfathiazole or chlortetracycline lead to changes in several reproduction related endpoints in Oryzias latipes, including up-regulation of vitellogenin gene level in male fish or fewer broods per pair. In addition, plasma E2 concentrations increased significantly after 14 days of exposure to 50 or 500 mg/L sulfathiazole, or 40 mg/L chlortetracycline in adult male medaka (3-4 monthes old). Based on the results of this study, certain pharmaceuticals could affect steroidogenic pathway, alter sex hormone balance, and eventually damage the reproduction in organisms.

TU 165

Sub-lethal effects induced by the non-steroidal anti-inflammatory drug (NSAID) ibuprofen on the freshwater bivalve Dreissena polymorpha

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Pharmaceutical compounds are considered emerging environmental pollutants, since hundreds of these molecules are commonly revealed in the aquatic environment in the high ng/L to low µg/L range worldwide. Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of therapeutic molecules that share similar pharmacologic properties and are widely used for control of pain and inflammation. Among these, ibuprofen (IBU; ((+/-)-2-(p-isobutylphenyl) propionic acid with R and S isomers), being one of the core medicines included in the "Essential Drug List"of World Health Organization, is produced, sold and used in great amount worldwide. Because of its considerable selling, often over-the-counter, large prescription volume, and high excretion degree as parent compound and/or in form of metabolites, IBU has been identified as one of the main pharmaceuticals present in the aquatic ecosystems. Notwithstanding, at present, few studies have evaluated its potential toxicity toward non-target organisms.
Sub-lethal effects induced by this NSAID were investigated by using a multi-biomarker battery applied to the freshwater bivalve *Dreissena polymorpha*. According to a semi-static in vivo approach, zebra mussels were exposed for 96 h to increasing environmentally relevant IBU concentrations (1, 9 and 35 nM), perfectly comparable to those currently revealed in aquatic ecosystems. Cyto-genotoxicity was evaluated by the single cell gel electrophoresis (SCGE) assay, the DNA Diffusion assay, the micronucleus test (MN test), and the lysosome membrane stability (Neutral Red Retention Assay) on mussel haemocytes. In addition, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the phase II detoxifying enzyme glutathione S-transferase (GST) was measured in the cytosolic fraction extracted from a pool of entire bivalves in order to reveal a possible oxidative status unbalance of treated-specimens. The biomarker battery pointed out a slight cyto-genotoxicity on zebra mussel haemocytes at low environmental levels (1 nM), while higher IBU concentrations were able to significantly increase both genetic (apoptotic and MN cells) and cellular damage. In addition, IBU seems to induce moderate effects on the activity of antioxidant and detoxifying enzymes, as shown by the notable oxidative status unbalance of exposed bivalves.

TU 166

Ibuprofen: an early life-stage toxicity test with the fathead minnow (pimephales promelas)

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Human pharmaceuticals are regularly detected in the aquatic environment, especially those produced in very large quantities. The guideline for human medicinal products, finalized in 2006, is used to estimate the environmental risk of human pharmaceuticals and requires data that includes chronic toxicity data for Algae, Daphnia and Fish. Such data are often missing for products with a market authorization before 2006. Alternatively literature data, frequently of poor quality, were used instead, leading to greater uncertainty and often to a debate about the general usage of these data. This is the case for Ibuprofen as well. Therefore we conducted an Early Life Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*) according to OECD 210. Fathead minnow embryos were exposed to a geometric series of six test concentrations and a negative (dilution water) control under flow-through conditions. The exposure period included a 5-day embryo hatching period, and a 28-day post-hatch juvenile growth period. Mean measured concentrations were 0.0091; 0.029; 0.092; 0.30; 0.93, 3.0 mg/L respectively. Four replicate test chambers were maintained in each treatment and the control group, with one incubation cup in each test chamber. Endpoints measured were hatching success, survival and growth respectively. There were no statistically significant treatment-related effects on hatching success, survival or growth at concentrations ≤3.0 mg/L. Consequently, the NOEC, based on all parameters measured, was 3.0 mg/L, the highest concentration measured. The LOEC was >3.0 mg/L

TU 167

Changes in global gene expression profiles and development of biomarker gene(s) in larval zebrafish exposed to selective serotonin reuptake inhibitors (SSRIs)

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The selective serotonin reuptake inhibitors (SSRIs) are a group of pharmaceuticals used in human medicine to block serotonin reuptake on cell membranes and elevate the levels of the neurotransmitter serotonin. Some SSRIs and their metabolites have been detected (sub to low ppb levels) in surface waters receiving inputs from wastewater treatment plants. In non-target organisms such as fish, SSRIs can act on the neuronal and neuro-endocrine signalling system and potentially influence higher levels of biological organization including reproduction and behaviour. To obtain a better understanding of the biochemical pathways that are influenced by SSRI exposure in fish, we evaluated global gene expression profiles for two representative SSRIs (fluoxetine and sertraline) to determine if the expression pattern of specific genes could be used as indicators of SSRI exposure in fish. Larval zebrafish Danio rerio were exposed to two concentrations (25 and 250 ppb) each of fluoxetine and sertraline for 5 days prior to extraction of total RNA for global gene expression analyses based on the Affymetrix GeneChip® Zebrafish Genome Array platform. No mortality or changes in fish behavoir were observed during the exposure, however alterations in global gene expression indicated treatment effects on gene regulation. The number of genes with significantly altered expresion > 1.5 fold relative to the control was 482 genes for 25 ppb fluoxetine, 242 genes for 250 ppb fluoxetine, 66 genes for 25 ppb sertraline, and 90 genes for 250 ppb sertraline. Ten genes were differentially regulated in all treatments relative to the control, suggesting that both SSRIs share some similar molecular pathways during larval zebrafish exposure. Of particluar interest in GO analysis under biological process of fluoxetine exposure was that many differentially expressed genes identified by microarray were involved with stress responses. The observed quantitative relationship between common or different transcriptional responses among treatments led to identification of potential biomarker genes and these genes include: acetylcholinesterase and FK506 binding protein 5. We are currently investigating changes in expression of these and other genes influenced by SSRI exposure in zebrafish by quantitative reverse transcriptase PCR, and these results will lead to enhanced understanding of the effects of environmental SSRI exposure in fish and development of biomarkers of exposure to SSRIs.

TU 168

Cyclic volatile methylsiloxane (cVMS) residues in fat, muscle, and liver tissue of American mink (Mustela vison) obtained from Lake Pepin, Minnesota USA

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The concentrations of cyclic volatile methylsiloxane (cVMS) materials were measured in mink tissue (fat, muscle, and liver) taken from organisms collected from the marsh areas of Lake Pepin, Minnesota, USA. This large freshwater lake has previously been examined for the trophic magnification behavior of the cVMS compounds octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) in an aquatic food web consisting of benthic invertebrates and fish. Three male and one female mink were captured on Lake Pepin in November 2008 by a commercial fur trapper. The mink were dissected to remove the liver, perirenal fat, and a portion of quadriceps muscle from a rear leg. The stomach was also removed for content identification to identify dietary preferences immediately prior to death. For D4, Lake Pepin lipid adjusted aquatic organism concentrations ranged from approximately 300 to 900 ng/g-lipid in benthic invertebrates, to ~40 to 350 ng/g-lipid in fish, to <LOD in mink fat. For D5, lipid-adjusted Lake Pepin concentrations ranged from approximately 4000 to 18000 ng/g-lipid in benthic invertebrates, to ~174 to 3000 ng/g-lipid in fish, to a mean value of 44 ng/g-lipid in mink fat. Finally for D6, the Lake Pepin lipid-adjusted concentrations ranged from approximately 150 to 1300 ng/g-lipid in benthic invertebrates, to 10 to ~170 ng/g-lipid in fish, to a mean value of 5 ng/g-lipid in mink fat. The Lake Pepin mink data demonstrate continued trophic dilution of cVMS in higher trophic level species, a trend that is similar to that reported for cVMS in food webs terminating in seabirds (i.e., kittiwakes and eiders) and grey seals.

TU 169

Phytotoxicity, persistence and uptake of antimicrobial salinomycin in the plant Brassica rapa

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The phytotoxicity of the veterinary antimicrobial salinomycin, both in a pure form (96%) and in formulation as Sacox120 (120g of salinomycin/Kg), to the plant *Brassica rapa* and its persistence in soil was evaluated. Plants were grown for 14 days under controlled conditions in natural standard soil (LUFA 2.2) amended with concentrations ranging from 1.6 to 32 mg Sacox120/Kg dry weight of soil (DWS) and 0.095 to 7.68 mg salinomycin sodium /Kg DWS. LCMS analyses of salinomycin in soil was conducted to confirm the concentrations at the beginning and the end of the chronic exposure test as well as to investigate its stability and persistence over a 15-day period with no plant growth. Measurement endpoints included shoot and root length, shoot and root wet and dry mass, seedling emergence, and flower bud formation; assessment endpoints included the interpolated IC50s and IC25s for each endpoint. Growth of plants exposed to pure salinomycin was affected at concentrations between 0.35 and 3.71 mg/Kg DWS. The IC50s for growth of plants exposed to Sacox120 ranged between 23 and 38 mg/kg DWS. When the ICps for Sacox 120 were adjusted for the active ingredient, the ICps ranged between 2.7 and 4.5 mg Salinomycin/kg DWS, indicating that the other constituents in the formulated product had little influence on the efficacy of the active ingredient. Seedling emergence was either less sensitive or comparable to the growth metrics and production of flower buds was the least sensitive. Although toxicity can be soil, chemical, species, and endpoint specific, the effect levels were surprisingly consistent among growth endpoints. LCMS analyses of LUFA 2.2 soil at the beginning of the chronic exposure test confirmed the theoretical concentrations of salinomycin with average recoveries across the whole range of exposure concentrations being 90% and 83% for Sacox120 and salinomycin, respectively. At the end of the 14-day chronic exposure test salinomycin concentration had deceased to 5.8% and 6.2% of theoretical values. Decay curves for Sacox120 and salinomycin in LUFA 2.2 soil for two different concentrations (low and high) with no plants present showed no change in salinomycin concentrations over a 15-d period. These experiments demonstrated that the presence of plants and microbial flora have a significant effect on salinomycin concentrations in soil.

TU 170

Assessment of chronic effects of Ivermectin in Danio rerio using several parameters

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Ivermectin (IVM), a veterinary pharmaceutical, is widely used in livestock production and is a component of various parasiticides. No information is available about the chronic effect of IVM to fish species, despite the vast literature about IVM effects to non-target organisms. In this study it was performed a chronic test based on the OECD (2008) guideline - Fish Screening Assay for Endocrine Active Substances. The test species Danio rerio was used. Concentrations ranged between 0.25-25µg IVM /L, with a daily medium exchange. At test end, lethal and sublethal endpoints were measured: survival, growth, behaviour (swimming and feeding), biomarkers (catalase-CAT, cholinesterase-ChE, and glutathione-S-transferase-GST), and reproductive markers (gonads histopathology and vitellogenin-VTG levels). Results showed mortality at 25µg IVM/L. IVM affected growth, particularly in males. Swimming behaviour disturbances occurred in all tested concentrations: lethargic movement and erratic swimming, spending more time at the bottom of the aquarium. Feeding behaviour showed that organisms exposed to the highest concentrations were using more time for the first strike and for complete pellet ingestion. GST was inhibited also at the highest concentrations of IVM. Qualitative histophatological analysis showed no alterations in male and female gonads.Vitellogenin levels were affected in females,

with an inhibition at the highest concentration. Besides the assessment of effect concentrations of IVM to Danio rerio, the combined use of this series of endpoints contributed to a better understanding of the underlying mechanisms of toxicity of IVM.

TU 171

Effects of sequential exposure to Ciprofloxacin and Sulfamethoxazole in marine microbial biofilms

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Many human pharmaceuticals are emitted into the aquatic environment after usage and antibiotics belong to a commonly detected group. Designed to be effective at low concentrations, it is hence likely that environmentally realistic concentrations of antibiotics directly affect environmental microbes. Also, antibiotic resistance is increasingly recognized as a potential threat to human health. In particular there is concern that resistance will develop in natural bacterial communities and spread to pathogenic bacteria.

To test whether environmentally realistic concentrations of antibiotics affect natural communities and lead to tolerance developments, a flow through microcosm experiment was performed in 2010. Long-term effects of two antibiotics were studied on periphytic biofilms (communities of predominantly microalgae and bacteria), established in aquaria from the indigenous micro-biota found in the natural seawater in the Gullmar fjord on the Swedish west coast.

During the first two weeks the communities were continuously exposed to either Ciprofloxacin (CIP) or Sulfamethoxazole (SMX) at nominal concentrations of 1nmol/L. Afterwards the exposure regimes were changed, so that communities previously exposed to CIP were exposed to SMX (1, 14, 200nmol/L) instead and vice versa for communities originally exposed to SMX. This second phase lasted five days.

At the end of each exposure regime the communities were sampled and assessed with respect to various ecotoxicological endpoints. Chl a content and pigment patterns were used to describe the effects on the algal part of the communities, while effects on bacteria were investigated using bacterial production (3H-Leucin incorporation) and bacterial catabolic profiling (Biolog Ecoplates). Samples were also taken to study the genetic profiles of the exposed communities.

TU 172

Toxicity assessment of oxytetracycline at different trophic levels

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Use of antibiotics in the aquatic environment has become an important concern. Oxytetracycline (OTC) is one of the most common veterinary antibiotics used worldwide. OTC is utilized in fish and shrimp farming, and for other animal intensive farming systems, as a growth promoter or for bacterial disease treatment. Several field studies have showed that OTC can persist in the sediment of aquatic environments for more than180 days. Considering the extensive use and high environmental persistence of OTC, this study aims at assessing the lethal and sublethal toxicity of OTC for species of different trophic levels. To achieve these objectives a battery of acute toxicity tests with algae, rotifers, crustaceans and fish were performed following OECD and ToxKit protocols. Following the acute toxicity assessment, different assays were performed for sublethal effect assessment. Representative biomarkers from different metabolic pathways were chosen (catalase-CAT, lactate dehydrogenase-LDH, cholinesterase-ChE, and glutathione-S-tranferase-GST) and teratogenic endpoints were determined using Danio rerio embryos. The results of OTC acute toxicity show Thamnocephalus platyurus, a freshwater species, as the most sensitive species with a 24h-LC50 of 0.042 mg/l (CI: 0.039-0.044) and Artemia salina, a saltwater species, as the most resistant with a 24h-LC50 870 mg/l (CI: 797-987). No difference was found on morphological development of zebrafish early life-stages but a significant ($p<0.05$) delay in hatching time was found for embryos exposed to concentrations above 150 mg/l. The enzymatic markers analyzed showed inhibition of oxidative stress enzyme (96h-LOECCAT = 5mg/L) and effects of OTC on detoxification phase II (96h-LOECGST =10mg/L) pathways. In comparison with the 96h-LC50 of 330 mg/l (CI: 314-345) for zebrafish embryos the sublethal endpoints analyzed seem to be more accurate in effects assessment of OTC. Linkages between the sublethal (enzymatic and developmental parameters) effects found in this study indicates that with parameters at higher levels of organization are essential for better understanding of the effect of OTC on organisms and the environment. Moreover, studies on environmental risks of OTC, even at lower concentrations focusing on long term effects applying other methods such as chronic toxicity tests, mesocosm assays, and mixture exposures should be carried out.

TU 173

Multi-generation life-cycle toxicity assessment of triclosan using Daphnia magna

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Triclosan is a ubiquitous compound in wastewater treatment plant effluents and biosolids and is commonly detected in surfaces waters throughout North America and Europe. The acute toxicity of triclosan (TCL) toward aquatic organisms has been relatively well studied but information on long-term, low level exposures at environmentally relevant concentrations is needed. The objective of the present study was to evaluate the toxicity of triclosan in a multi-generational test with the waterflea *Daphnia magna* in a laboratory exposure. *Daphnia* were exposed to nominal concentrations of TCL ranging from 0.5 to 250 µg/L in 21-d tests for five generations (we have completed the second generation). Brood randomly collected from five replicate beakers after the 21-d exposures were used to seed to next generation. Endpoints measured included survival, number of offspring (total and per adult), and cumulative number of offspring. After the first generation, a 21-day LC50 value could not be calculated due to high adult survival; after the second generation, an LC50 of ~140 µg/L was determined with a NOEC of 100 ug/L suggesting increased sensitivity in pre-exposed animals. Time to first brood increased significantly, and the number of offspring decreased significantly with increasing concentration during each of the first two generations but relative LC50 and NOEC values were comparable. No effects were observed at concentrations <100 µg/L indicating that triclosan may pose minimal long-term risk to aquatic invertebrates at environmentally relevant concentrations.

TU 174

Ecological risk assessment of selected antifungal and antimicrobial agents in small urban creeks in Tokushima, Japan, with unsewered drainage area

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Pharmaceuticals and personal care products (PPCPs) are widely used in our daily life as drugs and cosmetics. Sewage and household effluent containing PPCPs, which are designed to show certain physiological effects, may have harmful effects on some aquatic organisms. Among these compounds, four antifungal or antimicrobial agents, triclosan, triclocarban, 4-chloro-3-methylphenol (PCMC) and 4-chloro-3,5-dimethylphenol (PCMX), used in products such as antiseptic soaps and cosmetics, were selected and their ecological risk was estimated in small urban creeks in Tokushima, Japan, highly contaminated by treated or untreated sewage. The concentrations of these compounds and other antibacterial and antifungal agents (e.g., chlorothalonil, 2-phenoxyethanol, 3-methyl-4-isopropylphenol) were monitored using a GC-MS using trimethylsilyl derivatization. The sunlight photolysis and river water die-away test were conducted to examine the persistence of these compounds in the aquatic environment in addition to the sorption of these compounds to determine the partitioning between aqueous and sediment phase. Furthermore, acute toxicity of these compounds were evaluated using fish (Oryzias latipes), daphnia (Daphinia magna), green algae (Pseudokirchneriella subcapitata), and chironomid (Chironomus yoshimatsui) while short-term chronic toxicity were evaluated using fish eggs (Danio rerio), daphnid reproduction (Ceriodaphnia dubia), NOEC of green algae, and chironomid.

First, triclosan was detected as high as 155 ng/L. The other antifungal/antimicrobial agents, triclocarban, 3-methyl-4-isopropylphenol and 2-phenoxyethanol were also detected in most of the sampling sites while the other agents were under the detection limit in most of the sampling sites. The results of biodegradation experiments suggest relatively slow biodegradation of triclosan and triclocarban while those of photolysis experiments suggested relatively rapid photodegradation of the two. Triclosan was found to be very strongly toxic for algae with 96h-EC50 of 2.8 µg/L but moderately toxic for daphnid and fish, and the measured concentration/predicted no effect concentration (MEC/PNEC) ratio exceeded 1 in water and the MEC/PNEC ratio was in the range between 0.1 and 1 in sediment. Triclocarban showed the similar trend. For the other antifungal/antimicrobial agents, MEC/PNEC values were far below 0.1 and no severe risk for aquatic organisms is suspected.

TU 175

Cytostatic drugs - are they environmentally relevant?

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Pharmaceuticals are emerging micropollutants of concern. Cytostatic drugs are discussed as high risk substances in regard of human health. In addition they are considered as problematic for the environment. Chemotherapy drugs are of potential environmental relevance due to their poor biodegradability. The toxicity of chemotherapy drugs to humans is well known whereas very little is published about the toxicity to the environment.

The demand for chemotherapy treatment is increasing from year to year. It is expected that the consumption of cytostatic drugs will rise not only for human use but also in veterinary medicine. Therefore, an increase of these substances in the environment is also expected in the future.

The aim of this work is to survey the ecotoxicological potential of this special group of human pharmaceuticals. Since 2006 data requirements for the environmental risk assessment of human pharmaceuticals are mandatory for the authorisation procedure within the EU. So far, applicants have provided a multitude of acute effect data but only a limited number of long term effect and fate data within the authorisation process. Fate and effect data from applicants as well as data from publicly available literature will be analysed regarding acute and chronic toxicity and biodegradability. Furthermore, for some most widely used cytostatic drugs the relevance for the environment will be emphasised based on recent consumption data and measured environmental concentrations.

TU 176

The neutral species of the weak base trimethoprim is more toxic to willow trees (Salix viminalis) than the cation

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The acute toxicity of the veterinary antibiotic trimethoprim (TMP) to willow trees was tested at three different pH levels in hydroponic solutions with TMP concentrations of 1, 10, 100 or 1000 mg/L. The pH variation was achieved by using ammonium (pH 4.3, low) or nitrate (pH 6.4, medium) as nitrogen source, and by additional tritration with KOH (pH 8.15, high). TMP is a weak base that dissociates at pKa 7.2. A statistically significant higher toxicity of the neutral form was observed, i.e. a higher toxicity at medium and high pH than at low pH. A toxic effect of the neutral form was observed at an external concentration of 100 mg/L, while the toxicity of the cation appeared only at the concentration of 1000 mg/L. The result of the study shows that the toxicity of TMP to willow trees is low, but also that the toxicity of weak bases varies with pH.

TU 177

Ecotoxicity tests to evaluate the toxicity of some anticancer drugs released in hospital waste water

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Most pharmaceutical compounds are excreted partially transformed or even unchanged via urine and faeces of patients under medical treatment. As hospital effluents reach the municipal sewer network generally without preliminary treatment, they can reach environment and affect animals, plants, but also human health via drinking water. The aim of this research is to validate biomarkers to evaluate the potential toxicity of drugs (ciprofloxacin, tamoxifen) frequently used in cancer chemotherapy in range of concentrations found in waste water. In the present study, several bioassays have been optimized : (i) cellular viability of human cell exposed in vitro to ciprofloxacin or/and tamoxifen, (respectively 0.3 µg.L-1 to 0.3 mg.L-1 and 5.6 ng.L-1 to 56 µg.L-1) during 24-72h ; (ii) growth inhibition of the aquatic plant Lemna minor. Plants were exposed during seven days at the same concentration as the cells. Ciprofloxacin and tamoxifen even at low doses are toxic for

human cell and plant models. Effects were different depending on the relative proportion of the drugs applied to cells, suggesting different target and action of these compounds.
Other bioassays will be optimized, in particular the 32P post-labelling technique to determine DNA adduct reflecting genotoxic effect.

TU 178
Cytochrome P450 (CYP) 3A activity in Atlantic salmon (Salmo salar) liver microsomes
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Cytochrome P450 3A (CYP3A) is involved in hepatic metabolism of a number of endogenous and exogenous including commercially available drugs. This isoform is well studied in mammals, whereas considerably less information is available on CYP3A in fish. However, the role of fish CYP3A in detoxication of aquatic pollutants and pharmaceuticals potentially implies the possibility of using CYP3A as a biomarker in aquatic pollution monitoring.
This study investigated an applicability of the marker substrates of mammalian cytochrome P450 (CYP) 3A for fish. For this purpose, the effect of a selective CYP3A inhibitor ketoconazole on in vitro metabolism of several substrates was studied. Following compounds were investigated as possible substrates for CYP3A: 7-benzyloxyresorufin (BR), 7-ethoxyresorufin (ER), 7-benzyloxy-4-trifluoromethylcoumarin (BFC) and 7-benzyloxyquinoline (BQ). These substrates were incubated with a pool of liver microsomes from 4 fish (Atlantic salmon) with or without ketoconazole. The concentrations of ketoconazole in the incubations were 1, 10 and 40 µM . The product formation from those substrates was measured by HPLC. The mode of inhibition and inhibition constants (Ki) were determined by a GraphPad Prism version 4.0 for Windows, GraphPad Software (San Diego California, USA).
Ketoconazole was a potent competitive inhibitor of BR (Ki=45.3 µM) and BQ (Ki=1.6 µM) and non-competitive inhibitor of BFC metabolism (Ki=2.3µM) in the microsomes. The results indicate that BFC and BQ can be used as substrates to estimate CYP3A activity in fish liver microsomes.
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TU 179
Effects of sodium hipochlorite in different trophic levels
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Sodium hypochlorite (SH) is a chemical compound used on a large scale, frequently as a disinfectant or a bleaching agent. It is present in agriculture, chemical, paper, pharmaceutical and waste disposal industries, among others. Chlorine disinfectants in wastewater react with organic matter, giving rise to organic chlorine compounds which are toxic for aquatic organisms and are persistent environmental contaminants.
In this work we assessed the toxicity of SH to organisms belonging to different trophic levels. Effects of SH were evaluated in the growth of the algae Pseudokirchneriella subcapitata and Chlorella vulgaris (concentrations ranging from 0,1 to 100 mg/L), in the survival of Artemia fransciscana and Thamnocephalus platyurus (concentrations ranging from 0,05 to 10 mg/L) and in Danio rerio (zebrafish). Zebrafish assays include acute (concentrations ranging from 0,7 to 10,1 mg/L) and chronic (concentrations ranging from 5 to 500 µg/L) exposure of adult organisms and early life stages test in which embryo development was monitored. The biomarkers lactate dehydrogenase (LDH); GST,glutathione-S-tranferase (GST); Cholinesterase (Che) and catalase (CAT) were measured after zebrafish early life stages and adults exposure to SH.
Most sensitive species was T. platyurusand with a LOEC of of 0,5 mg/L, followed by P. subcapitata (LOEC =1 mg/L), C. vulgaris (3,2 mg/L), A. fransciscana (10 mg/L) and D. rerio (adult) (5,5 mg/L). Moreover, it was observed that SH induced an early hatching in D. rerio embryos exposed to concentrations above 6,4 mg/L. Preliminary results show that D. rerio embryos exposed to concentrations above 7.4 mg/L also presented a higher GST activity.

TU 180
Histology of liver and gills in mosquitofish, Gambusis holbrooki, after long-term exposure to sublethal concentrations of benzalkonium chloride
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Benzalkonium chloride is a detergent with effective germicide and preservative proprieties commonly used in several pharmaceutical and personal care products. The histopathological effects of benzalkonum chloride on the liver and gills of mosquitofish, Gambusia holbrooki, were studied by light microscope in tissues stained with hematoxylin and eosin. The fish were exposed to sublethal concentrations (0,025 mg/l, 0.1 mg/l and 0.4 mg/l) of benzalkonium chloride for 4, 10 and 28 days. Qualitative histological examination of both organs showed a variety of minor cellular alterations (e.g. lifting up of epithelium, intraepithelial oedema, fusion of adjacent secondary lamellae and hepatocytes vacuolization), also present in the control group. Almost changes are of minimal pathological importance, suggesting that the lesions were easily reversible as exposure to irritants ends. In addition, gill morphometrical indices were been used to test any physiological disturbance of the respiratory surface with exposure to benzalkonium chloride. However, statistical analysis did not find any relationship between the concentration and/or duration of exposure and the histopathological alterations, suggesting that benzalkonium chloride as little effect on gambusia.

TU 181
Preliminary risk assessment of organic UV filters and UV light stabilizers in coral reef ecosystems at Okinawa, Japan
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Organic UV filters are used in personal care products and polymer based products to protect human skin from UV-A and UV-B radiation as well as to maintain the product quality for a longer time. UV light stabilizers (UV absorbers) are used in technical products such as plastics, coatings, adhesives and construction materials. Recent studies have reported that these chemicals are released into aquatic environment and that some of them were detected in surface water, sediment, aquatic organisms and human breast milk. In addition, it was reported that organic UV filters in sunscreens cause coral bleaching by promoting viral infections. The aims of this ongoing research are 1) revealing organic UV filters and UV light stabilizers concentrations in seawater and sediment at coral reef lagoons adjacent to recreational area by passive samplers in order to evaluate impacts from recreational activities, 2) revealing their concentrations in coral reef organisms, especially predators of hard coral to evaluate hard coral exposure to them.

ET04 - Ecologically relevant endpoints

TU 184
Binding biomarker results with ecologically-relevant parameters in an aquatic macrobenthos community
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The effects of plant protection products (PPPs) on a freshwater macrozoobenthos community where studied in a semi-controlled system. The system consists in a series of artificial channels alimented by an unpolluted river (River Fersina, Trentino-Alto Adige region, northern Italy). The channels are filled with stones, creating a substrate comparable to that present in the river and are naturally colonised by a macrozoobenthos community. The structure of the community in the channel and in the river was monitored for a long time before the experiments, indicating a reasonable comparability.
A realistic PPP application scenario was developed in order to reproduce an exposure condition comparable to those likely to occur in the rivers flowing in intensive agricultural basins of the region. For each channel, a section upstream the PPP immission was taken as untreated control. A panel of biochemical parameters (acetylcholinesterase, glutathione-S-transferase, catalase, and alkaline phosphatase activities,) were measured on selected taxonomic groups of the community (Tricoptera and Plecoptera) after each PPP application.
Quantitative samples of the community were collected after the PPP applications in order to assess effects on the populations of relevant taxonomic groups and on the community structure. Biomarker responses were compared with population and community data in order to assess possible relationships between responses at different hierarchical levels.

TU 185
Effects of the pharmaceutical ivermectin on freshwater nematodes at the organismic, population, and community level
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Extrapolating effects of contaminants found at lower levels of biological organization to higher levels, such as ecosystems, is important for environmental risk assessments (ERAs), but it is often already difficult to link two proximate levels. Although these extrapolation uncertainties exist, combined studies at different levels provide complementary and ecologically relevant information that is valuable for ERAs.
In this presentation, effects of the veterinary parasiticide ivermectin on free-living freshwater nematodes are shown at three levels. First, single-species bioassays with two nematode species (*Caenorhabditis elegans* and *Panagrolaimus cf thienemanni*) were conducted to investigate how ivermectin's mode of action (interference with transmission of neural stimuli to muscles) affects reproduction of free-living nematodes and to compare their sensitivity towards ivermectin with other freshwater organisms. The derived lowest observed effect concentrations (LOECs) were 2.1 and 0.5 µg/L, respectively. Second, full life-cycle experiments using these LOECs were conducted to estimate the actual impact of ivermectin on life-cycle traits of nematodes, e.g., age at maturity and intrinsic rate of natural increase (r_m). A consequence of the mode of action became more obvious than in the bioassays: towards the end of the reproductive period, *C. elegans* juveniles hatched inside 38% of their mothers before the mothers died, because muscle relaxation might have inhibited egg-laying. Third, a microcosm experiment with an ambient meiofauna community was conducted over a period of 224 days to take into account indirect food web effects and bioavailability of ivermectin in sediments. For nematodes a no observed effect concentration ($NOEC_{Community}$) of 0.6 µg/kg dry wt was derived, which is close to predicted environmental concentrations (PECs) of ivermectin in sediments (0.45-2.17 µg/kg dry wt).
Effects of ivermectin could be demonstrated at all observed levels of biological organization. The bioassays and the life-cycle experiments yielded valuable fundamental information, indicating a risk for free-living freshwater nematodes, while the microcosm experiment confirmed the risk under more realistic conditions.

TU 186
Assessment of the reproductive and developmental toxicity of the herbicide Betanal® Expert and corresponding active ingredients on Daphnia spp.
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Pesticides usage is regulated taking into account environmental protection and ecotoxicological evaluation in the aquatic compartment prior placement in the market is generally required. The recommended workflow for ecotoxicological assessment tends to focus on the active ingredients rather than the commercial formulations of pesticides and on demanding references to the acute toxicity of the chemicals rather than considering life-history effects. The herbicide Betanal® Expert is a recently developed herbicide formulation that delivers the active ingredients (a.i.) ethofumesate (systemic benzofuranyl alkanesulphonate acting as growth inhibitor), phenmedipham and desmedipham (systemic bis-carbamates acting as photosynthetic inhibitors). The long-term toxicity of the commercial formulation as well as that of each a.i. to the freshwater cladocerans Daphnia magna and Daphnia longispina was addressed here, and reproductive effects were focused in particular. In this way, this study aimed (i) to provide experimental evidence on the reproduction injury induced by the herbicides; (ii) to compare the sensitivity of the model species D. magna and the indigenous D. longispina. Both the commercial formulation and the a.i.s were able to induce reproductive and developmental effects in both non-target species. Despite no great effects could be depicted in total fecundity (total number of eggs deposited in the brood chamber), concentrations below 1 mg L-1 were generally able to induce massive developmental effects in the progeny, thus the yield of unviable progeny. These include egg abortion, release of undeveloped embryos and release of dead neonates. Ethofumesate was the least toxic herbicide inducing the yield of unviable progeny following exposures to concentrations equal or higher than 1.9 and 4.6 mg L-1. The sensitivity of the standard D. magna was comparable to that of D. longispina as to the production of unviable progeny, being ethofumesate the single herbicide able to induce clearly distinct responses: D. magna and D. longispina released significant amount of aborted eggs following exposure to 1.9 mg L-1 and 4.6 mg L-1, respectively. Based on these

results one should conclude that reproduction injury operated by exposure to very low concentrations of the herbicides should translate into serious impairment of population growth. Such impairment could never be predicted on the basis of acute toxicity assessments as required by regulation.

TU 187

Gastropod interspecies difference in response to 17a-ethinyl-estradiol: a full life cycle dose-response experiment

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Effects of 17α-ethinyl-estradiol (EE2) on fish are fairly well documented. However, for lower trophic levels, such as macroinvertebrates, the knowledge is less and the studies that have been performed are over a short period of their life cycle. Accordingly we investigated the effects of EE2 on two common freshwater gastropods, *Radix balthica* and *Bithynia tentaculata*, over a full life cycle. The quantified life history parameters for P-generation were growth, mortality, time and size to first reproduction and for F1-generation, success of hatching. Intrinsic rate of increase was also calculated utilizing the life history parameters. The responses for *B. tentaculata* were of a general negative trend with increased EE2 concentration but for *R. balthica* there was none or even positive trends. *Bithyina tentaculata´s* lower growth rate, longer time to reproduction and decreased reproduction resulted in a lower intrinsic rate but for *R. balthica* there was no response in the intrinsic rate of increase at ecological relevant concentrations. We also analyzed the importance of duration of the treatment on fecundity and found that *B. tenataculata* was significantly affected but *R. balthica* was not. The result from this study showed that the responses not only differed depending on treatment, but also on the sensitivity of the two species and that duration is an important factor when evaluating response from EE2. These results can imply that *R. balthica* may benefit from the effects of EE2 at ecological relevant concentrations by being less negatively affected.

TU 188

Ecological endpoints of exposure to toxic substances on interstitial copepods (crustacea, copepoda)

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Poor information on the ecological effects of pollutants on meiofaunal species living in the groundwater environments is presently available. This contribution is aimed at assessing the sensitivity to toxic substances of interstitial copepods living in subsurface porous aquifers, with special reference to the hyporheic habitat. Copepods are by far amongst the most abundant and species-rich groups in the hyporheic habitat and for this reason they have been selected for sediment toxicity assessment, being plausibly predictive of the ecological risk for the hyporheic fauna as a whole. The effects of chemicals, potentially contaminating ground water, i.e. Ammonium (NH_4^+) and the pesticide Aldicarb, were assessed by analysing shifts and/or alterations of developmental and population dynamics of copepod species. Four harpacticoid species were selected; namely, *Bryocamptus zschokkei*, *B. minutus*, *B. echinatus*, *B. pygmaeus*. Mono-specific cultures were developed for each species. Harpacticoid copepods have holobenthic life-cycle and pass through six naupliar stages, followed by six copepodite stages, copepodite VI being the adult. The selected species were assayed at sublethal concentration of 13 mg/L and 0.6 mg/L for NH_4^+ and Aldicarb, respectively. A developmental, time-dependent, relative index (DRIC) was evaluated for copepods along eight weeks, by scoring each developmental stage, measuring time and number of organisms at each developmental stage. It was calculated as DRIC = ΣSi*Ni, where Si is the stage i and Ni the number of copepodites at the i-stage (n = 6). Data for partial and final DRIC were obtained. Time for reaching the adult stage and sex ratio were also analysed. Data obtained with Aldicarb tests indicated that final DRIC was significantly reduced at values higher than 60% without modify the polynomial trend, adult stage timing ranging from 6 weeks or longer than 6 weeks, if compared to control populations; sex ratio on female basis showed extreme values, ranging from 0 to 100%. Ecological effects of NH_4^+ was highly species-specific, being *Bryocamptus zschokkei* the more sensitive to this pollutant, as indicated by the final DRIC reduction close to 50%, and adult stage attained after eight weeks. Experimental results based on copepod species were also evaluated under the perspective of the environmental risk assessment for the hyporheic fauna.

TU 189

Age altered sub-organism's responses in chromium exposed Daphnia schodleri (Anomopoda: Daphniidae): sensitivity and antioxidant enzymes

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Daphnia schodleri is a natural occurring cladoceran in Mexican freshwater bodies, and its relative big size and short life cycle allow its use in ecotoxicological bioassays. Generally, younger and older life stages are considered to be the most susceptible and/or sensitive organisms because of several sub-organisms' level processes, such as ageing, in which antioxidant activity decreases and reactive oxygen species (ROS) cannot be totally neutralized. Moreover, aquatic communities are structured by organisms of different ages, in which physiological and biochemical responses differ in magnitude from each other. According to these statements, seven age groups of *Daphnia schodleri* (0, 3, 5, 7, 14, 21 and 28 d) were exposed to Cr (VI) in acute and non lethal bioassays. The results from acute toxicity bioassays were used to estimate the chromium LC_{50} values for every age group, which ranged from 0.11 to 0.61 mg L^{-1}, and a Gaussian distribution pattern was observed. Antioxidant enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) was assessed in two different experimental designs: a) using two sublethal concentrations (0.032 and 0.0064 mg L^{-1}); and b) two fractions (1/5 and 1/25) of previously determined LC_{50} for every age group. In the first experimental design, SOD and GPx activities showed a decreasing pattern with respect to their control groups; the CAT and GR levels were hardly modified by chromium exposure in neonates, but not in other groups. The second design results were totally different: no pattern was found among the age groups but not only neonates were affected by the toxicant. The two groups that could be considered similarly susceptible are the identified as pre-adults, the ones before the beginning of reproductive activity (5 and 7-d old). These results suggest that organisms' sensibility is not always related to susceptibility at suborganisms' level, since 7-d old organisms were the less sensitive in acute toxicity bioassays.

TU 190

Is a higher resistance to copper correlated with a faster recovery in daphnids?

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Natural populations are commonly exposed to sequential pulses of contaminants. If the input of a pulse of lethal levels of a contaminant causes the disappearance of the most sensitive genotypes from a population (genetic erosion), then, the ability of the impacted population to withstand a further pulse of the contaminant will largely depend upon recovery rate of the remaining organisms. This study aimed at evaluating if the genetically determined resistance is positively related with a faster recover capacity, which would guarantee a double advantage to the resistant genotypes. To achieve this goal, cloned lineages of Daphnia longispina O.F. Müller, exhibiting different sensitivities to lethal levels of copper, were exposed to sequential pulses of this metal, to evaluate specific recovery rates and their relation to resistance. For each cloned lineage, the intensity of pulses corresponded to the respective concentration of copper causing 30% of mortality after 24h of exposure (LC30,24h). Obtained results showed no positive correlation between genetically determined resistance to lethal levels of copper and recovery rates.
It was also observed that even during the recovery period, mortality continued to occur, suggesting that standard lethal assays sub-estimate toxicity (as endpoints are computed solely based in mortality occurring during exposure to the toxicant). Furthermore, the lack of a positive correlation between resistance and a faster recovery highlights the increased risk at which impacted populations are subject after being exposed to a pulse of lethal levels of a contaminant.

TU 191

Cellular energy allocation in Chironomus riparius: sensitivity and relevance

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The Cellular Energy Allocation (CEA) methodology was developed as biomarker technique to assess the effect of toxic stress on the energy budget of test organisms. It measures changes in energy reserves (total lipids, protein content and sugar content) and energy consumption (electron transport activity) and thus evaluates the metabolic costs induced in organisms by contaminant exposure. However CEA methodology is not commonly applied to benthic macroinvertebrates. Moreover there is a need for validation of these energetic biomarkers by assessing its sensitivity and reliability measuring it after exposure to contaminants with different modes of action and relevance by comparing it with endpoints measured at higher levels of biological organisation.
In this work we investigated the sublethal effects of the insecticide Movento® (spirotetramat) and Cadmium chloride on the midge Chironomus riparius. After exposure to a gradient of insecticide and metal sub-lethal concentrations, effects on CEA, growth, oxygen consumption rate, burrowing behaviour, emergence and adult size were measured. Results are discussed in terms of the sensitivity of the different endpoints and the advantages of using energetic biomarkers as a early warning indicator of adverse ecologic effects in freshwaters.

TU 192

Chitobiase activity as an indicator of altered survival, growth and reproduction in Daphnia pulex and Daphnia magna (Crustacea: Cladocera) exposed to spinosad and diflubenzuron

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Chitobiase is involved in exoskeleton degradation and recycling during the moulting process in arthropods. In aquatic species, the moulting fluid is released into the aqueous environment, and chitobiase activity present therein can be used to follow the dynamics of arthropod populations. Here, chitobiase activity was used for monitoring the impact of mosquito candidate larvicides on *Daphnia pulex* and *Daphnia magna* under laboratory conditions. Both species were exposed to spinosad (2, 4, 8 µg L^{-1}) and diflubenzuron (0.2, 0.4, 0.8 µg L^{-1}) for 14 days. *Bacillus thuringiensis* var. *israelensis* (*Bti*; 0.25, 0.5, 1 µL L^{-1}) was used as the reference larvicide. Chitobiase activity, adult survival, individual growth, and fecundity, expressed as the number of neonates produced, were measured every two days.
In contrast to *Bti*, spinosad and diflubenzuron significantly affected both species in terms of adult survival, and production of neonates. As compared to *D. pulex*, *D. magna* was more severely affected by diflubenzuron, at low and medium concentrations, with reduced adult growth and much lower chitobiase activity. Chitobiase activity was positively correlated with the individual body length, number of neonates produced between two consecutive observation dates, and number of females and neonates. In addition, the significant positive correlations between chitobiase activity measured on the last sampling date before the first emission of neonates and the cumulative number of neonates produced during the whole observation period strongly support the potential of the activity of this chitinolytic enzyme as a proxy for assessing the dynamics of arthropod populations exposed to larvicides used for mosquito control.

TU 193

Do eastern mosquitofish male harass less when exposed to environmentally relevant levels of 17β-estradiol?

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Behaviour is the outcome of many complex physiological and biochemical reactions. Animals adapt to changes in their environment through their behaviour and thus behaviour provides a sensitive early warning signal for the presence of human-induced compounds, such as endocrine disrupting chemicals (EDCs). Our study species, eastern mosquitofish, is widespread in Australia and found in sites heavily polluted by EDCs. Male mosquitofish use a modified fin known as a gonapodium during copulation to inseminate females, and previous research has found that females preferentially associate with males with longer gonapodiums. In this study, we exposed male mosquitofish to environmentally relevant concentrations of 17β-estradiol (E2) and examined how exposure affects the reproductive behaviour and gonapodium morphology of males. Specifically, we asked if E2 exposure decreases male copulation attempts and gonopodial display.

We set up a preference test, where female could first observe male behaviour (exposed and unexposed) and then mate with the prefered male. We measured the time and frequency of male behaviours: orientation, chasing, gonopodial display and copulation attempts, and the time female spent in close proximity with the preferred male. In addition, male mating success was assessed by calculating the number of live offspring and mating success tested against the morphology of the male's gonapodium. Results will be discussed within the context of male reproductive success, sexual selection and EDC exposure.

TU 194

Toxicity of the sugar cane herbicides Diuron and Tebuthiuron in zebrafish (Danio rerio) early-life stages

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Herbicides are widely used in agriculture and are known as a diffuse source of pollution of surface water and groundwater. The impact of herbicides use on non-target organisms is little known although mutagenic and genotoxic effects in fish exposed to herbicides have already been observed. Sugarcane is the predominant crop cultivated in the state of São Paulo, Brazil, with an area comprising approximately five million hectares. In the present study, the toxicity of diuron and tebuthiuron, two herbicides widely used in sugarcane cultivation, was evaluated using the Fish Embryo toxicity Test (FET), with *Danio rerio*, a tropical cyprinid used in toxicological research. The endpoints monitored included pigmentation, otholith, yolk absorption, pericardial oedema, tail deformation, hatching, coagulation and death. Five concentrations of each chemical were tested, ranging from 0.001 to 0.08 g L^{-1} of Diuron; and from 0.1 to 0.6 g L^{-1} of Tebuthiuron. At concentrations over 0.02 g L^{-1} of diuron and 0.24 g L^{-1} of tebuthiuron, exposed embryos presented a general development delay, pericardial oedemas, tail deformities and a delay in yolk absorption. Despite the low acute toxicity of diuron and tebuthiuron ($LC_{50\text{-}96\,h}$ = 0.024 (0.022 - 0.026) g L^{-1} and $LC_{50\text{-}96\,h}$ = 0.35 (0.17 - 0.53) g L^{-1}, respectively) observed for zebrafish early-life stages, deleterious effects at sublethal level could be observed. The endpoints used on this study allow a better understanding of the toxicity and mode of action of these two herbicides, suggesting that, chronic effects due to long term exposure to the herbicides can be very important, leading to significant alterations in the organisms and consequently disrupt the functioning of aquatic ecosystems. Thus, as these chemicals are widely used in the field, an evaluation of risk should be performed based on the monitoring of sublethal parameters in organisms belonging to several trophic levels.

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TU 195

Biomarkers as a tool to study different scenarios of exposure to potassium dichromate in zebrafish early life stages

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Potassium dichromate (PD) was classified by the European Chemicals Agency as a "Substance of Very High Concern"due to the environmental and human health risk posed. PD enters the air, water and soil through natural processes and human activities such as chemical, leather and textile manufacturing and may be an indicator of environmental contamination. PD is genotoxic, carcinogenic, neurotoxic and interferes with cellular metabolic activity. The level of chromium in water is generally low (levels found in rivers are often around 1 ppb) and thus, a proper analysis of risk is best achieved by evaluating long term effects and different exposure scenarios able to take into account different exposure periods, age of the organisms and capacity of recovery after an episode of exposure. Moreover, sublethal parameters such as biochemical, behavioral and physiological must be used so that effects can be detected.

The aim of this work is to evaluate the effects of PD in zebrafish (Danio rerio) early life stages using different exposure scenarios. The test was based on the OECD guideline 210 (Fish, Early-life Stage Toxicity Test) and used newly fertilized eggs. In the first scenario (A), eggs were exposed to PD concentrations between 0,37 mg L-1 and 10 mg L-1 during 28 days. In the second scenario (B), organisms were subjected to a period of exposure (14 days) to the same PD concentrations followed by a period where organisms were transferred to clean medium (14 days) to assess the potential recovery. In the third scenario (C), organisms were first kept in clean medium for 14 days and then transferred to the above mentioned PD concentrations for 14 days to assess influence of age in the response to the chemical. A semi-static test design was used in every exposure scenario, with test medium renewal each two days.

At days 7, 14, 21 and 28 of all scenarios the biomarkers cholinesterase, glutathione S-transferase, lactate dehydrogenase and catalase were measured in order to contribute to the understanding of the dynamic of the toxic response and the influence of exposure time, age and recovery potential on that response. Moreover, biochemical responses were linked to behavioral, developmental (hatching and anomalies) and physiological (length and weight) parameters which are ecologically very relevant and indicators of the organism fitness.

TU 196

The estrogen receptors in salmonid fish

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Estrogen receptors (ERs) and the genes that encode them are well conserved among vertebrates. They mediate physiological effects of hormones involved in growth, reproduction and development in both males and females. Compared to other vertebrates, salmonid fish species have twice as many ERs due to a unique and rather recent duplication event early in the salmonid lineage. Gene duplications are considered one of the major factors that enable species to rapidly adapt to various ecological challenges by providing novel sources for gene functions. The multiple ER genes could be expressed during either the fresh water phase or the marine part of their lives, helping adaptations to the different environmental conditions.

The activation of the ER is controlled by estrogen as well as their mimics. Endocrine disrupters, such as ethinyl estradiol in human contraceptives, interfere or bind directly to the ERs. Lately these compounds have become a major concern since they influence reproductive success. Affected species may develop a skewed gender distribution since genetic males could develop as intersex. As salmonids change life strategies repeatedly during their life cycle there could be an increase or decrease in sensitivity to xenobiotic exposure as the different hormone genes possibly play important roles in different environments.

We recently identified and quantified four different ERs in adult Atlantic salmon (Salmo salar). Our wild study populations originate from the Baltic Sea and the freshwater river Mörrum in Sweden. Our results confirm a complex system in transcription levels of the ERs diverging between locations, sex and tissues. We now investigate the ER gene complex in trout (Salmo trutta) in order to further clarify the distribution and thus the role of the different ERs in the salmonid life-cycle, migration phase and during embryonic development. The results will in the future help determine the impact of endocrine disrupters on the activity and functions of the ERs inside salmonid tissues, asking if it is an advantage or disadvantage to have several ERs?

TU 197

The respiration rate at suborganismal and organismal level in the ground beetle along two metal pollution gradients

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Surviving in a polluted environment is usually connected with changes in metabolic processes. An increase in respiration rate may results from energetically costly processes of combating contaminant exposure (e.g. cellular repair mechanisms, elimination of contaminants) but a decrease due to damage in respiratory enzymes may be also observed.

While some species of carabids are able to maintain viable populations in areas highly contaminated with metals, they may still incur physiological costs associated with metal exposure. To address the question about costs of living in polluted areas, we measured biomarkers linked to metabolism in *Pterostichus oblongopunctatus* collected along two metal pollution gradients in the vicinity of two zinc and lead smelters: Olkusz and Miasteczko Slaskie, S. Poland. Because the response of an animal to stress is a complex reaction, which depends not only on the type of a chemical but also is influenced by many internal and external factors, studies on two or more gradients, each with a number of differently polluted sites, allow for more general conclusions and give better control over possible confounding factors.

The whole animal respiration rate was measured as oxygen consumption with Micro-Oxymax respirometer, and cellular energy consumption was measured as the electron transport activity (ETS), which is linked to cellular respiration rate. We found significant effects of body Cd concentration, body mass and collection date on the respiration rates at the individual level. Although regression analysis revealed a significant increase of respiration rate with increasing body Cd ($p<0.01$), the interaction between Cd concentration and body mass was also found ($p=0.01$). The interaction was caused by a rather unexpected opposite relationship: the respiration rate increased with Cd concentration in the largest beetles but decreased in the smallest. We did not find significant relationship for the respiration rate at the cellular level. The lack of relationship between body Cd concentration and ETS may indicate that ETS is either not sensitive enough or is prone to rapid temporal changes in conditions - as is the case for most biochemical biomarkers. The effects on whole-organism respiration rate would thus represent long-term effects of chronic exposure to Cd, while ETS - the instantaneous respiration rate in response to actual conditions at the time of sampling the cells.

TU 198

Allometric slope to assess stress factors on biological communities

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Biological food webs are strongly organized in hierarchical levels, as shown by their several multitrophic interactions. Studies focusing merely on single food chains are known to be difficult to extrapolate to higher hierarchical levels, like entire biological communities. Therefore, allometry (i.e. the relation between abundance and body mass of organisms in biological communities) provides unique tools to encompass single chains and to capture the energy flow and performance of entire food webs. Since the allometric slope of any food web is related to the relative distribution of differently sized species, it can be used as indicator for eco(toxico)logical disturbance.

By exploitation and management of soil systems, for instance, humans induce huge changes in the environmental conditions as a whole. Input of nutrients and toxicants alter the composition of soil food webs, as observed by the relative importance of bacterial vs. fungal pathways or smaller vs. larger invertebrates. These shifts in the abundance of organisms with different body size result in changes in the allometric slope of the soil food web. By comparing allometric slopes between affected soils and reference sites ("target"), the impact of the anthropogenic disturbance on the performance of food webs and the soil quality can be determined. Large deviations from normal operating range (NOR) indicate major disturbances in the soil food web.

In studies on soil systems in the Netherlands, we show that the elemental levels for phosphorus, copper, and zinc in the soil influence the allometric slope of food webs. The influence of increase in soil phosphorus concentrations on the functioning of soil communities can be explained by the change from fungal to bacterial pathways and the larger demand for phosphorus for fungivore invertebrates. However, comparable allometric changes under increasing soil copper and zinc content are recorded as well. A biological explanation for this last change is less clear, but chemical correlations between cations are plausible. Overall, we show that the allometric slope in soils reflects (1) the elemental conditions in the soil and (2) the functioning of food webs. Allometric slopes can therefore be used to assess the actual effects of stress factors on biological communities.

TU 199

Metallic pollution affects small mammal assemblages: evidence from a large smelter-impacted area

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Effects of pollutants on small mammals have mainly been studied at individual level while effects on populations and communities might be more relevant endpoints to assess long-term impacts of pollution on ecosystems. In field studies interested in impacts of pollutants on species assemblages, discriminating the influence of natural environmental variables (landscape, habitat) from the influence of pollution is an essential issue. In the present study, potential effects of metallic pollution on small mammal assemblages were studied over a large smelter-impacted area (40 km2, Northern France), where soils have been polluted for decades by Cd, Pb and Zn. Animals were trapped (8640 trap-nights) during autumn in woody habitats (copses, hedgerows, tree plantations, woods...) in 4 landscapes (agricultural lands, urban areas, shrublands and woodlands) each along a soil pollution gradient. Concentrations of metals were measured in soils where traplines were set. Metal concentrations were correlated, and here Pb was used to represent soil pollution. Lead concentrations in soils ranged from 73 mg.kg-1 to 42,000 mg.kg-1. A total of 1,338 individuals from 11 species were captured. The influence of landscape, habitat and Pb on assemblage structure parameters was analyzed using generalized additive models, placing the variables in this order and attributing a smoother to Pb. Results showed that total abundance differed between landscapes and between habitats, and in agricultural and urban landscapes, increased

with pollution until ≈ 1000 mg.kg-1 of Pb in soil, and then decreased. Richness differed between landscapes, and, in urban landscape, increased until ≈ 1000 mg.kg-1 of Pb, and then decreased. Evenness varied only with soil pollution, showing a constant decrease. We found effects of pollution on assemblage structure independent from landscape and habitat, and hypothesise that pollution may affect small mammals by direct (toxic) and/or or indirect (suitability of habitats, availability of food) effects. Therefore, assemblage structure could vary because of the disappearance of species sensitive to metals and/or dominance of species poorly sensitive to habitat quality (opportunistic generalist species). Analyses of metallic pollution influence on assemblage composition are in progress, which may allow determining which species are mainly affected and which are the characteristics of small mammal assemblages in metal-polluted lands.

TU 200

Reproductive effects of ingestion of fungicide- and insecticide-coated seeds on red-legged partridge

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Birds from agricultural environments are susceptible to suffer lethal intoxications after ingestion of pesticides incorporated into plants or invertebrates, and so toxicity of agrochemicals has been proposed as a potential cause for avian declines in agricultural areas from Western Europe some sublethal effects, especially those related wit hreproduction, may also compromise population viability. Certain fungicides like vinclozolin, thiram or maneb have been reported as endocrine disruptors, and thus are likely to reduce reproductive success. Cereal seeds are usually coated with some of these pesticides before sowing, and these seeds constitute a main portion of the diet of some species during sowing seasons in autumn and late winter. The aim of this study was to analyse the effects of the ingestion of coated seeds by red-legged partridges (*Alectoris rufa*) on reproduction process and viability of their chickens. We tested three currently used chemicals, an insecticide (imidacloprid) and two fungicides (difenoconazole and thiram). Each experiment consisted in two groups of six couples of partridges each. One of the groups was given wheat seeds treated with the recommended dose for seed coating, whereas the other group was given seeds treated with a concentration twice as high as the recommended one. Treated seeds were administered for a 10-day period that concluded 20 days before the beginning of the reproduction. Cages were checked daily for egg-laying monitoring. Eggs were incubated and the following variables were retrieved: clutch size, egg length and width, shell thickness, fecundation and hatching rates, and chick survival and growth (i.e., mass and length) at days 0, 8, 16, 24 and 32 after hatching. Egg size was significantly reduced by difenoconazole ($F_{2,130}$=10.163; p<0.001) and imidacloprid ($F_{2,91}$=11.283; p<0.001), whereas thiram reduced eggshell thickness ($F_{2,66}$=6.224; p=0.003). None of the chemicals affected fecundation or hatching rates, but chicken survival was significantly reduced by the three compounds, with mortality rates >50% in all cases (*vs* <20% mortality of controls). Chicken growth rates were unaffected by the pesticides, although control chickens tended to show higher sizes when compared to other treatments. The last egg was laid 98 days after the end of the exposure. These observed lagged effects on chicken survival suggest a potential for reproduction disruption of coated seed ingestion

TU 201

PCBs and ecotoxicological data. In silico analysis in relation with in vivo and in vitro data

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Due to urbanization and agro-industrial growing, coastal ecosystems like Seine estuary, are increasingly exposed to the input of anthropogenic contaminants. Among the contaminants, Polychlorinated biphenyls (PCB) are industrial chemicals causing widespread persistent contamination in water and sediment. The toxicity of these compounds is well known on the vertebrates but few data exist about their ecotoxicity on aquatic (marine) species. In Seine estuary, some studies showed the presence of PCB in marketable and edible species (mussels for example). It is therefore essential to obtain new toxicity data for marine species with high economic potential for the Basse-Normandie region in France. In REACH, the use of in silico and in vitro methods is encouraged to minimize the cost of experimentation. Two main objectives of this study are: 1) application of in vitro and in vivo methods, in order to obtain new toxicological data of the PCBs on aquatic species; 2) definition of (Q)SAR models in order to predict the toxicity of the PCBs. Using in silico approach, a classification of the PCBs based on their structural characteristics and initial classifications (DL and NDL) has been firstly carried out. This classification leads to a selection of derivatives for in vivo and in vitro studies. In vivo acute toxicity (endpoints) of 18 PCBs on Daphnia magna and 14 PCBs on Selenestrum capricornutum was determined. In vitro studies were focused on the effects of three PCBs after 10 days of primary culture on enzyme activities and viability of a marine mollusk (Haliotis tuberculata) hemocytes: PCB153 (the most predominant PCB in environment), PCB 28 (PCB with the highest in vivo acute toxicity) and PCB77 (one of the most toxic PCB (DL) for the vertebrates). In vitro and in vivo results show that the different congeners of PCB have different toxicity profiles.

TU 202

Development of q-PCR approaches to assess water quality: Effects of cadmium on gene expression of the diatom Eolimna minima.

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Aquatic ecosystems are nowadays highly impacted by anthropogenic activities and by the various resulting pollutions. Among these pollutions, heavy metal contaminations are of particular concern because of their acute toxicity and their potential accumulation and transfer along the food chain.

In this context, the development of tools for assessing water quality is a fundamental issue. The numerous qualities of periphytic freshwater diatoms communities as bio-indicators have already been shown and they are used in numerous indices for water quality evaluation as the Biological Diatoms Index (BDI, Coste *et al*. 2009) regularly applied in European territories.

Nevertheless theses indices have shown their limits in water quality assessment considering toxic pollutions. Therefore news approaches are called to emerge in the assessment of water quality, and investigations in the field of genetics could be very promising.

Therefore in our study, the impact of cadmium is evaluated at a genetic level by q-PCR on 8 genes of interest after exposure in laboratory conditions of *Eolimna minima*, diatom populations cultivated in suspension under controlled conditions by following their growth kinetics and their Cd bioaccumulation.

Population growth rates reveal a high impact of Cd at a concentration of 100µg Cd/L with a total inhibition of growth. These results are linked with the high bioaccumulation values calculated after 14 days of exposure, 57,0±6,3µg Cd/g dw and 734,1 ± 70µg Cd/g dw for exposures of respectively 10 and 100µg Cd/L .

Genetic responses reveal the impact of Cd on mitochondrial metabolism and chloroplasts photosystem of *E. minima* exposed to 10 and 100µg Cd/L with an induction of cox1, 12S, d1 and psaA after 7 days of exposure for the concentration of 100µg Cd/L and of nad5, d1 and psaA after 14 days of exposure for both conditions.

The first tests made on three different diatom species in suspension (*Eolimna minima*, *Achnanthidium minutissimum* and *Nitzschia palea*) with primers designed for *Eolimna minima* suggest high potential for their application to a larger range of diatoms species and prospects of application on natural biofilms.

Our study is a first attempt to the use of q-PCR for assessment of toxic pollution by genomic biomarkers on river benthic diatoms. The results obtained presage interesting perspectives, but the techniques developed need to be optimized before their use on natural biofilms for the development of new diagnosis tools of water quality.

TU 203

Genotoxicity assessment in gonads, liver and gills of zebrafish (Danio rerio) by use of the comet assay and micronucleus test

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Over the last decades, there has been a decline in fish populations in European and Northern American freshwater ecosystems. Anthropogenic chemical pollution is supposed to play a major role in this respect. Especially for the assessment of ecological risks of genotoxins in the aquatic environment, it is important to understand the relationship between genetic damage in endangered species and detrimental effects at the population level. For this end, it is necessary to be able to detect DNA fragmentation and micronucleus formation not only in somatic cells, but also in generative tissues of individual fish exposed to genotoxins. In toxicology, the comet assay or single cell gel electrophoresis has frequently been used with mammalian germ cells. In contrast, in ecotoxicology, the comet assay has almost exclusively been applied to cells from various somatic tissues. In the present study, adult zebrafish (Danio rerio) were therefore exposed in vivo under semistatic conditions to several concentrations of the known alkylating genotoxin methylmethanesulfonate (MMS) for two weeks. Genotoxic effects were investigated with the alkaline comet assay using primary cells from gonads, liver and gills. In addition, the micronucleus assay was used to identify stable chromosomal aberrations caused by genotoxicity in histological sections of the same organs. In primary hepatocytes, gill and gonad cells, MMS caused an increase of the tail moment. Likewise, the micronucleus frequency was elevated by MMS in gills and gonads, but not in liver. In conclusion, it could be demonstrated that the alkaline comet assay as well as the micronucleus test are appropriate for the detection of genotoxicity in primary gonad cells and histological sections of the gonads, respectively, of both sexes of the zebrafish. The investigation of population-relevant effects is underway.

TU 204

Study of DNA lesions stability and detection of oxidative DNA damage by hOGG1-comet assay in Dreissena polymorpha

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Freshwater bivalve molluscs are considered as effective indicator of environmental pollution. The comet assay, allows the detection of DNA damage like the strand breaks and alkali-labile sites in different types of individual cells. The main oxidative lesion, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), can be detected by the comet assay coupled with a human DNA repair enzyme hOGG1, involved in base excision repair. The aim of this study is to use the comet-hOGG1 assay in gill cells of *Dreissena polymorpha* to quantify 8-oxodG and to determine their stability. Mussels were exposed during 24h to BaP, MMS and Cd and DNA damage levels were measured during 3 days. This study shows that BaP induced DNA damage, with a dose/response relation, and 8-oxodG. The basal level of DNA damage is reached on day 3 indicating an effective DNA repair. The 8-oxodG level detected in MMS treated mussels is weak during the exposure. Cd induced 8-oxodG 6h after the beginning of the exposure. The level of 8-oxodG is higher with the higher Cd concentration but it was detected later. This study revealed that the comet-hogg1 assay is sensitive enough for the measurement of 8-oxodG in exposed mussels.

TU 205

Characterization of growth, gene expression and behavioural effects of exposure to ozone-treated and untreated oil sands process water on Chironomus dilutus larvae

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Global energy demands continue to increase, driving a shift from conventional oil sources to further exploitation of alternative sources. Canada currently hosts the second largest proven oil reserves, with the majority located within the Athabasca oil sands of Alberta. Oil sands production has raised concern over greenhouse gas emissions, habitat loss and wildlife impacts. Water use is often overlooked, but is a key issue associated with oil sands development. To extract the petroleum pre-cursor from the oil sands, a process is used involving addition of hot water and caustic soda and results in oil sands process water (OSPW). This water is alkaline, saline and produced at a rate of approximately 3 m3 per 1 m3 of oil sands processed. Companies are held to a zero-discharge policy so over a billion m3 of OSPW are held in settling ponds and this will increase as production continues.

OSPW contains high concentrations of naphthenic acids (NAs). These are classified as carboxylic acids with the formula CnH2n+z O2 and have been implicated as the primary cause of the

acute and chronic toxicity of OSPW observed across multiple taxa. To effectively treat OSPW for future release or reclamation, an approach is required that targets NAs. Ozonation can target NAs with greater molecular weights - those NAs that tend to be most persistent. To assess the effectiveness of ozonation to eliminate OSPW effects on relevant organisms, we initiated exposure studies with the benthic invertebrate Chironomus dilutus. OSPW was treated with either 30 or 80 mg/L of O3, and both ozonation levels were examined for effects on C. dilutus larvae following a 10-day exposure. Survival was not significantly impacted, but OSPW-exposed larvae were 64-77% smaller than their respective controls ($p<0.05$). Organisms exposed to 30 mg/L ozonated OSPW were no smaller than the controls ($p=0.486$). Larvae in 80 mg/L ozonated OSPW were only 40% smaller than the controls ($p <0.001$), so ozonation generally mitigated growth inhibition. In both assays, larvae exposed to untreated OSPW exhibited disordered case-building behaviours, but ozonation also eliminated these effects. The attenuation of behavioural anomolies and inhibited growth in the ozone-treated OSPW exposures suggests that NAs may be contributing to the effects observed. We are quantifying global gene expression in C. dilutus exposed to untreated and ozone-treated OSPW and aim to correlate molecular changes and whole-organism level effects.

TU 206

Ecological atlas of Azerbaijan

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"Ecological Atlas of Azerbaijan"represents uniform integral cartographical product at which drawing up under our management were used results of long-term researches of scientists of republic. Atlas represents the arch of the extensive information for research of ecological conditions in republic for the purpose of its optimisation, working out and realisation of a rational state ecological policy, ecological education, educate of various strata of society and as relevant endpoint increase of ecological culture. Value of the atlas for an assessment of ecological conditions in Azerbaijan within the limits of world environmental problems as a whole is great. The special thematic maintenance of the atlas is presented in 11 sections: atmosphere, hydrosphere, lithosphere, soils, biosphere, landscapes, plantar cover, fauna, natural accidents, society ecology, legal bases of ecology of Azerbaijan. The atlas contains more than 110 maps. For increase in information capacity of the atlas maps are accompanied by explanatories, tables, schedules and diagram's. Almost all maps of Azerbaijan are given in 1:1500000 scale, that considerably facilitates their comparison at the analysis of maps of the various maintenance. This scientific product represents an ecological relevant endpoint.

ET09 - Laboratory and field measurements and alternative approaches in bioaccumulation

TU 210

Mercury bioaccumulation and biomagnification from diffuse sources in tropical estuaries (Ceara, Brazil)

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Mercury (Hg) is a toxic metal of greater global toxicological concern. Imposed environmental legislation lead to a significant reduction in emissions from point sources, such as industry, and diffuse sources, such as the improper discharge of waste from these activities into the environment. However, prevailing environmental legislation is still insufficient to deal adequately with the Hg legacy mainly from diffuse source emissions.

Food consumption is known to be an important route of exposure and bioaccumulation for MeHg and trophic level is a very significant factor influencing Hg and MeHg concentrations in aquatic organisms, while increased temperatures speed up the methylation of Hg. Consequently, tropical ecosystems (such as rivers, estuaries and coastal regions) even receiving chronically low loads of Hg may experience biomagnification processes (driven by dissimilar uptake and depuration Hg rates) along the trophic chains, whereas bioaccumulation is expected to increase in response to the environment high temperatures. Therefore, the process of bioaccumulation of Hg in fish is expected to go faster and in shorter periods of time in tropical estuaries.

In this study, the fish *Ulaema lefroyi* (a benthic feeder that uses its highly protrusible mouth to forage for infaunal invertebrates, feeding primarily on bivalves, copepods, other crustaceans and polychaetes) was sampled and the content of Hg and MeHg was quantified in muscle tissue, liver and stomach content. Results are presented and discussed, indicating that Hg bioaccumulation and biomagnifications related mainly to remobilization processes results are expected to contribute to the knowledge of the biogeochemical processes that control the dynamic of mercury in the tropical environment.

TU 211

Comparing mercury bioavailability and bioaccumulation in tropical and temperate ecosystems (Ceará, Brazil and Ria de Aveiro, Portugal)

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Mercury contamination is nowadays a worldwide problem in various ecosystems, presenting a high risk to biota over the world including human. A potential warming up climate scenario is expected to increase Hg methylation, therefore is extremely important to evaluate how different climate factors can affect mercury bioavailability. In this study, mercury bioavailability and bioaccumulation in tropical (Ceará river estuary, Brazil) and temperate (Ria de Aveiro estuarine lagoon, Portugal) ecosystems are compared using surface sediments, water and epibenthic fish, under dissimilar climate environments. Mercury was quantified by pyrolyse atomic absorption spectrometry in tissues, surface sediments and SPM filters. Certified reference materials TORT-2, Dorm-3 and Dolt-3) were also used for quality control. As reference site was used the non-contaminated Pacoti estuary (Fortaleza, Brazil). Results indicate different levels of bioavailability and bioaccumulation suggesting dissimilar mercury kinetics.

TU 212

A novel approach for reporting concentrations of mercury in marine mammal muscle tissue

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When muscle tissue mercury values were normalized to 75% muscle water content, the mean, median and range values were drastically lowered. Although dry, wet, and fresh weight values are much higher than normalized values, reporting and running statistical analyses on these would give a false sense of THg accumulation. Using normalized THg values allows for a clearer indication of the amount of mercury the animal accumulated before stranding. A stranded animal whose bodily fluids are depleted will have a higher concentration of mercury than when healthy. Normalizing tissue weights for total mercury represents a universal method for clarifying results whether the tissue sample comes from a healthy or a decomposed animal.

TU 213

Lifespan mercury bioaccumulation in Pomatoschistus microps: a field study in two temperate coastal ecosystems (Ria de Aveiro and Mondego estuary, Portugal)

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Pomatoschistus microps (Kroyer, 1838) is one of the most abundant fish species in estuaries, lagoons and shores of Europe, being an important intermediate predator in the trophic webs. Regarding its importance and with the purpose of verifying its role in the mercury transfer along the estuarine food webs, lifespan mercury accumulation was assessed in this species in different contamination scenarios. Sampling was made in Ria de Aveiro, ecosystem that received mercury rich effluents continuously for five decades, what created a well-defined anthropogenic mercury gradient, and in Mondego estuary, assumed as a mercury free ecosystem. Mercury content of fish was in accordance with mercury contamination of the water and sediment, showing higher values near the contamination source. A mercury accumulation pattern along lifespan was denoted in all sampling sites, although showing different trends according to the environmental contamination. The differences between sampling sites are more evident in the mercury content of the youngest age classes, what suggests a rapid accumulation of the metal in these fishes. Organic mercury content was also assessed, following the same accumulation pattern than total mercury and representing over 90% of total mercury.

TU 214

Percentage of trace elements and ultrastructural analysis before hatching on wild and captive avian eggshells from Thailand: evidence from scanning electron microscope

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Fifty species from five orders (Passeriformes, Charadriiformes, Columbiformes, Ciconiiformes and Gruiformes) of before hatching avian eggshells were collected from Thailand. Results from EDX/SEI of SEM in all species of avian eggshells revealed the energy spectrum of the X-rays character emitted from the element of non metal (oxygen, O; carbon, C; silicon, Si; chlorine, Cl; phosphorus, P; sulfur, S), alkaline metal (sodium, Na), alkaline earth metals (magnesium, Mg; calcium, Ca), other metal (aluminum, Al), and transition metal (molybdenum, Mo). The percentage of all elements was found and has the following order: O (50.12-60.19 %)>Ca (17.48-29.48%)>C (16.67-28.33 %)>Al (0.08-0.75%) >Si (0.03-0.53%) >Mg (0.03-0.52%) >Na (0.08-0.40%) >P (0.03-0.22%) >S (0.02-0.19%)>Mo (0.03-0.11%) >Cl (0.04-0.09%). There was not a significant difference of elemental composition between 15 species of avian eggshells. We found high percentage of O, Ca, and C (97.39-99.62%), which the composition of calcium carbonate (CaCO3), and essential for avian embryonic development. Especially, Mg (0.52-0.07%), Na (0.13-0.40%), P (0.02-0.22%) were found in all avian eggshell species. These elements are major inorganic constituents of the avian eggshell which directly related to an increase in eggshell hardness. Interestingly, we also found Mo contamination in eggshell of Copsychus saularis and Nectarinia jugularis. SEI showed normal character on 3 layers of 15 eggshell species. The thickness of 3 layers was found and has the following order: a middle palisade layer > an inner mammillary layer> an outer cuticle layer. There are 2 types (ambiguous shape or knobbed shape) of mammillary layer. While, the morphology of palisade layer is spongy shape. It has porosities which connect from cuticle layer to mammillary layer. The total eggshell thickness of all species was ranging from 45.68+13.31 to 162.92+40.41 micrometer and correlated with Neoaves. This finding showed the utilization of SEM is a powerful and comfortable to analyze percentage of trace elements accumulation, metal contamination, and classified avian eggshells correlated with the avian evolution.

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TU 215

Bioaccumulation of Molybdenum in the aquatic environment: literature and laboratory/field-generated data

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Information on the bioaccumulation of a substance in the aquatic environment can be used for different scientific and regulatory purposes (e.g., derivation of a $PNEC_{oral}$, setting water criteria based on the maximum tolerable daily intake of a substance through fish consumption). As part of the REACH requirements for >1000 tonnes substances, information on the bioaccumulation of molybdenum was collected from open literature and assessed on its relevance and reliability. Available data suggested homeostatic control of molybdenum in fish, i.e., decrease of bioaccumulation with increasing Mo-levels in the aquatic compartment. The overall robustness of the data-set, however, was rather poor with all exposure levels well below the $PNEC_{aquatic}$ for Mo. As a result, the aquatic $MTC_{hh,food}$ (Maximum Tolerable Concentration of a substance in the surface water that does not cause any harmful effects to human health via fish consumption) that was initially derived by Dutch authorities for Mo - using existing bioaccumulation data - was 2-3 orders of magnitude below the freshwater PNEC of 12.7 mg Mo/L as reported in the Chemical Safety Report of several Mo-substances. In an effort to expand the amount of reliable bioaccumulation data and to refine BAF-based water criteria, the International Molybdenum Association conducted two research studies in 2009-2010.

In the first study the internal Mo-content (total + organ-specific internal levels) was evaluated in various fish species (eel, stickleback, perch, carp bream, roach) that grew up in the discharge water collector tanks of a molybdenum trioxide/ferromolybdenum producing company, containing a mean Mo-level of 1.03 mg Mo/L. A second 120d-study under laboratory conditions exposed rainbow trout to two different Mo-levels (1 and 12.7 mg Mo/L), for a period of 60 days followed

by a depuration period of similar length.
Both studies generated bioconcentration factors well below 1 when muscle tissue was considered. Concentration levels in the muscle tissue of fish taken from both experiments remained below 0.2 mg/kg dry wt. Total concentration levels that were determined for rainbow trout during the exposure period were situated between <0.20 and 0.53 mg Mo/L. The outcome of these studies clearly support the inverse relationship between exposure concentration and bioaccumulation factor for molybdenum, and also demonstrate that molybdenum in fish is adequately regulated up to the $PNEC_{aquatic}$ of 12.7 mg Mo/L.

TU 216

Behavioural response of damselfly larvae captured in small ponds and their biomarker response in relation to accumulated micro pollutants

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A wide variety of micro pollutants might be present in surface waters. Some of these pollutants are only present in the environment for a small period of time, especially pesticides, which makes monitoring difficult. Moreover, little is known on the bioavailability and their related effects of accumulated micro pollutants. Therefore accumulated levels of a wide range of micro pollutants in field captured damselflies were measured and related to functional endpoints and biomarker responses.
We selected 7 ponds through Flanders (Belgium) based on expected differences in type and amount of pollution. From each pond around 40 individuals of damselfly larva species Ischnura elegans were captured. The collected organisms were placed in vivo for 24h, each in individual containers filled with water from there ponds. In each location the larvae-bodies were measurement on 15 different pesticides and 9 heavy metals.
To verify if the accumulated concentrations of the pollutants result in an ecological relevant effect, functional endpoints were measured. In fact general activity and feeding rate were scored over a fixed time period. The general activities were filmed and total distance, speed and amount of movements were counted. The feeding rate was assessed by scoring the number of juvenile waterfleas (Daphnia magma) ingested over a time period. As biomarker responses, Acetylcholinsterase (AChE), Glutathione S-transferase (GST) and energy budget (Ea) were measured in the head of individual damselfly larvae.
Five pesticides are accumulated in the larvae, Isoproturon, metalochlor, terbutylazine, linuron and dimethoate. The metal load is present in all samples with a great difference between de polluted and unpolluted sites. The GST activity and some energy budgets give a significant correlation to the accumulated metals and significant differences occur between the ponds for metal accumulation and biomarker responses.
Furthermore our results indicated that the accumulation of micropollutants can increase the induction of biomarkers and affect the general activity of the damselfly larvae Ischnura elegans.

TU 217

Antioxidant enzyme activities responses in freshwater biofilm in a metal polluted system

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Antioxidant enzyme activity (AEA) responses to metal toxicity have been studied in previous laboratory and microcosm experiments. Results indicated that AEA could be used as an "early warning system"and also AEA were proposed as biomarkers of metal adaptation. However, no field studies have so far assessed AEA in natural biofilms.
The main goal of this study was to assess seasonal patterns of metal exposure and toxicity in a metal polluted freshwater system based on biofilm AEA activities. We expected that AEA response in biofilms will be dependent on environmental variability, assuming metals to be the most important source of variability.
To reach this goal an annual monitoring study was carried out in the Osor River (NE Catalonia, Spain). It is located in a former mining area and receives elevated metals inputs (mainly Fe, Mn and Zn). Three sites were selected: Non-Impacted site (NI), before the mining source with a background Zn concentration, Site1 (S1) downstream the mining source causing an increase of Zn concentration, and Site2 (S2) farther downstream.
Physical and chemical parameters were analysed. Dissolved and bioaccumulated Zn, Mn and Fe were determined using ICP-MS analysis and free metal ions and labile species were calculated with the vMINTEQ. AEA analysed in biofilms included CAT, APX, GR, GST and SOD. The relative abundance of the groups of photosynthetic organisms was determined by means of a PhytoPAM fluorometer.
The free metal Zn concentrations and bioaccumulated Zn showed the same pattern over the monitoring period and were inversely related to the water discharge.
AEA in biofilms displayed different patterns over the year. In the NI, AEA did not follow the metal concentrations. In the S1, APX, GR, CAT and SOD decreased when free metals and bioaccumulation concentrations increased, suggesting an inhibition caused by accumulated metals. GST did not relate to metal exposure or accumulation. AEA dynamics might be explained also by other environmental factors such us light, as well as by spatial and temporal species compositional changes.
We can conclude that AEA responses in biofilms respond to metal accumulation. However, despite being very sensitive, AEA do not linearly correlate to the metal concentrations. Thus, the use of AEA measurements combined with other biomarkers in monitoring metal impacted sites can improve the predictive capabilities for ecological risk assessment.

TU 218

Mercury accumulation in laboratory-reared Chironomus riparius and in indegenous chironomids' assemblages

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Mercury is a priority pollutant characterized by a strong tendency to bioaccumulate in living organisms and biomagnify along trophic chains. These features follow from the transformation of inorganic mercury into the corresponding methylated form, mainly via bacterially-mediated mechanisms controlled by several environmental variables. From a risk-assessment point of view, mercury therefore requires an approach based on establishing its bioaccumulative potential in resident biota rather than the typical toxicity-based approach commonly used for other trace elements. In the case of small organisms (e.g., freshwater benthic invertebrates), collecting an adequate amount of biomass of resident biota for measuring Hg accumulation can become quite problematic. Resident organisms may also be absent due to e.g., strong pollution or cross-sites comparison hindered by the impossibility of collecting the same organisms at all monitoring points. Bioaccumulation experiments with laboratory-reared organisms therefore become attractive provided that they can accurately simulate Hg accumulation in indigenous biota.
We have exposed reared larvae of *Chironomus riparius* to sediments collected at various locations from an aquatic system impacted by Hg pollution. Assemblages of indigenous chironomids have also been collected and are currently being analyzed to validate laboratory results. In the laboratory experiments, adults of chironomids emerged after exposure to field-collected sediments have also been analyzed for Hg content. This type of measurement represents another useful tool of a combined laboratory-field approach to estimate the Hg load exported from contaminated aquatic systems to the surrounding terrestrial environments.

TU 219

An evaluation of bioaccumulation data for Hexachlorobenze (HCB): can these data be used to convert biota standards into water based risk limits?

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For HCB, the EC has set quality standards in biota, but in the Netherlands a preference exists for quality standards based on water concentrations. To calculate these, reliable values for bioaccumulation are a must. Data on bioaccumulation, biomagnification and bioconcentration of HCB, both from the field and the laboratory, were assessed. To calculate biota standards into water standards, either bioaccumulation factors (BAFs) or the ratio of the bioconcentration factor and the biomagnification factor (BCF x BMF) can be used, where the BAF represents the quotient of the BCF and the BMF. Where BAFs are often determined in the field, BCFs are mostly determined in the laboratory. For the purpose of recalculating quality standards, only studies with water concentrations expressed as dissolved concentrations are considered valid.
The evaluation resulted in 19 valid values for laboratory BCFs, partly with water-based exposure and partly with dietary exposure, with a geomean of 12800 L/kg (5% lipid-normalised). Regarding biomagnification, 19 values for the biomagnification and 8 values for the trophic magnification factor were considered valid. From these data, an overall BMF of 3 can be deduced. In contrast, only 10 valid values for bioaccumulation factors were retreived from literature, with a geometric mean of 221000 L/kg (5% lipid-normalised). BAF measurements show a variation of more than one order of magnitude. Normally, BAFs correlate with trophic level or age of the fish, but for HCB this is not the case. An explanation for this deviation of what is expected from theory is lacking. Even at lower trophic levels (algae, small zooplankton), accumulation of HCBs already far exceeds what is expected through equilibrium partitioning. This affects BAFs at higher trophic levels as well. When comparing BCF values for fish multiplied by the BMF (12800 [GREEKX] 3 = 38400 L/kg) to the observed BAF values for fish (geomean 221000 L/kg), there appears to be a large gap between laboratory data and field data. While the confidence in the laboratory data is high, these data seem to be not relevant for the field situation.
Thus, from a scientific point of view, using biota standards could be preferred over recalculating them into water standards, because this involves the least uncertainties. Nevertheless, sampling of individual fish or fish species is accompanied with some additional uncertainty as well.

TU 220

Moving aquatic bioaccumulation assessments to the next level: Progress made and challenges ahead

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Since it was formed in 2005, the HESI Bioaccumulation Project Committee has successfully advanced the science of bioaccumulation ('B') in several areas: developing in vitro methods as part of weight of evidence approaches to 'B' assessment; publishing peer reviewed papers on the state of 'B' science; advancing models to better screen organic chemicals for 'B'; and funding research in basic fish physiology to better understand ADME processes. In addition, the HESI committee has collaborated with SETAC and others to sponsor workshops bringing together academia, industry and regulatory authorities to discuss and advance 'B' science.
A workshop was held in February 2011 to highlight recent and ongoing research efforts, to demonstrate progress that has been made in the field to identify key gaps in current 'B' knowledge, and to discuss how recent progress in 'B' science can be integrated into the regulatory environment. Topics included integration of modeled predictions with laboratory measurements, extrapolation from lab to field data, a review of ADME processes and determining method domain of applicability across chemicals and species, and abbreviated BCF studies. In addition the potential incorporation of these methods into regulatory 'B' assessments and what is needed to best communicate the scientific advancements to the risk assessment community was discussed. This poster summarizes the key discussions in the workshop. The ultimate output will provide guidance to the best path forward to further develop the research and continue to move the science of bioaccumulation forward.

TU 221

Uptake and effects of particle-bound polycyclic aromatic hydrocarbons (PAH) from spiked sediment suspensions in rainbow trout (Oncorhynchus mykiss, Walbaum)

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In context of the ongoing scientific discussion about the potential ecotoxicological impacts of flood events, it is of vital importance to understand the detailed mechanisms of contaminant uptake from suspended particles and related effects in aquatic biota. As part of the interdisciplin-

ary project Floodsearch II, which was supported by the German Excellence Initiative, rainbow trout (*Oncorhynchus mykiss*) were exposed to suspensions of natural sediment from the River Rhine. Prior to suspension, the sediment was spiked with a mixture of the polycyclic aromatic hydrocarbons (PAH) pyrene, phenanthrene, chrysene, and benzo[*a*]pyrene at environmentally relevant concentrations. A control treatment without addition of PAHs was also included in the experimental design. The nominal concentration of suspended solids was 10 g L^{-1} in both experiments. After 1, 2, 4, 6, 8 and 12 days of exposure, important physico-chemical parameters, concentrations of PAHs in water and suspended matter, as well as biomarkers of exposure to the analytes in rainbow trout (n=10; biliary metabolite concentrations, CYP1A protein content in liver tissue, as well as lipid peroxidation) were investigated. Instrumental chemical analyses revealed that analyte concentrations in suspended solids decreased over time, while the degradation rate was highest for phenanthrene, followed by pyrene and benzo[*a*]pyrene. This indicates that the loss was most probable due to microbial metabolization. Concentrations of biliary PAH metabolites in fish increased only slightly in the treatment without addition of PAHs, while mean levels increased to 166 µg ml^{-1} for 1-hydroxypyrene (control value 4.6 µg ml^{-1}) and 17 µg ml^{-1} for 1-hydroxyphenanthrene (control value 0.1 µg ml^{-1}) in the spiked treatment within two days, followed by a subsequent decrease over time. With a latency of two days, this peak of metabolism was followed by a peak of lipid peroxidation that indicates the oxidative stress caused by PAH metabolites. CYP1A protein levels are currently being evaluated using Western Immunoblotting. The results of this study clearly indicate the importance to account for the temporal variability of physiological markers and to support exposure experiments of aquatic biota to particle-bound pollutants with analytical data.

TU 222

Investigating variation in bioaccumulation potential of hydrophobic organic chemicals caused by lipid composition diversity

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Bioconcentration factors (BCFs) reported in the literature are often highly variable for a single hydrophobic organic chemical (HOC). Though in some cases the variation may be caused by experimental artifacts (e.g. incomplete phase separation or nonequilibrium conditions), true mechanisms may affect bioaccumulation of HOCs in such a way that BCFs should perhaps be considered case-specific rather than generic parameters. Bioaccumulation is a product of equilibrium partitioning between an organism and its surrounding aqueous environment and is therefore determined by the characteristics of the interacting phases. For instance, temperature and pH of the aqueous medium and biotranformation capacity of the organism have been shown to affect bioaccumulation of HOCs. Another, less intensively studied factor is lipid composition. Fatty acid pools are known to be highly variable between species, but even intraspecies differences may occur due to a temperature-dependent mechanism regulating homeoviscosity of cellular membranes. In the present study, both inter and intraspecies variation in bioaccumulation were investigated by measuring sorption of PCBs to (1) biological homogenates generated from diverse aquatic organisms (i.e. snail, midge larva, water flea, blackworm, mussel, stickleback, eel, and carp), and (2) homogenates of blackworms and sticklebacks acclimatized to different environmentally-relevant temperatures (4 - 24 °C). Results indicated that lipid-normalized homogenate-water partition coefficients may differ for up to 0.86 log units between species. For both blackworms and sticklebacks, lipid-normalized partition coefficients for differently acclimatized batches were also significantly different, although differences were minor. However, there was no clear relation with acclimatization temperature. These results indicate that BCF values may not generally be applicable to any species for the purpose of risk assessment of HOCs, even when disregarding the interspecies variation in metabolic capacity. Studying homogenate-water partition coefficients provides clear advantages above using living organisms, as it enables measurements of sorption to biological material without the interference of complexing factors, such as biotransformation, kinetics, and growth dilution.

TU 223

Toxicokinetic and toxicodynamic modelling for Daphnia magna exposed to acetylcholinesterase inhibitors

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Carbamates are well known as acetylcholinesterase (AChE) inhibitors, but, in general, less toxic than organophosphate pesticides (OPs) due to the relatively fast reactivation rate of AChE. In the presence study, we demonstrate a toxicokinetic (TK) and toxicodynamic (TD) model for carbaryl to describe the relationship between the internal concentration in test species, Daphnia magna, and the inhibition of AChE activity at several time points during the 48 h exposure. The internal concentration of carbaryl is measured as the estimate of inhibitor concentration at the target site, whereas the production of 1-naphthol is also monitored as a dominant transformation product (TP) of the parent compound. The TKTD parameters for carbaryl are determined by fitting data from in vivo and in vitro experiments. The results are compared to those for diazinon (Kretschmann et al., 2010), one of the representative OPs which exhibit irreversible AChE inhibition.

TU 224

Bioaccumulation, biotransformation and elimination kinetics of organic chemicals in Gammarus pulex

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We investigate the bioaccumulation, biotransformation and elimination kinetics of 15 organic chemicals (Aldicarb, Carbofuran, Carbaryl, Malathion, Chlorpyrifos, 2,4-Dichloroaniline, 2,4-Dichlorophenol, 1,2,3-Trichlorobenzene, 4,6-Dinitro-o-cresol, 2,4,5-Trichlorophenol, Pentachlorophenol, Ethylacrylate, 4-Nitrobenzyl-chloride, Sea-nine and Imidacloprid) from diverse chemical classes and covering a wide range of physicochemical properties (log Kow from 0.33 to 4.96) in the freshwater amphipod Gammarus pulex. This study consists of metabolite screening and identification tests (24h exposure), biotransformation kinetics experiments with a 24h exposure followed by a depuration phase of variable length and toxicokinetic modeling. Further, a comparison of rate constants and bioaccumulation factors derived in this study (i.e. with biotransformation accounted for) with those from a previous study based on total 14C measurements (without consideration of biotransformation) was undertaken.
Known biotransformation products as well as the parent compound were identified by spiking unlabelled standard material of these to samples of control organisms and identification of these peaks via UV-detection. In case no reference standards were available or biotransformation products were unknown, extracts were analyzed after HPLC separation and electrospray ionization in either positive or negative mode with high resolution mass spectrometry using a LTQ-Orbitrap. Four compounds (1,2,3-Trichlorobenzene, Imidacloprid, 4,6-Dinitro-o-cresol and Ethylacrylate) were not biotransformed. For the other eleven parent compounds we detected 19 biotransformation products in total (up to three per parent) of which we identified nine.
Toxicokinetic models were fitted to the time-course of internal concentrations of parent and metabolite compounds. Comparison with bioaccumulation parameters based on total 14C-body residues from a previous study showed correlation of uptake rate constants within one order of magnitude around the 1 to 1 line, confirming that uptake rate constants are independent of biotransformation. Bioaccumulation factors are overestimated by the total 14C-study by a factor of six. Elimination rate constants for compounds which were biotransformed very fast were much smaller compared to those from the total 14C-based study because there the elimination rate reflects elimination of the biotransformation products.

TU 225

Sources and data quality of the existing data for bioconcentration

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To protect environmental and human health, REACH legislation requires data, e.g.. for bioaccumulation the bioconcentration factor (BCF) in aquatic species, preferably fish. BCF is requested for all compounds produced or imported above 100 tonnes/y, and it is useful for compounds produced or imported above 10 tonnes/y to perform PBT (Persistent, Bioaccumulative, and Toxic) assessment and Chemical Safety Assessment. For PBT assessment two thresholds for BCF are set: 2000 and 5000 L/kg. Below 2000 L/kg a compound can be considered non bioaccumulative, above 5000L/kg it is very bioaccumulative and between 2000 and 5000 L/kg it is bioaccumulative.
In this work the experimental variability and data quality of five different public data sets and databases containing BCF data were analysed. One data set (Dimitrov et al., 2005) contains only single, reliable data, two contain mostly single data (Fu et al., 2009 and Footprint database) and two contain single or multiple data (CEFIC LRI and Arnot & Gobas, 2006 databases). In the CEFIC LRI database a Klimish reliability score is reported for some compounds. It should contain only reliable data. In the Arnot & Gobas database a global score is indicated, based on six different criteria. We analysed the experimental variability of a data set obtained combining the reliable data from the previous sources.
In general, in all databases/data sets with more than one value for each compound, the variability is greater than the difference between the two thresholds established by REACH. Moreover, for some compounds, the experimental variability is high, even more than three orders of magnitude (e.g. for DDT experimental variability is of 3.57 log units).
Financial support by the OSIRIS project (GOCE-CT-2007-037017) is gratefully acknowledged.

TU 226

Applicability and performance evaluation of QSAR models for bioconcentration in fish

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Under the REACH legislation, human and environmental protection requires carefully assessing each compound produced or imported in EU. To reduce the number of animal tests (in particular tests involving vertebrates) and the cost, REACH promotes the use of all existing information and alternative methods (e.g. in-vitro, QSAR or read-across).
Goal of this work was to evaluate in which cases QSAR models can replace in-vivo tests. For this reason several QSAR models, developed within OSIRIS project (i.e. ChemPropTM and BCF regressions model for monovalent ionic compounds by Fu et al., 2009) or freely available (i.e. EPISuite, T.E.S.T., CAESAR, CORAL and logP-based equations) were tested. Performance and applicability of the models has been evaluated using a large dataset. The models were analysed both as regression models (in particular error distribution and correlation) and as classification models.
It is interesting to notice that the results of the different models do not always overlap. This offers the opportunity to identify strategies for the careful integration of different methods.
Besides the use of combination of the results on a purely statistical way, we will address the possibility to define a more reliable use of the model, referring to the applicability domain.
Financial support by the OSIRIS project (GOCE-CT-2007-037017) is gratefully acknowledged.

TU 227

Read-across model to estimate the BCF in fish from data for similar compounds

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A validated BCF (fish) dataset for 473 compounds, collected within the former EU project CAESAR and made available by project partners within the EU integrated project OSIRIS, is exploited as the data base for an read-across approach. The model decomposes the training set and the test compound into atom-centred fragments (ACF) and selects the nearest neighbours of the test compound from the data base through the ACF similarity. The BCF data for these chemicals then are used to predict the result. With different levels of similarity, users of the model may switch between increased accuracy or a more general applicability. The performance of the model is shown via leave-one-out statistics. The implementation in the software system ChemProp provides the modelling results and details as well as the coverage of the applicability domain and an uncertainty estimation for the model result. Financial support from the EU Integrated Project OSIRIS (contract No. 037017) is acknowledged.

TU 228

Integrated testing strategies (ITS) for bioaccumulation: in vitro modules

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Under REACH, assessment of the bioconcentration potential is required for chemicals with a log Kow greater than 3 if it is produced or imported in quantities greater than 100 t/year. The

gold standard for the determination of the bioconcentration factor (BCF) is the OECD Test Guideline 305 with fish, which is demanding in terms of resources, costs and number of animals. Integrated? Testing Strategies (ITS) for bioaccumulation assessment take advantage of a variety of information sources in order to estimate whether a chemical is bioaccumulative (B, BCF > 2000) or very bioaccumulative (vB, BCF > 5000). Here, we discuss the potential contribution of in vitro methods to an ITS for fish BCF.
A major process influencing bioaccumulation of xenobiotics in fish is the endogenous metabolism or biotransformation, which will result in a lower BCF value than expected on the basis of the lipophilicity. In vitro methods provide information on the rates of biotransformation . Since the liver is the main site of xenobiotic metabolism in fish, in vitro assays to measure metabolic turnover of xenobiotics should be based on metabolically competent preparations from fish liver, either subcellular fractions such as S9 (supernatant 9000) and microsomes, or isolated liver cells. The metabolic clearance rates generated by such in vitro systems need to be scaled to the whole fish. To this end, physiologically based models can be used that convert the in vitro clerance rate of the liver preparations into the in vivo clearance rate, Km, of the fish. The Km can then be incorporated into existing BCF models to predict the in vivo BCF. Currently, in vitro-based BCF predictions are available only for a small number of chemicals, but these data look promising. In conclusion,the role of in vitro methods within a BCF-ITS would be to provide additional evidence for those cases when in silico-based predictions of the BCF lead to equivocal results.
Financial support by the OSIRIS project (GOCE-CT-2007-037017) is gratefully acknowledged.

ET14 - Natural toxins in ecotoxicology

TU 234

Effects of botanical insecticides on the egg parasitoid trichogramma cacoeciae marchal (Hym. Trichogrammatidae)

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Parasitoids of the genus Trichogramma occure naturaly worldwide and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops. Laboratory studies were carried to investigate the side effects on both the susceptible life stage (adults of parasites) and Less susceptible life stage (parasites within their hosts) of Trichogramma cacoeciae to two formulated products of each of two botanical insecticides: Azadirachtine (Neemazal T/S Blank and Celaflor) and Quassin (alcoholic or water extracts). The results showed that by exposing adults T. cacoeciae to residues of Neemazal formulations on glass plates, the tested preparations were either harmful (Neemazal-Blank) or moderately harmful (Celaflor). The two Quassin formulations tested were harmless. When treated host eggs were offered to adults T. cacoeciae, all tested chemicals were almost harmless. In a further test, host eggs parasitized at different time intervals (1-8 days), were sprayed at the same day. The results indicated that only Neemazal T/S-Blank formulation was slightly to moderately harmful reducing adult emergence. The results showed, in general, that both Azadirachtine and Quassin were relatively safe to the tested parasitoid and could therefore be used in combination with Trichogramma releases. The results of this study can be utilized in designing a less hazardous biopesticides control strategy to combat noxious insect pests with less negative impact on beneficial natural enemies as well as the surrounding environment.

TU 235

Evidences for genotoxicity of paralytic shellfish toxins produced by Gymnodinium catenatum in white seabream (Diplodus sargus)

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The re-emergence of the toxin-producing dinoflagellate *Gymnodinium catenatum* in 2005 after a ten years hiatus of absence in the Portuguese coast was the leitmotiv to the present investigation on the effects of paralytic shellfish toxins (PSTs) in fish. Although fish are frequently exposed to PSTs, the number of studies on its toxicity is low. In particular, reports on genotoxicity evaluation of PSTs are almost inexistent and the few available data are controversial. Thus, the aim of this study was to investigate the induction of cytogenetic damage, measured by the erythrocytic nuclear abnormalities (ENA) assay, in peripheral blood of white seabream (*Diplodus sargus*) exposed to a combination of PSTs produced by *G. catenatum*. *D. sargus* is a valuable species for Mediterranean aquaculture and abundant in littoral waters from Eastern Atlantic to Mediterranean Sea where blooms of G. catenatum occur and consequently toxin events. Toxins were extracted from a 15 L G. catenatum culture and their profile was determined by HPLC-FLD, showing the following compounds in decreased order of relative abundance (in a molar basis): C1+2 (34.3 %) > B1 (23.6 %) > C3+4 (17.1 %) > B2 (16.2 %) > dcSTX (4.1 %) > dcGTX2+3 (3.1 %) > dcNeo (1.5 %) > GTX2+3 (0.1 %). Fish (38 ± 2 g) were intracoelomically (IC) injected with a 0.5 mL single dose of PSTs (1.60 µg STXeq kg-1), using physiological saline solution (0.9 % w/v) as vehicle. A control group received only the vehicle solution. Fish were sacrificed at 2 and 6 days after IC injection, blood was collected from for ENA evaluation and liver was removed for the quantification of PSTs in composite samples.
PSTs concentration in fish liver after 2 days of exposure was 15.2 µg STXeq kg^{-1}. Following 6 days, a slight decrease to 12.2 µg STXeq kg^{-1} was recorded. Although a combination of several PSTs were IC injected to fish, only dcSTX that initially constituted 4.1 % of the toxins was detected in fish, suggesting ability to metabolize PSTs and or to selectively sequester dcSTX. ENA frequency increased significantly both at 2 and 6 days after exposure in comparison with control fish. Current data demonstrated the genotoxic potential of PSTs in fish pointing to an ecological risk associated with the natural exposure of fish to *G. catenatum* blooms.
Acknowledgement
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TU 236

Effects of microcystin-LR on the physiological performance of Daphnia magna and its offspring

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Due to eutrophication and other factors, the frequency of cyanobacterial blooms has increased worldwide. Besides being a nuisance, they can produce toxins harmful to the ecosystem and human health. Some of the most common and potent cyanobacterial toxins are the microcystins MC, especially the variant MC-LR. As a consequence of blooms, the freshwater planktonic communities are affected, particularly the herbivores populations such as those from *Daphnia* spp. The cyanobacterial factors driving this effect are mechanical interference on feeding, nutritional inadequacy and toxicity. The physiological responses of *Daphnia* to MC have been widely studied; however a consensus on the tolerance and its transfer to the offspring has not been reached. Despite the importance of such interaction and the transfer of toxins to higher trophic levels, the effect of MC to *Daphnia* and its offspring is unclear.
Aim of our study is to address these questions, how are *Daphnia* and its offspring affected by MC-LR at the biochemical level? What are the consequences for the physiological performance? We analyzed the effect of MC-LR exposure on antioxidant and biotransformation enzymes and its relation with metabolic enzymes, comparing different age groups and offspring of treated *Daphnia*.
We exposed neonate and adult *D. magna* to MC-LR to determine the effect on enzymes catalase CAT, glutathione-S-transferase GST, pyruvate kinase PK, malate dehydrogenase MDH, lactate dehydrogenase LDH and the parameters thiobarbituric reactive substances TBARS and lactate. Additionally, we exposed *D. magna* to different schemes of exposure and collected its offspring for further exposure to compare their response with those from non exposed parents.
Results show MC-LR effects on CAT and GST. CAT activity increases in neonates exposed to the highest concentration but not in adults, GST has a concentration dependant response to MC-LR. TBARS indicated oxidative stress at high concentrations, pronounced in adults correlated to the lack of antioxidant enzyme activity increase. LDH is inhibited, impairing this metabolic pathway. Based on the ability to maintain enzyme activities and buffer oxidative stress, neonates may cope better with MC-LR than adults. The transgenerational effect of MC-LR depends on the scheme of exposure; there is a differential response of the offspring, particularly on CAT according to the exposure scheme of the parents.

TU 237

Comparison of the transcriptomic response of a Cd-sensitive and a Cd-tolerant Daphnia pulex isolate to cyanobacterial stress

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We investigated gene expression of a Cd-tolerant and a Cd-sensitive *Daphnia pulex* isolate chronically exposed for 16 days to the toxic cyanobacteria *Microcystis aurigunosa*. Cyanobacterial blooms of this species, which are predicted to become increasingly prominent under climate change conditions, are known for its production of microcystin (a toxic cyclic hexapeptid). Life table tests revealed an increased tolerance of the Cd-adapted isolate to *M. aeruginosa*. The microarray experiment followed a 2x2 full-factorial design: Cd-tolerant isolate vs Cd-sensitive isolate. (control diet vs. 50% *M. aurigunosa* in diet). Microarray analysis of the gene expression patterns resulted in 4250 uniquely upregulated genes in the sensitive isolate exposed to *M. aeruginosa*. A subsequent metabolic pathway analysis revealed at least two significantly upregulated pathways in the Cd-sensitive isolate (but not in the Cd-tolerant isolate), i.e. biodegradation and energy metabolism. These results, in combination with microcystin accumulation data, suggest that the higher sensitivity to *M. aeruginosa* of the Cd-sensitive isolate may be due to a higher microcystin accumulation in this isolate, This research benefits from and contributes to the Daphnia Genomics Consortium.

TU 238

Effects of cyanobacterial extracts and cyanotoxins on the growth of microalgae

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Toxin-producing cyanobacteria occur in aquatic ecosystems worldwide and their cyanotoxins may have adverse effects on aquatic organisms. However, not much is known about the potential effects of cyanotoxins on microalgae, an ecologically important group which plays an essential role at the base of aquatic food chains. The purpose of this work was to study the effects of Microcystis aeruginosa and Aphanizomenon ovalisporum cell extracts containing respectively the cyanotoxin microcystin-LR (MC-LR) and cylindrospermopsin (CYL) and pure MC-LR and CYL on the growth of three microalgae species, the freshwater algae Chlamydomonas sp. and Chlorella vulgaris and the marine alga Nannochloropsis sp. The effects of cyanobacterial extracts and pure toxins on microalgae growth were respectively investigated after 4 and 7 days of exposure in the concentration range 0.005-2.5 µg.ml-1 and 0.5-20 µg.ml-1 using microplate assays. Our results demonstrate different susceptibilities of microalgae to cyanotoxins. Pure MC-LR induced more pronounced effects on microalgae growth than M. aeruginosa cell extract. Pure MC-LR strongly inhibited the growth of microalgae species at concentrations higher or similar to 2.5 µg.ml-1 after 4 and 7 days of exposure, whereas the M. aeruginosa cell extract stimulated only the growth of microalgae species at the highest concentrations during the same exposure period. On the other hand, A. ovalisporum cell extract caused more pronounced effects on microalgae growth than pure CYL. The A. ovalisporum cell extract strongly affected the growth of microalgae at concentration of 2.5 µg.ml-1 after 4 and 7 days of exposure, while growth of C. vulgaris was only slightly inhibited by pure CYL at 10 and 20 µg.ml-1 after 4 days of exposure. Our results suggest microalgae growth response are species specific and dependent on the type of toxin. However, cyanotoxins are not likely to affect microalgae growth at environmentally occurring concentrations. This indicates that so-discussed allelophatic effects against microalgae are unlikely to occur, at least concerning microalgae growth.

TU 239

Transfer of the cyanobacterial toxin BMAA via irrigation from water to an agricultural plant (Triticum aestivum)

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BMAA (β-N-methylamino-L-alanine) is a neurotoxic non-protein amino acid produced by different species of cyanobacteria. It can occur as a free amino acid or protein-bound. It is discussed if BMAA is a possible cause of the "Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex"(ALS/PDC), since protein-bound BMAA has been found in the brain tissue of ALS/PDC patients.
Triticum aestivum is one of the most important corns on the world market. If BMAA contami-

nated water is used for irrigation, BMAA may be absorbed by the plants. Hence, even a transfer of BMAA to animals and humans via the food chain is possible.
It was the aim to examine whether BMAA is absorbed from wheat plants when irrigated with BMAA containing water. (1) After irrigation of germinated wheat for 48 h with different concentrations of BMAA (0, 0.01, 1, 10 and 100 mgL^{-1}) sprouts and roots were analysed for free and protein-bound BMAA by LC-MS/MS. As rising oxidative stress is expected, activity of antioxidant enzymes peroxidase (POD) and catalase (CAT) were determined in both plant compartments at the same time point. (2) Sprouts and roots of plants irrigated with 100 mgL^{-1} BMAA were additionally analysed for free and protein-bound BMAA, as well as for POD and CAT activity after 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 h.
The exposure to 100 mgL^{-1} BMAA showed that until 3.5 h there are higher amounts of free BMAA in roots than in sprouts. After 48 h this pattern is reversed with higher concentration of free BMAA in sprouts compared to roots. The results clearly evidence that BMAA in transported within the plant. The content of protein-bound BMAA was lower than that of free BMAA, thus rising with increasing concentrations of BMAA in the exposure medium. Furthermore, plants exposed to 10 and 100 mgL^{-1} BMAA had two-fold higher amounts of protein-bound BMAA in sprouts compared to roots after 48 h.
POD activity was slightly increased in the sprouts of plants from all treatment groups, whereas only the highest concentration provoked a POD activity increase in roots. CAT activity increase was more pronounced. CAT activity was elevated after only 1.5 h in sprouts of plants irrigated with 100 mgL^{-1} BMAA. Moreover, especially in sprouts there was a concentration dependent CAT activity increase yielding to the assumption that oxidative stress was generated due to the BMAA exposure.
In conclusion, irrigation is a potential way to transfer cyanobacterial toxins to the terrestrial environment.

TU 240

Transfer of cyanobacterial toxins into edible plants via irrigation with lake water - a Chinese case study

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The rising pollution of water bodies goes along with an increase of cyanobacteria in the microbiotic community. The ability of cyanobacteria to produce toxins is well known and many cases of poisoning of animals and humans have been described worldwide. Thereby the exposure routes were mainly via drinking water. Another possible risk is the irrigation of edible plants with cyanotoxin contaminated water, since plants are able to take up these toxins. This scenario takes place e.g. at the lake Chao (China). In this study the cyanotoxin content in vegetables (spring onion, pak choi and courgette) grown on the shore of lake Chao was investigated. Microcystins (MC) were measured in the cyanobacterial bloom containing lake. The continuous irrigation of the plants with lake water led to high accumulations of MCs in all vegetables. The MC concentrations in the vegetables were above the TDI ("tolerable daily intake") recommended by the WHO.

TU 241

Harmful algae in the Venice Lagoon and in the Po River Delta (northern Adriatic Sea, Italy)

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An integrated approach for the structural identification and quantification of algal toxins, in Northern Adriatic Sea, specifically in the Po river delta and in the Venice lagoon, by applying a combination of analytical techniques such as optical and scanning microscopy (OM, SEM), and Liquid Chromatography coupled with High Resolution Time of Flight Mass Spectrometry (HPLC-HR-TOF-MS), is presented. The proposed approach has been preliminary applied to the investigation of harmful algae occurrence and distribution in the above mentioned coastal areas.
The performed sampling sessions showed that potentially harmful algae such as Dinophysis caudata, D. mitra and D. sacculus were present during summer period in most of areas directly influenced by seawater, such as the Venice port entrances and the Po river delta, but not in the inner Venice lagoon parts. Nevertheless, quantitative observations demonstrated that their abundance were always significantly below the conventional limit (~200 cells/L) for which poisoning events could occur. The presence of Pectenotoxin2 (PTX2) in phytoplankton cells extract was anyway confirmed by HPLC-HR-TOF-MS at Venice lagoon port entrance, so indicating the potential release of toxins in detectable amounts even at such low cell concentration levels.

TU 242

Temporal variations of microcystins and anatoxin-a in San Roque reservoir (Córdoba-Argentina)

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The occurrence of cyanobacterial toxic bloom is well known worldwide. However, reports on the occurrence of cyanotoxins in South America are scarce. San Roque reservoir is an artificial lake, formed by a dam, which is the main source of drinking water for Córdoba city (1.5 million inhabitants). Fishing, swimming, and sailing are additional activities practiced in its waters. The main goal of this study was evaluating temporal variations of three common microcystins (MC-LR, -RR and -YR) and anatoxin-a in San Roque reservoir, looking to identify environmental factors that could promote the presence of these cyanotoxins in this water body. Cyanotoxins were quantified by HPLC-ESI-MS/MS in samples collected monthly from February, 2006 to March, 2007. MCs and anatoxin-a were detected in 71% and 38% out of analyzed samples, respectively. Highest levels of MCs were observed during summer, while anatoxin-a was mainly present during autumn. The occurrence of MC-RR and MC-LR was similar but MC-RR reached higher values than MC-LR. MC-YR was found in 4 samples, being the main cyanotoxin present in these samples. MCs levels showed positive correlation with Microcystis sp. and Pseudoanabaena sp. biovolume ($p<0.05$, $r=0.51$ and $r=0.55$ respectively), while anatoxin-a concentration showed positive correlation with Anabaena sp. and Oscillatoria sp. biovolume ($p<0.05$, $r=0.40$ and 0.48, respectively). Discriminat analysis showed that the presence of MCs in the reservoir could be favored at pH close to 8.0, in addition to low proportions of Chlorophyta and Crysophyceae in the phytoplankton. On the other hand, the dominance of anatoxin-a could be favored at higher levels of pH and chlorophyll-a, lower temperature and total phytoplankton biovolume, with total inorganic nitrogen close to 500 µg L-1. Low proportion of Criptophyta, Cyanophyta and Crysophyceae in the phytoplankton also favored the presence of antoxin-a. The co-occurrence of both toxins (MCs and anatoxin-a) was detected when the pH was over 7.6, with dissolved oxygen close to 9.2 mg L-1 and chlorophyll-a around 61 µg L-1. MCs levels exceed guidelines values recommended by WHO for either recreational or drinking water. This is the first report on the presence of anatoxin-a in Argentinean freshwaters, although anatoxin-a levels were below the guidelines established in other countries (i.e. New Zealand- 6 µg L-1). Further studies are necessary to fully assess environmental factors causing the observed temporal variation.

TU 243

Rapid detection of Pacific-ciguatoxin-1 (P-CTX-1) in blood of coral reef fish, mice and humans using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

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Ciguatera fish poisoning (CFP) is a kind of food intoxication caused by ingestion of coral reef fish contaminated by ciguatoxins (CTXs). Among the CTXs, Pacific-CTX-1 (P-CTX-1) is the major toxin in the muscle of carnivorous fishes from the Pacific Ocean, contributing to around 90% of the total lethality. Recently, cases of CFP have increased globally, probably due to greater international trade in and consumption of coral reef fish. To protect human health and minimize economic losses by the fisheries industry after CFP outbreaks, rapid screening of ciguateric coral reef fish is crucial. Analytical methods are currently available for the detection of P-CTX-1 in fish muscle. However, quantification of CTXs in blood has the advantages of being non-destructive and allows for repeated sampling for continuous monitoring, and blood CTX concentrations may serve as a surrogate for estimating levels in fish tissue. This study aims to develop a chemical method for quantifying P-CTX-1 in blood from coral reef fishes using sonication, together with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Using the optimized conditions, matrix spike recoveries of P-CTX-1 in blood of nine coral reef fish species ranged from 76–98%. The variation among samples related to repeatability and reproducibility of the developed method was < 11%. Linear correlation coefficients for both solvent- and matrix-based calibration curves were above 0.997 and matrix effects in blood extracts were much lower than those in fish muscle extracts. The limit of quantification (LOQ) of P-CTX-1 in fish blood was determined to be 0.5 ppb; and it can be improved by the employment of a more sensitive HPLC-MS/MS. The whole analytical method required 12 hours for eight samples. The robustness of the present method was examined using blood from mice and humans so as to assess its potential for clinical diagnosis of CFP in animal models as well as humans and the matrix spike recoveries were found to be 77±7% and 71±10%, respectively. To validate the optimized method, blood will be collected from coral reef fish and mice that will be exposed to ciguateric fish extracts. This experiment will provide information about the absorption, distribution and elimination of P-CTX-1 in fish and mice.

TU 244

Rapid, simple and efficient method of extraction of microcystins LR, RR and YR from fish tissues

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Cyanobacteria are aquatic microorganisms which can be toxic to aquatic life and also to humans via food. In the recent years, the occurrence of cyanobacterial blooms has increased especially because of the eutrophication of lakes and rivers. Among the toxins they produce, there are the microcystins (MC). These are hepatotoxic cyanotoxins very stable and persistent in the environment. Microcystins can thus accumulate in flesh and organs of fishes and potentially transfer along the food chain.
The objectives of this study were to develop a rapid, simple and efficient method for microcystin extraction from fish tissues for MC-LR, -RR and -YR. In this study, we particularly focused on fish liver, muscles and blood. The liver is the target organ for microcystins, the muscles are usually the only part consumed by humans which makes them the principal vector of human exposure and blood allows the distribution of microcystins through the fish. We also verified whether it is adequate to lyophilisate the tissue before extraction or not.
In this study, samples of liver, muscles and blood were incubated in the presence of spiked standards of MC-LR, -RR and -YR. For samples of liver and muscles, one half of the samples was lyophilized and the other half was frozen pending extraction. We chose a solid-liquid extraction with methanol 100% and 80%. The detection method developed is a high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) using multiple reaction monitoring (MRM).
The extraction method developed allows a high recovery rate (min. 80%) for MC-LR, -RR and -YR extracted from non-freeze-dried liver and muscles and from the blood. For the extractions from freeze-dried tissues, the recovery was lower and varies between 26 and 67%. This method also allows a high and stable recovery rate for a large range of microcystin concentrations (15 - 240 ug.L-1) for each microcystine and each tissue.
The extraction and detection methods we have developed are simple, fast and efficient compared to other previously published methods which require either the preparation of a specific internal standard and protein precipitation, or extraction in acidic water at high temperature in a filtration system based on sand. After validation in vivo, this method of analysis will be used to study the accumulation of microcystins in tissues of trout and perch. This will ultimately improve estimates of human exposure to microcystins through fish consumption.

TU 246

A model for emergency response and risk management of drinking water reservoirs affected by cyanobacteria blooms

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Hydrologic extreme events related to climate changes may contribute to the presence of blooms of toxic phytoplanktonand seriously impact the quality of surface water reservoirs used for drinking water supplies.
The occurrence of an extraordinary bloom of cyanobacterium Planktothrix rubescens, with maximum algal density exceeded 150 million cells/liter, was observed since the beginning of 2009 in the Occhito basin, (Apulia, Italy) most probably as the consequence of an flash cycle of drought and flooding. The event was used for a pilot study to evaluate a feasible model for risk management.
Emergency response related to the possible presence of harmful algae are mainly focused to mitigate the risk of toxins presence in distributed drinking water and to efficiently communicate risk information to the target population and authorities, according to the 'water safety plans' ap-

proach by World Health Organization.
Screening and confirmatory methods were evaluated in terms of reliability, practicability , response-time and cost-effectiveness in order to propose fit-for-purpose analytical tools for a multi-stage approach to control water basins affected by cyanobacterial blooms.
Response actions have included:
a) identification and quantification of cyanotoxins in raw, treated and distributed water;
b) specific evaluation of raw water treatments , combining granulated activate carbon (GAC) with conventional treatment practices, i.e. pre-oxidation, flocculation, sand filtration and post disinfection;
c) risk communication on different media channels, including press communicates and a dedicated web site within the Apulia Region Portal.
The above measures, as implemented through an intensive collaboration amongst the main stakeholders, were effective in successfully managing the health risk for the affected populations. Risk management plans for short-long term period were implemented involving specific investment for new flexible treatment system, investigation on environmental parameters inducing/affecting/regulating bloom formation in the basin related to seasonal changes, specific training activities for local environmental and health authorities.
Finally, general national criteria and procedures for a comprehensive risk assessment and risk management approach with reference to cyanotoxins in drinking water resources have been developed and validated.

TU 247
Epigenetic toxicity of cyanobacterial extracts: effects of ozonation and chlorination
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Cyanobacteria and their toxins may present a hazard for drinking water safety. We examined *in vitro* potencies of *Microcystis* sp. water bloom to downregulate gap junctional intercellular communication (GJIC) and to activate mitogen-activated protein kinases (MAPK) in rat liver stem-like cells WB-F344. Both parameters are potentially associated with tumor promotion. The extract induced the acute inhibition of GJIC and activation of MAPKs ERK1/2 and p38 within 30 min of exposure at non-cytotoxic doses. Cytotoxicity was observed only at the highest doses after a 24-h exposure. A known tumor promoter, microcystin-LR, was detected in the extract by HPLC-DAD, but this cyanotoxin has been shown not to affect GJIC/MAPK in this *in vitro* model. Thus, the effects observed in our study were caused by not-yet-identified chemicals of the cyanobacterial origin. The inhibition of GJIC, activation of ERK1/2 and p38, as well as cytotoxic effects were completely eliminated when the extract was treated with ozone for 30-min. Chlorination with sodium hypochlorite had any significant effect neither on tumor promoting activity nor cytotoxicity, even when the extract was chlorinated for 24 hrs. Although chemical identity of the responsible agents remains to be elucidated, the information on the rapid elimination of the tumor promoting activity and cytotoxicity of cyanobacteria by ozone might have a particular importance for the selection of the best technology for drinking water treatment from cyanobacteria-contaminated reservoirs.

TU 248
Identification of Protein Biomarker(s) Associated with Pacific Ciguatoxin (P-CTX-1) Exposure in Grouper (Epinephelus coioides)
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Ciguatera is a form of food poisoning caused by consumption of reef fish contaminated with ciguatoxins (CTXs). Outbreaks of ciguatera have had both public health and socioeconomic impacts more than a century. CTXs are extremely potent"the lethal potency of the most toxic CTX, P-CTX-1, is 100 times greater than that of tetrodotoxin. Ciguatera is not limited to tropical regions, but also occurs in non-endemic regions because of the expanding international trade in tropical fish species and the lack of practical and accurate testing methods for CTXs. Reliable biomarkers for ciguatera and/or CTX exposure in fish and humans have not been characterized to date. The identification of biomarker(s) related to CFP can support the development of more specific detection methods for the toxin, and greater understanding of resistance and biotransformation mechanisms in ciguateric fish. This study compared the effects of P-CTX-1 exposure on protein expression profiles in grouper (*Epinephelus coioides*) GL-a liver cells and in juvenile fish. Stable isotope labeling with amino acids in cell culture (SILAC) was used with a non-gel-based proteomic approach and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for performing differential display analysis in the GL-a cells, while two-dimensional gel electrophoresis (2-DE) was used for analysis of the juvenile groupers. Heavy, [$^{13}C_6$ L-arginine (R_6) 4,4,5,5-$_2H_4$ L- Lysine (K_4) and $^{13}C_6{}^{15}N_4$ L-arginine (R_{10}) $^{13}C_6$ L-Lysine (K_6)] and light [normal L-arginine (R_0) and L-lysine (K_0)] amino acids were incorporated into the cells during culturing prior to experimental exposure. The juvenile groupers were fed with grouper larvae pre-injected with P-CTX-1. The proteins were extracted from the GL-a cells and juvenile groupers after exposure and digested for analysis by nano flow liquid chromatography quadrupole time-of-flight mass spectrometry (Nano-LC-QToF-MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS) respectively. Six proteins were identified with induction higher than two-fold in 0.3ng/g P-CTX-1 exposed cells. The mechanisms of these protein changes and their potential to be a biomarker(s) will be investigated.

TU 249
Effects of combined mixture of microcystin-LR and cylindrospermopsin on the growth of Chlorella vulgaris
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Cyanobacteria (blue-green algae) can simultaneously produce a variety of potent toxins in aquatic ecosystems including microcystin-LR (MC-LR) and cylindrospermopsin (CYL). The combined effects of these toxins, that can be found in water bodies, to aquatic communities are poorly understood and are of particular concern as a potential risk to human and animal health and to the environment. The purpose of this work was to study the joint effect of the cyclic peptide MC-LR with the alkaloid CYL on the growth of freshwater alga Chlorella vulgaris using a full factorial design. The two reference conceptual models for mixture evaluation based on the effect of individual compounds, concentration addition (CA) and independent action (IA), as well as the deviations to synergism/antagonism, dose-level and dose-ratio dependency were applied to the growth data. Experiment was carried out in 96-well polystyrene microplate and the effects of the binary mixture on growth of C. vulgaris were evaluated after 4 and 7 days of exposure in a concentration ranging from 0.5 to 20 µ.ml-1. The IC50 value for each toxin was compared to values calculated in the presence of the other toxin. In addition results obtained for the binary mixture were compared with those expected from the exposures to individual compounds and significant differences were observed. This work highlights the importance of this study on the effects of combined exposure to toxins and understanding their potential risks to aquatic communities.

TU 250
Marine algal toxins and epigenetic effects: does okadaic acid induce alterations in DNA methylation in the mussel Mytilus edulis?
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Okadaic acid is a marine toxin, produced by different species of dinoflagellates such as *Prorocentrum lima* and *Dinophysis* sp. These species can proliferate to form a harmful algal bloom, resulting in filter-feeding shellfish accumulating these toxins. Human consumption of contaminated shellfish results in diarrheic shellfish poisoning. This is probably caused by the inhibiting effect of okadaic acid on protein phosphatases. Okadaic acid is also a known tumor promoter. It has been shown that this toxin affects DNA methylation in mammalian cells, with possible consequences for gene regulation and expression. In this study, the effects of okadaic acid on DNA methylation are tested in the mussel *Mytilus edulis*. This species is directly exposed to toxin producing algae and is known to accumulate okadaic acid. Global DNA methylation was measured by an LC-MS/MS method, which was previously successfully used to determine DNA methylation levels in the water flea *Daphnia magna*. Specific methylated sites were analyzed by an enzyme based method 'Amplification of intermethylated sites'. Results of this ongoing study will yield important insights in the toxic mechanism of okadaic acid in *Mytilus edulis*. Alterations in an epigenetic system such as DNA methylation may also give rise to transgenerational effects, which will be subject of further studies.

LC03 - Increasing robustness of LCA methodology

TU 254
How simple is it? - testing options for simplification on a LCA of mobile phone
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The possibility to perform full-scale LCAs for new ICT products and upgraded models is very limited. Thus there is a need for simplification, but the methodological choices should be made with care and implications should be considered. The study presented here was performed to identify simplifications that could be made in the assessment of ICT products and different simplifications were tested on a previous LCA on a mobile phone.
From studying the results for the different impact categories and how they vary between different life cycle phases we learned that by only considering Global Warming Potential we can not make a satisfying environmental assessment of a mobile phone. To select a few impact categories only was neither an easy task based on the study results. Many of the impact categories varied to a too large extent. Furthermore, based on the current study we do not suggest the possibility of excluding any life cycle phases. However, some specific processes seem to be the major reasons for environmental impact of material acquisition and manufacturing. Thus, a possibility for simplification through focusing on some process within the life cycle may be feasible. Processes include gold production, electricity generation and chip production. Testing the possibility to use data for electronic components and all other processes from the Ecoinvent database, the results for global warming potential showed a high correspondence with the original figures which indicated that the use of generic data would give relevant results. However, for the other impact categories the results were not as promising as major differences were shown for most of them. Some first conclusions are at this stage that it is not enough to consider only global warming potential when ICT products are to be assessed from an environmental perspective. There are some processes in material production and manufacturing that seem to be of major importance. In line with earlier studies the electricity generation proves to be an important issue. However, the possibility to draw general conclusions on a set of process to focus assessments on need to be confirmed in further studies and through other case studies performed in the ICT field. Finally, as there is always the possibility that data gaps and uncertainties in current "full"LCAs will lead to the recommendation of simplifications which are invalid, more comprehensive LCAs always need to be made to complement the simplified assessments.

TU 255
Investigation of LCA simplification approach: the wind power electricity case
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Scientific publications related to the electricity generation systems from Life Cycle Assessment (LCA) show great variability in their results and conclusions. Thus it leads policy makers to consider LCA somehow as an inconclusive method. Moreover, LCA is usually considered in the industry sector as time and energy consuming.
This study concerns the onshore wind power electricity greenhouse gases (GHG) performances. It aims at developing a correlation facilitating access to these performances as a function of key parameters.
A thorough LCA literature survey and analysis of the ecoinvent 2.1 wind turbine (WT) LCAs have highlighted the importance of a limited numbers of parameters.
These, have been classified into 3 categories: technical (related to the intrinsic WTs characteristics such as weights or power curve), geographical (related to the wind conditions on the implantation site) and methodological (the lifetime defined arbitrarily).
A 17 WTs sample has been selected (from 800kW to 4.5MW) which is assumed to be representative of wind turbines installed since 2003, and forecasted for the near future. The WT are

characterized by their component weights, tower heights and their power curves. The WTs inventories have been built considering the main assumptions highlighted by the literature survey and the ecoinvent inventory assessment. For instance, we kept the same lifetime for both the moving and fixed part . We did not consider the end of life because this is too systems specific (regarding the possible scenarios).Then the electricity production for the WTs has been calculated according to their power curve and the mean wind speed.
The correlation relating GHG impacts per kWh produced by each turbine to the key parameters has then been defined across the whole sample. A confidence interval (based on the relative standard deviation) has been found out to vary according to wind speed. For a 20 years WT lifetime, the GHG performances vary from 6.8 g CO2 eq/kWh ± 10.6% (vwind= 9 m/s) to 38.3 g CO2 eq/kWh ± 14.8% (vwind=4m/s). For average wind condition in Europe (6 m/s) the correlation results (13g CO2eq/kWh ±12.2%) are in accordance with the literature average results (13.5 g CO2 eq/kWh). Moreover, varying the life time of WTs from 10 to 30 years have induced a high variability of GHG performances from 8.7 to 26.1 gCO2eq/kWh ± 12.2% (for vwind= 6 m/s), GHG performances of WTs are therefore found to be very sensitive to this methodological parameter.

TU 256

Development of a life cycle assessment tool for construction, maintenance and use of civil works

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LCA assessments and experiences have become common practice in the building sector in the last years. However the number of studies carried out is still rather scarce in the linear infrastructures sector. A LCA tool for linear infrastructures has been developed within the CLEAM project framework, with the aim of making easier the application of LCA in the civil work sector and to provide a complete evaluation of the environmental impacts generated throughout the whole life cycle of an infrastructure.
The aim of the project is to simplify the assessment of environmental impacts in the sector of civil works and set the ground for evaluating constructive alternatives from the perspective of LCA thinking. With this in mind, the tool has been developed following two objectives: i.e. (i) simplifying data introduction and (ii) making the interpretation of results more user-friendly.
To fulfil the above objectives, the LCA tool for linear infrastructures is organized around a commercial budgetary database (Preoc 2009), upon which the LCA inventory has been developed. This inventory is the backbone of the developed tool. To assess environmental loads, the Ecoinvent Data v.2.0 has been used. As for the quantification of impacts, the ReCiPe midpoint and endpoint methods have been applied. Finally, as support for the evaluation process, the SimaPro software v.7.2 has been used.
The assessment has taken into consideration the manufacturing, construction, maintenance and use phases of the infrastructure's life cycle. In relation to the manufacturing phase, the manufacture of materials used in the infrastructure has been considered. For the construction phase, machinery, waste management and transport needs for the works have been evaluated. For the maintenance phase, material manufacturing as well as machinery have been considered. Finally, for the use phase, traffic emissions, lighting and traffic lights energy consumption have been considered.
The generated LCA tool allows assessing environmental impacts while considering the entire life cycle of the infrastructure. Furthermore, this easy to use and powerful evaluation tool allows comparing construction alternatives, as well as identifying critical materials or specific components and/or life cycle phases, even for the less experienced users. Hence, the twofold objective of this project is achieved.

TU 257

The project BioEnergieDat: a German LCA data base for decision support on bioenergy

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LCA studies on bioenergy are based on diverse methodological choices and factual specifications, resulting in discrepancies in data sets of identical technologies or products. Assessment of technologies for robust decision support fosters the need for further harmonization of methodological procedures, validation of input data and assumptions on specific processes. In the context of the German energy and climate policy, the need of an adequate database on bioenergy reflecting German framework conditions has been identified. A two-year's project for a database on energetic use of biomass for Germany has been launched by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) in September 2010. The project with the akronym BioEnergieDat is carried out by a consortium of seven scientific partners led by the Karlsruhe Institute of Technology (KIT). It is based on previous work and experiences of the German Netzwerk Lebenzsyklusdaten and is aimed at a validated database for use within the German strategy on biomass, but due to a modular concept also for all applications of LCA and diverse related instruments like Carbon Footprint. It is intended to take over information from technology development as well as to provide methodological support for assessment of technology.
BioEnergieDat will generate harmonized and validated datasets for bioenergy based on the specific framework conditions of Germany, to be used as decision support for the assessment of technology as well as for environmental strategy development. The concept shall support methodological development as to specific questions of bioenergy like substitution effects or land use, it shall foster the evolvement of data quality procedures, and it shall provide novel IT solutions for efficient, reliable and fast exchange of data and information, supporting not only national but also international networking. First results of the project work will be presented as well as the embedding in the research programme on biomass energy use. The relationship of the project to the Sustainable Biomass Strategy in Germany will also be shown.

TU 258

Enhancing the data basis for LCA through process simulation: the case of lignocellulosic ethanol production in Sweden

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One of the application areas of LCA is to assess the environmental impact of emerging technologies in order to support strategies and policies for technological development. However, in many cases this is restricted by a lack of robust data caused by the immaturity of these technologies.
A possible solution to this constraint is the use of process simulation, whose mass and energy calculations combined with LCA can be used to support strategic decision making for emerging technologies. For example, process simulation can generate data on the water consumption of a process as well as the level of pollution for the water leaving the process. Combining these data with additional data regarding water availability and quality and integrating both into an LCA can support strategic decisions i.e. the sustainability of industrial infrastructure intending to use the simulated process.
In our study we want to demonstrate the potential of using process simulation for the environmental assessment of emerging technologies, using the example of lignocellulosic ethanol production in Sweden. This is of particular relevance, since lignocellulosic ethanol production is currently not set up in an industrial scale in Sweden and therefore the study can deliver highly valuable information on how this technology could be further pursued and developed by the private and public sector in Sweden. In addition, our study uses the data generated for lignocellulosic ethanol to assess the environmental impact of producing bio-ethanol based polyethylene in Sweden from a local feedstock, which at a later time and under a wider scope could be used to support strategic decisions about where to build plants for the biomass based production of this widely used plastic.
In general, the use of process simulation in this case study is intended to further the use of process simulation for LCA purposes and consequently to make LCA more reliable and open the path to a wider set of application areas.

TU 259

Considerations about using European LCA-database in a not European LCA-application: case study of waste destination in Brazil

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In Rio de Janeiro Municipality 2,000 t of C&D (Construction and Demolition) waste are daily disposed at a MSW (Municipal Solid Waste Landfill). There is almost no recycling of C&D waste in the city. A LCA was carried out to investigate seven scenarios involving four alternatives for the C&D waste destination: (a) recycling at the source through small mills; (b) recycling at a stationary facility; (c) disposition in an inert landfill and; (d) disposition in a MSW. The results of the LCIA (Life Cycle Impact Assessment) have indicated that the scenario with less environmental impacts was the scenario with 20% recycling at the source and 80% disposition in an inert landfill. Nevertheless, it can be argued about the consistency of this result, because almost all data was secondary and from an European Database. In this work, having as case study the C&D waste destinations in the city of Rio de Janeiro, it will be discussed that in the absence of a national LCA Database (as in the case of Brazil) the use and the adjustment of data sets from an European Database can be a good option, considering however that the interpretation of the results must be based on different sensitivity and uncertainty analysis.

TU 260

The use of Genetic Algorithms to solve the allocation problems in the Life Cycle Assessment

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One of the most controversial issues in the development of Life Cycle Assessment (LCA) is the allocation procedure, that consists in the partition and distribution of economic flows and environmental burdens among to each of the products of a multi-output system.
Because of the use of the allocation represents a source of uncertainty in the LCA results, this paper presents a new approach based on Genetic Algorithms (GA) to solve the multi-output systems characterized by a rectangular (and thus non-invertible) coefficients matrix, without using computational expedients such as the allocation procedure.
The GA is a population-based stochastic global search technique inspired from the biological principles of natural selection and genetic recombination. Starting from a codified random set of possible solutions of a problem (population), the GA simulate the evolution of the population through genetic operator (as inheritance, selection, genetic mutation) up to obtain the optimal solution.
The evolution of a component of the population (single solution of the problem), its probability to procreate and to hand down its gene pool depend on its fitness: a solution is assessed as acceptable if it minimizes or maximizes a specific objective function.
The objective function is based on two principles: the respect of the bonds and the analysis of the performance of the solution. The genetic research represents a balance between "exploitation"(local research) of the optimal available solution and "exploration"(global research) of the space of the research.
The paper applies a GA to a multi-output productive process of essential oils, natural and concentrated juices from oranges and lemons.
The results obtained for the case study taken into consideration showed that the application of GA allows to respect the energy and mass balances for the examined system. Moreover, low differences between the inventory vectors obtained by using the GA and those obtained with the solutions based upon the traditional computational technique of the allocation have been observed. Since the real solution of the inventory vector is unknown, the authors are not able to compute a proper performance indicator for the implemented algorithms. However, considering that the differences of the obtained GA solutions from the traditional solution are not overwhelming, this methodology is worthy of further investigations.

TU 261

Towards an integrated approach between Material Flow Analysis and Life Cycle Assessment

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The requirement of predictive methods has increased over the past decades in response to the environmental and socio-economical emergencies. Despite the huge amount of approaches and tools, it seems quite difficult to compare results obtained from different procedures. It is widely accepted that an integrated approach of disciplines and methodologies could help to cover all the complexities related to a problem in a life cycle thinking perspective.
In this work the potential of applying Material Flow Analysis (MFA) approach to Life Cycle Analysis (LCA) is discussed, aiming at proposing a systematic strategy of identifying Sustainable Practices. MFA is a systematic assessment of the flows and stocks of materials within a system defined in space and time. Emphasis is placed on linkage between sources, pathways an the intermediate and final sinks of a material (i.e. substances as well as elements). In these sense, MFA allows to identify the depletion or accumulation of substances early enough to take countermeasures towards more sustainable solutions. Furthermore, by means of MFA approach even small

changes, that imply difficulties in measuring effects in short time scales but that could lead to long term damage, may become evident.
MFA can be regarded as a method to establish the inventory for an LCA. This is especially true when LCA is applied to systems rather than to single goods. Indeed, the results of an MFA are quantities of flows and stocks of materials according to measurements, and the principle of mass conservation. In these sense, these are objective quantities (aside from analytical and numerical uncertanties). The interpretation and evaluation of MFA results, which may be conduct by means of LCA, is instead a subjective process, since it is based on social, moral, economical values.
Proposing an integrated approach between MFA and LCA will lead to overcome critical aspects that usually affect environmental studies. In particular, the impact assessment of LCA strives for assessing as many as possible substances and compounds to guarantee completeness while MFA is directed towards reducing the number of substances of study as much as possible to maintain trasparency and manageability.
Finding the best solution to fit results from MFA and LCA may lead to greater benefits in waste management, resource conservation and topics relating with Industrial Ecology issues.

TU 262
Hybrid IOLCA model to determine carbon footprint of products and services to estimate their true cost - A case study of pulp and paper sector in Spain
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Nowadays, there is an increasing concern over the anthropogenic greenhouse gas (GHG) emissions more than ever. Different scientific researches reveal the fact that the way how products / services are produced and consumed is one of the major drivers of this accelerating pace of human related GHG emissions. Therefore, the need to change this unsustainable consumption and production patterns becomes increasingly urgent. One of the possible solutions to alter the current consumption and production patterns to sustainability is to account for the true costs of products / services, which reflect the actual social and environmental costs and benefits. Environmental tax which is proportional to products'/ services' carbon footprint reveals their true costs by internalizing all the external loads that are not included in the economy. This is then reflected in the actual prices and increases the prices of unsustainably made products. The traditional life cycle analysis (LCA) is the most product/service-specific; probably it is the best approach to calculate carbon footprint of products and services. However, the current methods of LCA suffer from problems of subjective system boundary selection, availability of data and time requirements.
An alternative approach to LCA is Environmental Input-output (EIO). It has some advantages over LCA as it takes into consideration all the upstream processes, but it still lacks strength to assess carbon footprint of products and services at a micro system level. Therefore, the best proxy would be given by a hybrid IOLCA which combines the best features of both LCA and EIO approaches. In this study we develop a Hybrid IOLCA model to estimate life cycle GHG intensity of products and services to determine their true costs. Paper production sector of the Spanish economy is analyzed by using hybrid IOLCA, which uses calculated LCA emissions of different paper products where LCA data is available and IO-calculated data for the rest. Results from the Hybrid IOLCA model are then compared with those obtained by pure LCA and EIO. Finally the carbon footprint of products and services are translated into environmental tax and the overall effects of tax introduction on the price of the products/services are then assessed.

TU 263
Life cycle analysis of advanced biofuels: accounting for coproducts and process residues impacts
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Lignocellulosic materials and microalgae are promising sources for alternative fuels production in the transport sector. Expectations are that 2nd and 3rd generation biofuels have a more sustainable performance than the biofuels currently available, but significant uncertainty remains about how well they will perform.
Life Cycle Analysis (LCA) appears to be the appropriate tool to evaluate the environmental impacts of these developing technologies. Results of such environmental assessments may be applied to immature technologies in order to support sustainable process design and offer reliable results for policy making. However, the generic LCA methodology needs to be adapted to deal with specific issues related to advanced biofuels.
This work presents two case studies where these issues are treated:
- LCA of cellulosic ethanol from agricultural residues

The European Directive on the use of energy from renewable sources states that 'agricultural crop residues, including straw, bagasse, husks, cobs and nut shells (...) shall be considered to have zero life-cycle greenhouse gas emissions up to the process of collection of those materials'. In this work, different possibilities to account for agricultural residues impacts are explored.
- LCA of microalgae biodiesel

Microalgae LCA is complicated by the fact that the carbon dioxide (CO2) necessary for microalgae growth needs to be fed into the system (it is not captured directly from the atmosphere). When the CO2 source is flue gas from a power plant, most of LCA practitioners consider it a process residue and no emissions are affected to it. As in the cellulosic ethanol case study, other possibilities are detailed.
The various possibilities to account for residues impacts explored in this work are: mass, energy and economic allocation; system expansion (substitution method recommended by ISO LCA Standards in the case of coproducts impacts accounting); consequential approach (comparison of life-cycle flows between conventional and alternative processes).

TU 264
Integrated design of a sustainable nursery school: energy and environmental evaluations with LCA
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The aim of the work was the design of a wooden nursery school in Italy, following sustainability guidelines. The choice of building materials, following a Life Cycle Assessment perspective, allowed the achievement of optimal energy efficiency and a consequent reduction of environmental impacts.
LCA analysis has been applied to a portion of the building and the energy demand for operation calculated considering an operation phase of 20-25 years, during which it is reasonable to discharge any extraordinary repairs; for the sake of brevity, in the operation phase scenario no ordinary maintenance has been considered as well. The energy efficiency and the environmental impacts have been studied focusing only on the construction and operation phases, while dismantling and demolition have been left out of this study. Energy use for building operation has been calculated with the CENED+ software, i.e. building primary energy requirements considering heating, domestic hot water and lighting. LCI analysis has been carried out exploiting ITACA database and alternatively ECOINVENT database. When using the latter database calculations were made using SimaPro software. However, the two approaches are correlated because ITACA database, which is an Italian database of building materials, heavily relies on ECOINVENT database. Fourteen impact categories are accounted for the materials listed in ITACA; instead, CML2 method has been used to perform LCIA with Simapro. Outcomes of the LCIA performed according to these two approaches enable a meaningful comparison only on seven impact categories; however, a critical analysis of impacts is required because conflicting results are found. For instance, both tools evaluate the positive contribution given by wood to the greenhouse effect, due to the CO2 storage in it. However, discordant results come from the two approaches: with ITACA, wooden materials give a negligible positive contribution to the Climate Change, if compared to the total effect arising from the whole construction phase. On the contrary, using directly ECOINVENT with the support of Simapro software, the beneficial effect of wood products is much more evident. Being ITACA ultimately based on ECOINVENT, this discrepancy is difficult to explain if one does not consider the different geographical contexts; indeed, a consistent amount of ITACA data values, among which transports and country energy mix, are modified to take into account the Italian context.

TU 265
Importance of linkage between LCA methodology developments and their applications in practice
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Efforts are continually performed to improve the Life Cycle Assessment (LCA) methodology, with a strong focus on both the life cycle inventory (LCI) and the life cycle impact assessment (LCIA) phases. However, those methodological developments may not always be applied in practice, e.g. due to delays or improper implementation into LCA-software. In such situations, the consistency of the results obtained by the LCA practitioner may be threatened. The present study uses an update in the normalization references for the EDIP methodology to quantify, through two examples, the influence that such problems may pose in LCA practice. In the first instance, discrepancies observed when employing an old - but still in use - set of normalization references (emission year: 1994) and an updated one (emission year: 2004) are estimated for all commonly assessed impact categories. The second example analyses the modelling of pesticides in current LCI databases, which traditionally treat applied pesticides as emissions to agricultural soil whereas, the agricultural soil belonging to the technosphere, only the fraction of pesticides reaching the biosphere is to be considered in the impact assessment. Consequences from this inconsistent modelling approach on toxicity-related normalized impact scores are quantified using a European emission inventory. In both examples, it is demonstrated that an inadequate application of LCA may result in large shifts of focus in the environmental profile of the background load as expressed in the normalisation references, some specific impact categories being significantly overestimated while others underestimated. To ensure the validity of the results, upon which recommendations for decision-support processes are built, the use of frequent updates and checks on the proper application of the LCA methodology is therefore advocated.

TU 266
LCA applications in Turkey
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Life cycle assessment (LCA) quantifies the environmental impacts and energy requirements of products over their full life cycle. It is also called a cradle to grave approach, which compares the similar products with respect to the environmental burdens. Although LCA is not something new, the special interest has increased since 1990`s. LCA is increasingly being used as a decision support tool by governments, companies, and authorities in order to improve the environmental performance, pollution prevention strategies and environmental management systems. LCA studies include four phases (goal and scope definition; life cycle inventory analysis; life cycle impact assessment and interpretation) according the ISO 14040 series. LCA methods are improved in developed countries for new impact categories and characterization models using the international standards framework contained in the ISO standards. Developed countries widely recognize that environmental consequences of a product should be evaluated by considering the impact results for each of its life cycle. LCA is applied a wide range from production to service such as energy generation, waste management, bottle production etc. over the world. On the other hand developing countries like Turkey are in the inception phase with a rising awareness on LCA. Life cycle impact assessment phase cannot be conducted easily due to the lack of valuation and weighting data, but the number of the studies is increasing day by day. This study outlines the current situation of the LCA studies for different sectors (automotive industry, gasification technologies, mining, building technologies, packaging, moulding, iron and steel industry, biofuels and agricultural sector) in Turkey. The difficulties for the life cycle inventory analysis and life cycle impact assessment phases in the studies are outlined and the future of the studies and the developments are also presented for Turkey. The aim of this study is to be an example for the developing countries, which increase the use of the LCA in their countries.

TU 267
The EPD® approach as meeting point between robustness and communicability of LCA studies: the certification of an internal LCA process
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As also suggested by the SETAC Meeting organisation, many LCA practitioners have proposed several approaches regarding "hot topics"such as allocation, cut off rules, biomass, etc... Often, researchers performing LCA aim to improve the methodology, while, on the other hand, applicants in the entity of industrial actors typically aim for strategy building or communications impact.
This paper wants to describe the manner in which a large company currently integrates the life cycle approach into its policies and views while conserving both the robustness of the elaborations and the suitability for communication purposes.
BARILLA is currently one of the top Italian food groups. It produces more than 100 products in about 50 plants around the world. The company has been using the LCA since the beginning of 2000. Since 2008, life cycle thinking made its way into Top Management of the company, as an instrument to thoroughly study the production chain and localise the most substantial environmental impacts
In virtue of these issues, Barilla has placed LCA approach robustness and importance at the top of its priorities, also developing a specific strategy: in 2010 an internal LCA process was imple-

mented and certified in compliance with the International EPD® regulations. In this view, Barilla has decided to implement its EPD internal process, which is based on three principle elements:
- the LCA databases, in which all the most important raw materials, production plants, packaging materials and other useful information are studied;
- the Product system that represents the product group model calculation tool;
- the Product specific data, related to the production of a specific product.

The overall system is verified twice a year by an accredited Certification Body.
It is foremost that all adopted rules and hypotheses be established it utmost clarity in order to continuously increase the suitability and robustness of the LCA implemented by companies. Since databases are a key element, their availability and updating shall undergo constant improvement through a specific updating procedure. Furthermore, Barilla seeks to publish as much information as possible on the environmental impacts of the food production chain by employing tools such as the Double Pyramid paper, which studies the relationship between the nutritional and environmental features of food.

TU 268
How potential carbon policies could effect producer grain cultivar selection: a LCA analysis of USA rice
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Given the Obama administration's push to reduce carbon emissions, increasing consumer demand for environmentally friendly products, and industry pressure from companies like Wal-Mart to reduce net GHG emissions, the likelihood of implementing of some form of a carbon policy in the USA is increasing. Little research on how a potential government policy or increased consumer demand for lower GHG emissions in agricultural products could affect the varietal selection of crops. This study attempts to fill this apparent gap in the literature by addressing how a climate change policy/views that caused producers to internalize costs of GHG emissions would affect the cultivars producers choose.
Given the introduction of hybrid rice in the USA there could be large environmental benefits from its widespread adoption. Hybrid rice can yield 15-20% more than conventional cultivars with roughly the same input requirements. This study estimates a net carbon footprint for 14 of the most commonly sown rice cultivars in Arkansas in 6 locations throughout the state. The results should provide producers, millers, and buyers an idea of the relative difference in GHG emissions by cultivar so they can adapt to a potential carbon tax/offset policy or to changes in consumer demand. This research has global implications since roughly 50% of the world's population consumes rice daily.
Preliminary Results
To estimate GHG emission per acre, a range of input requirements were gathered for types of rice as well as cultivars for six counties in Arkansas. Monte Carlo simulation was used to model uncertainty in production. Using a LCA, carbon was estimated for both direct and indirect emissions. Carbon emissions were estimated per acre and per bu of rice. From a cap and trade standpoint, the ratio of CE/bu appears to be the driving factor in which cultivars will experience a loss/addition of acreage. From a carbon offset standpoint, the estimates generated in this study do not indicate, even under high carbon prices, that an offset market will change varietal selection by producers. Given that rice is the largest emitting row crop produced in the United States, it is likely that acreage would decrease given a cap and trade policy that includes agriculture. It would appear that consumer and industry demand for "greener"products and/or a cap and trade type policy that included agriculture, but not a carbon offset policy, could ultimately effect which rice varieties producers sow in the USA.

TU 269
Accuracy vs. robustness: challenges of including LCA into legislations/standardization schemes for sustainable biofuels
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The Swiss regulation on tax exemption for biofuels as well as the European renewable energy directive (RED) or the standardization scheme of the Roundtable on Sustainable Biofuels are based on LCA calcula-tions for the evaluation of the biofuels.
Detailed guidelines for the calculations are necessary to allow comparability of the results, to facilitate the applicability and to reduce the burden for the economic operators. To be complete, the guidelines should de-scribe the level of data collection, the rules for allocation to co-products and give guidelines for the calcula-tion of emissions in the agriculture. The legislations use different approaches regarding to the guidelines: whereas the Swiss legislation provides a questionnaire where the information has to be filled in by the opera-tor, the RED only provides general guidelines on the calculations, so that there are great efforts undergoing in the European Union to define them more precisely.
By defining very detailed guidelines and using standard factors to provide robust and comparable results, the legislations might fail to provide the right incentives, as environmentally sound practices, e.g. in agriculture, might not show in the calculations. On the other side, it has been shown that different assumptions in LCA studies might lead to very different results, so that a harmonization of the calculations is needed to obtain comparable results. A compromise between accurate results reflecting well the actual production pathway and robust results which can be compared with other pathways is therefore necessary.
A further difficulty in the assessment of biofuels is the fact that certain factors which can have a great influ-ence on the results like N2O-emissions or indirect effects are still burdened with a high uncertainty in calcula-tion or in the methodology. The main challenges for the implementation of LCA are i) to refine global models or define global valid approaches for emission modelling in agriculture, ii) to find the right level of detail with-out increasing excessively the data-collection burden for the economic operator and iii) to develop accepted approaches for dealing with indirect effects.

LC05 - Life Cycle Management approaches for different industrial sectors (LCM)

TU 274
Life cycle assessment applied to events: the case study of Gymnaestrada
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Life cycle assessment was originally designed to evaluate environmental impacts of products. This methodology was extended to be applied to organizations. And lately, it has been adapted to be applied even to events.
The World Gymnaestrada, organized in Lausanne, Switzerland, in 2011, is the largest mass sport even in the world with more than 23'000 participants to be expected. However, this event will also generate increase of the city population of 20 %.
The organizing committee is interested to understand the environmental consequences of this event and would like to enroll in a sustainable development approach.
The evaluation of the overall environmental assessment of the event is part of the wide range of measures taken in order to implement these principles of sustainable development.
The goals of this project are to:
- Realize the environmental assessment of the World Gymnaestrada 2011
- Contribute to the establishment of a sustainable development mind for this event
- Value the impacts reduction measures towards the collaborators
- Create an evaluation tool that allows directing choices for the event
- Associate these elements to the communication of the event
- Contribute to create a life cycle assessment based tool for events

The results of the assessment, the tool and actions identified will be presented.

TU 275
Allocation procedures effects on greenhouse gases emissions in energy systems industrial sector: a case study of hydrotreated vegetable oil
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The allocation procedure in a multi-input/output process is one of the most significant matters in Life Cycle Assessment methodology. This modus operandi allows the partition of the environmental burdens associated with a multi-output process into its product and co-products.
Oil hydrotreating units in refineries are aimed at reducing the sulphur content of mineral fuels in order to achieve compliance with standard specifications. Currently, this process is one of the best available technologies to obtain biofuels from vegetable oil. Several co-products are obtained in this hydro-reaction such as liquid petrol gases, methane, propane, butane or naphtha, so an allocation procedure must be carried out for sharing the environmental charges.
This study presents the greenhouse gases (GHG) emissions of a hydrotreated vegetable oil (HVO) from co-processing with mineral diesel in a hydrotreating facility of a Spanish oil refinery. Two different allocation procedures have been considered in order to compare the results in the hydrotreatment unit output: energy allocation and system expansion (or substitution).
Results show that GHG emissions are lower in the case of the substitution (around 3-4 g CO2 eq/MJ in its entire life cycle) if a hypothetical 100% HVO is considered. However, European Directive 2009/28/EC recommends an energy-based content allocation in the case of biofuel GHG emission balances.
Finally, it is worth remarking that both results highlight that this renewable diesel fuel could attain an excellent environmental performance in terms of GHG emissions savings (roughly 50-60%) in comparison with the above mentioned directive standards.

TU 276
Life Cycle Assessment of daylight devices: the case of light pipes
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The benefits of the use of daylight devices for health are well known. Moreover, a better environmental performance than conventional lighting is expected; however, the increasing spread of these devices may lead to concerns about the actual environmental impact produced by them. In this context, this research deals with the environmental impact of one type of solar systems: light pipes. These systems are based on the principle of multiple reflections on specular surfaces: the system collects and transports daylight through a device, which is composed of three fundamental units: capture unit, conduct unit and diffusion unit. It is known that when they are used to produce light for a given area, there is no direct consumption of energy. However, this does not mean that they do not cause any impacts to the environment throughout their entire life cycle. As a methodological tool, LCA can evaluate, from an environmental viewpoint, the life cycle of a light pipe, so that more information about the potential impacts generated in the life cycle phases of a light pipe (including production, transport and end-of-life) can be obtained.
This study was conducted with reference to a hypothetic light pipe of 80-cm length and 25-cm diameter. The data on materials and dimension of the components were extracted from specific literature. The environmental analysis reveals that the highest impact is potentially caused by the production phase of the light pipe; more precisely, one critical component is the aluminum tube. This means that if a light pipe manufacturer wants to improve the environmental performance of his/her product, it could be interesting to develop researches aiming at identifying a more environmentally-friendly tube material. This study has some remarkable limitations: the data collection was mainly based on scientific literature and commercial databases; moreover, some of the raw materials were replaced by similar ones because of the lack of data. Therefore this must be considered as a preliminary study. Further research with actual data and more accurate data collection is advised.

TU 277
Life Cycle Management approach for the Sassuolo ceramic district
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The purpose of this study is to assess the environmental impacts of the different products and to evaluate possible actions to be taken to reduce them, including-market related considerations to verify their economic benefits.
The functional units chosen for this study is 1 m2 for each type of ceramic tiles representing the entire italian production. The LCA study has been performed using the SimaPro 7.1 software and four impact assessment methods: Eco-Indicator'99, IMPACT 2002+ and EPS 2000. The system boundaries for the analysis include the upstream phases, from raw material extraction, and arrive to the finished product packaging, thus obtaining "a cradle to the gate"overview. This choice has been forced by the lack of aggregated data regarding the use and disposal of ceramic tiles. LCI has been performed by using aggregated data regarding raw materials and energy consumption and emissions in air, water and ground. These data have been derived from a report regarding the ceramic district in the area of Sassuolo. Machineries and plants have not been considered in the present study.
The production step responsible for the main part of the damage is the firing phase, which is difficult to change because of technological reasons. The emissions related to the raw materials transport have a great influence on the overall environmental damage, though this is the aspect on which new suggestions have been advanced.
The proposal consists in replacing a part of raw materials using local minerals available. Before

assessing the new environmental impact, some experiments have been performed to verify how much of the original raw materials could be replaced without compromising the technological features of the product. Then a comparison has been made, resulting in a lower damage of about 15%.
To verify the economic benefit of such a change in raw materials composition, the Kraljic matrix has been applied. As a result of using local raw materials, a left shift has been obtained, from the 3rd (bottle-neck materials) to the 4th quarter (non critical materials).
This study shows how the environmental improvement strategies suggested by LCA have also economic benefits, and how LCA can be used within a LCM approach to support decision makers toward a more sustainable management of businesses.

TU 278
Incorporating ecodesign into a SME: future ISO 14006
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The forthcoming ISO 14006, Guidelines for incorporating ecodesign into environmental management systems, is intended to provide how to guide companies to incorporate ecodesign into a management system. This standard will be partially based on the Spanish standard UNE 150301, developed in 2003. The main goals of this standard are among others: minimise the environmental impact of products/services, promote the life cycle approach, systematise the continuous improvement in the framework of ecodesign, help the market to become aware of the need for products/services incorporating the environmental requirement, and provide a label to those companies involved in ecodesign.
This article analyses the implementation of the UNE 150301 in MACER S.L., a Spanish small and medium enterprise (SME) in the metal mechanic sector. This company is specialised in design, manufacture and repairing/maintenance of moulds for presses in ceramic tile companies. The implementation of this standard has involved the following activities: the analysis of the manufacturing and repairing process of moulds; annual data collection of production, raw materials, energy consumption, waste generation, etc.; allocation of data to unit processes such as drilling, milling, turning, grinding, electrical-discharge machining (EDM), wire EDM, welding, rubberised, etc., by means of the electricity consumption measurement directly on machines; modelling the life cycle inventory for each raw material, auxiliary material and unit process; modelling the life cycle of a mould including all its components; obtaining environmental indicators; identification of significant aspects from the environmental point of view; setting environmental improvement targets and actions for achieving them; and preparation of the required documentation for certification according UNE 150301.

TU 279
Review of simplified LCA approaches and tools: defining a streamlined tool for food LCAs addressed to SMEs
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Life Cycle Assessment (LCA) is a tool which has been increasingly used in order to identify the environmental profile of goods and services. However, for a number of reasons, including its high resource-intensity, LCA has not adequately spread as a practical tool, especially over Small- and Medium-sized Enterprises (SMEs). Therefore, the need to identify simplified, even though reliable, methodologies within the LCA framework has increased. In the framework of a recently-started Italian nationally-funded inter-university research project on improving sustainability and competitiveness of the agri-food chain with innovative environmental management tools, specific objectives and tasks have been established concerning Streamlined LCA in food supply-chains. This paper aims at describing those objectives, as well as presenting some preliminary results.
One overall objective of this project is to identify and/or develop an appropriate simplified LCA tool or approach to be addressed to SMEs in the food sector. In order for this to be accomplished, the first step has been to perform a comprehensive scientific literature review of approaches and tools proposed so far in the framework of LCA for different products, seen from a broad perspective. Later on, after having obtained a thorough knowledge on the various approaches and tools available; this will be the starting point to develop a tool which can be best suited in the agri-food context.
This paper presents and discusses some preliminary results of the undergoing research.

TU 280
Symbiotic exchanges and hazardous wastes in chemical industry. A 'path-dependent' case study
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The Eco-Industrial approach promotes the transition from traditional industrial clusters to Eco-Industrial Parks (EIP) [1-2], exploiting geographical proximity, existing synergies and peculiar socio-economic relation with the territory. This paper presents an hypothesis of symbiotic exchanges to redevelop a chemical industrial site located in Abruzzo Region: Bussi chemical site (BCS). It is one of the oldest Italian cluster and it involves five production units for a total of 220 employees. The sectors of activity are: basic chemicals, pesticides, micronized silica, production and distribution of electricity. This cluster reveals great opportunities in the re-use of by-products among two production chains: the reuse of the unreacted sand in the silicate sector and the sulfuric acid recovery for the detergency sector. The possible scenarios that could be implemented are: i) the exploitation of symbiotic internal exchanges; ii) the location of new industrial plant in the chemical site - this activity concerns the recovery of precious metals (as cobalt, vanadium, nickel, molybdenum) from used catalysts deriving from chemical and petrochemical industry; iii) the involvement of another regional cluster (Automotive industry) - in this case the exchanges could include: extraction of metals from electroplating sludges and used catalysts; exchange of product and by-products deriving from BCS, used as raw materials in the Automotive industry.
Preliminary results demonstrate great opportunities to improve economic and environmental performances of the cluster. An important reflection concerns the social acceptance that could represent a crucial limit in the transition process. Local community demonstrated a strong opposition to any proposal of redevelopment of the site related to the treatment of waste or by-products and to the establishment of new companies involved in these activities. This is due to the fact that, in the past, Bussi chemical site was protagonist of an environmental scandal for the presence of an illegal dump of toxic materials. The re-industrialization of the site following the EIP model met a distrust of local population used to conceive the production cycle as a linear cycle. Revolutionize this system, introducing a closed loop model, highlights the difficulty in conceiving wastes as a resource, not any more as cost to the company [3].

TU 281
Environmental impact of striped catfish production in the Mekong Delta (Vietnam)
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Intensive striped catfish production in the Mekong Delta has in the recent years raised many environmental concerns. To support policy making, we conducted a Life Cycle Assessment (LCA) to determine the environmental impact of producing striped catfish. The LCA was complemented with an assessment of biodiversity loss, water use, and flooding hazards. The goal and scope for the assessment, and the assessment results were discussed in a series of stakeholder workshops. The results Master Plan 2020.
The LCA covered all processes up to the exit-gate of the fish farm, and focused on global warming, acidification, eutrophication, human toxicity, and marine (MAET) and freshwater aquatic ecotoxicity (FWET). Except for eutrophication and FWET, the feed production, which largely took place outside Vietnam, dominated most of the impacts in the LCA. Among feed ingredients, rice bran dominated global warming and acidification, while wheat bran dominated eutrophication, mainly due to their quantities used. Fishmeal production, transport and energy processes dominated MAET. Grow-out farming in Vietnam dominated eutrophication and FWET. The water nutrient discharge was high in relative terms, but it hardly modified river water quality compared with that before sector expansion (0.01% increase while 0.0005% of river water passed through the ponds). Environmental impacts can be reduced by managing sludge, producing feed ingredients locally, processing feeds with lower Feed Conversion Ration and lower content of fishery products in the feed.

TU 282
Environmental impact assessment of rapeseed and barley cultivation for fallow land in winter season using LCA
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High input to the arable land contributed to increasing productivity, at the same time caused global environmental problems. In this context, rape cultivation has been asked to reassess its positive point such as utilization of winter fallow field. In this study, environmental impacts of rape cultivation in winter fallow after harvesting paddy rice were assessed and compared with barley cultivation as an competitor using LCA.
we collected raw data for input materials such as fertilizer and pesticide, and energy consumption rate by analyzing the type of agricultural machinery and working hours by 1 ton rape and barley as functional unit. Environmental impacts were evaluated by using Eco-indicator 95 method for 9 impact categories. Sensitivity analysis was conducted for crop responses to fertilization level and increasing productivity.
To produce 1 tonne of barley and rape, the potential of greenhouse effect in barley (1.85E+02 kg CO2-eq.) was higher than that in rape (2.16E+02 kg CO2-eq.). The environmental impacts of ozone depletion, acidification, heavy metals, carcinogens, summer smog and energy resources showed the higher potential in rape than barley. In case of eutrophication, impact of barley was higher than that of rape. For the sensitivity analysis, scenario 1 (crop responses to fertilization level) showed the environmental burden was not increased with the amount of fertilization only at the optimum crop responses to fertilization (N3). By increasing the productivity of rapeseed by 1 ton per ha, a quarter of greenhouse gas emission potential was reduced.
With these results, environmental load from rape cultivation in winter fallow land was higher than that from barley. However it is assessed that planting high productive cultivar and site-specific fertilization by recommendation might reduce the environmental burden by 30 %.

TU 283
Finnish methodology for carbon and other footprints for food products
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The "Foodprint", Footprint of food -research programme started in late 2009 following the initiative of active Finnish food companies. The programme is planned to be completed in May 2012, and is funded by the Finnish Funding Agency for Technology and Innovation (Tekes) and participating companies.
The public Foodprint Tools project comprises of four phases. The first phase aims at describing a generic methodology and requirements for carbon (and other) footprint(s) of food products. Other work phases will be more detailed, concerning data collection, data quality requirements, actual tools to assess environmental burdens in agriculture etc. Some specific characteristics are described below.
a. Concerning attributional vs. consequential modelling approaches, the attributional approach is chosen to allow comparability of footprints and to create as concrete modelling backbone for companies as possible.
b. The impact categories considered in this initiative are climate change, eutrophication, acidification and primary energy.
Data quality is one of the main topics under investigation, and plain division of data to primary and secondary data is inadequate. In many situations it is impractical to collect primary data, and well-founded approximations are needed. In the methodology topics of adequate data sources, data to be collected directly from the supply chain and data to be collected from national statistics, databases etc. shall be handled in detail. The data which needs to be collected is planned to relate to data which primary producers already collect in Finland for other purposes.
Allocation is perceived as one of the main challenges in LCA studies, and therefore a special focus is given to it. Work is underway to develop general principles for choosing appropriate allocation methods and for avoiding allocation (e.g. through system expansion). Some examples of allocation situations are going to be further explored through the case studies.
Other issues to be further investigated are: different land-use impacts (carbon storage, sequestration, soil carbon change, land conversion) and the need to verify activity and emissions data. Also, one area of improvement and generation of defaults are emission factors for electricity production. It is proposed to use as specific emissions factors related to the actual electricity supplier. This means that when the production profile is known, defaults for different production types shall be used.

NM01 - Detecting, quantifying and characterizing engineered nanomaterials in the environment and in biological systems

TU 286

Nanoparticles in food: analytical methods for detection and characterisation

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Nanoparticles in Food
Analytical methods for detection and characterisation

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"The Scientific Committee [of EFSA] makes a series of recommendations; in particular, actions should be taken to develop methods to detect and measure ENMs [engineered nanomaterials] in food/feed and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.". This citation illustrates the current situation with view to the analysis of engineered nanoparticles (ENP) in food. Nanotechnology applications for the food sector are intensively investigated and developed and nanomaterials are already in use as food additives or in food contact materials while at the same time very limited knowledge is available on the potential impact of ENP on consumers' health. Exposure of the consumer to ENP cannot be determined due to the lack of appropriate analytical methods.
This gap is addressed by the FP7 project NanoLyse. The NanoLyse project focuses on the development of validated methods and reference materials for the analysis of ENPs in food and beverages. The developed methods will cover relevant classes of ENPs, i.e. metal, metal oxide/silicate and encapsulate ENP. Rapid imaging and screening methods allow the distinction between samples which contain ENP and those that do not. These methods will be characterised by minimal sample preparation, cost-efficiency and high throughput. More sophisticated, hyphenated methods allow the unambiguous characterisation and quantification of ENP. These include elaborate sample preparation, separation by field flow fractionation and chromatographic techniques as well as mass spectrometric and electron microscopic characterisation techniques. The developed methods will be validated using the well characterised food matrix reference materials produced within the project. Interlaboratory method performance studies will demonstrate the applicability and soundness of the developed methods. The techniques and methods developed in this project for food could also be applicable to biological and environmental matrices (possibly after matrix tailored modifications).

TU 287

Secondary characterization of TiO2 nanoparticles in biological media by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM)

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Advantages and limits of Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) techniques for secondary size characterization of nanoparticles in biological media were addressed and discussed. Both techniques were employed to investigate size distribution of nano-TiO2 (Degussa-Evonik P25) in biological media commonly employed for in-vitro toxicological studies. Titanium dioxide nanoparticles were dispersed at physiological pH up to 5 mg/ml, stabilized with foetal bovine serum, a biologically compatible dispersant, according to specifically developed protocols, and their size distributions and stability vs. agglomeration were investigated in a wide set of biological media (DMEM, DMEM-HG, RPMI-1640 and DMEM-F12-HAM, with and without FBS). The influence of sonication step on the dispersion procedure has been also discussed. The obtained stock dispersions, when prepared according to the developed protocol, resulted to be stable at least 22 h despite the relatively high particle concentration level. Moreover, when diluted in all biological media tested, the obtained stock dispersions exhibited a very stable (at least 48 h) and similar size distribution, confirming the applicability of the proposed protocol for in vitro nanotoxicology testings.

TU 288

Ecotoxicity of fluorescent silica nanoparticles in a battery of freshwater test species

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The anticipated increase in nanoparticle production makes exposure of the environment to these materials more and more likely. Assessing the benefits and risks of nanomaterials requires a better understanding of their chemistry, mobility, bioavailability, and ecotoxicity in the environment. Nanoparticles currently in use, or nearly so, in industry are studied as a priority. Amorphous silica nanoparticles are already used commercially in foods, have significant industrial relevance to Ireland via their use in the IT sector, and are thus likely to have an early appearance in the environment, making them a strategic starting point. By labelling these particles, representative biological and environmental fate as a function of size will be studied by tracking and imaging methods.
In this project, the ecotoxiciticy of well-characterized 50 nm and 100 nm plain and fluorescently labeled amorphous silica nanoparticles on a test battery of aquatic organisms representing four trophic levels are investigated. The tests used are validated and standardized short-term methods for estimating the acute and chronic toxicity of toxicants to bacteria, algae, invertebrates and fish. Preliminary results show no acute toxicity of concentrations up to 1000 ppm of the different types and sizes of silica nanoparticles to the different species and further testing is on-going to predict the chronic effects of silica nanoparticles on Daphnia magna reproduction.

TU 289

Stabilisation of nanometric titanium dioxide in artificial seawater for in vivo ecotoxicity bioassays

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Bioassays investigating nanometric titanium dioxide (n-TiO_2) ecotoxicity on marine organisms are often affected by a low reproducibility, which is mainly caused by the tendency of nanoparticles to agglomerate and rapidly sediment in artificial seawater (ASW) [1]. As a consequence, the organisms are exposed to time-dependent nanoparticle concentrations as well as to agglomerates with a variable size distribution [2]. In this work, the ability of alginate to stabilise n-TiO_2 dispersions in ASW at various nanoparticle concentration levels (1, 10, 100 mg/L) was investigated. Alginate is a polysaccharide naturally produced during the degradation of brown algae and, being not toxic, is widely used in the food industry as emulsifier and gelling agent [3]. Investigation by Dynamic Light Scattering (DLS) measurements showed that dispersions prepared in 0.5 g/L alginate-ASW solutions presented agglomerates with a constant (up to 72 h) and reproducible average hydrodynamic diameter of approximately 270 nm. The stability of dispersions at 100 mg/L was further examined with a stability analyser under centrifugal forces, giving a sedimentation rate of 0.14%/h. The sonication step was also investigated in order to obtain the smallest agglomerates. The employed ASW was characterised (salinity, conductivity, and total organic carbon content) prior to evaluating alginate harmful effects on the marine microalgae *Phaeodactylum tricornutum* and the anostracan crustacean *Artemia franciscana*.

TU 290

Natural and engineered metal oxy-hydroxides behavior in a wetland system receiving mining influenced waters and waste water from a treatment plant

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Concern over environmental contamination by engineered nanoparticles (ENPs) has arisen due to the increase in the nanotechnology industry. Limitations on risk assessment are directly related to a lack of direct information on ENP stability. The absence of this information can be circumvented by studying natural NPs (NNPs) that can act as surrogates for examining the environmental behavior of ENPs. Mining influenced waters (MIWs) are excellent systems for study due to high concentrations of natural nanoparticulate hydrous iron oxides (HFO) precipitates. Constructed wetlands are engineered wastewater treatment systems, routinely used in remediation of these waters, being designed to encompass natural biological, chemical and physical processes (akin to the ones occurring in natural wetlands), thus enhancing water quality. One of these systems was installed in a sub-alpine stream (located in Jefferson County, Colorado, United States of America), receiving MIW from a number of mining sites together with effluent from a Wastewater Treatment Plant (WWTP). The effectiveness of the wetlands for removing NP-associated metals was investigated through natural and lab prepared iron oxides. Effect of the water composition on surface charge of NPs in sections of the system receiving MIW-only (the creek) and MIW mixed with wastewater effluent (constructed wetland) were investigated for the NNPs and compared to ENPs (NiO, Au, and CNTs). We observed temporal and spatial variations in the several MIWs, finding surface charges ranging from slightly positive, a rarely seen result, to slightly more negative. The considerable aggregation of HFO is consistent with these findings. Results suggest a variation of ENP stability over space and time.

TU 291

Effects of organic carbon content and soil ageing on the fractionation of silver nanoparticles in soil

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Due to sewage sludge application on soils, terrestrial ecosystems are very likely to be exposed to silver nanoparticles (AgNPs) and it is thus important to understand the behavior of Ag NPs once in contact with soil components. The aim of this work was to compare the behavior of silver under three forms, silver nitrate, citrate stabilized AgNPs (C-ANPs) and uncoated AgNPs (P-AgNPs), in two soils with contrasting organic matter content, and over time.
The physical and chemical properties of the studied soils as well as the nanoparticles size, shape, crystallographic structure and specific surface area were characterized. Soil samples were spiked with silver nitrate, C-AgNPs or P-AgNPs, and let for ageing 2 hours, 2 days, 5 weeks or 10 weeks before they were submitted to sequential extraction. The ionic silver solution and the two AgNPs types were radiolabeled so that we could detect and quantify silver by gamma spectrometry by measuring the 110mAg tracer in the different sequential extraction fractions. We thereby obtained for each silver form, soil type and time point a distribution of silver in the different fractions.
Silver was generally more mobile in the mineral soil, although the fractionation patterns were very different for the three silver types in both cases. Over 20% of the total C-AgNPs concentration were water soluble in both soils (<5% for AgNO3 and P-AgNPs) the first two days after spiking, but the fraction decreased to trace levels thereafter. This was compensated by an increase in the reducible fraction. Regarding P-AgNPs, 80% were not extractable at all, but contrary to AgNO3 and C-AgNPs, the water soluble and ion exchangeable fractions did not decrease over time in the mineral soil, and even increased in the organic soil.

TU 292

Bioaccumulation of sediment-associated gold in different forms to the deposit feeder, Capitella teleta

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There is much concern about the fate and effects of engineered nano-particles (ENPs) given their rapidly increasing applications in industrial and household products. Gold (Au) ENPs are used in a wide variety of personal care products and in materials science. Au and other metal ENPs, released into the environment, intentionally or accidently, may end up in the aquatic environment via sewage treatment plants, waste handling or aerial deposition. There are concerns that metal ENPs may have higher reactivity than other forms of metals due to their small sizes and high surface areas, leading to greater bioavailability and thus toxicity in aquatic organisms. However, studies of metal ENP bioavailability in the aquatic environment are limited.
We exposed the deposit feeding polychaete, Capitella teleta, to sediment-associated metal Au in different forms (particles and ions) to assess their relative toxicities. Au particles were tested at 4nm, 15nm, 40nm and 100-200nm sizes. After 2 weeks of exposure, almost all worms were alive and no significant difference in their growth rates was observed among Au treatments. Bioaccumulated Au concentrations in worm tissue will be presented and compared to relevant literature data.

RA08 - PBT substances

TU 295

Exposure to organochlorine compounds at the early stages of use of DDTs for indoor residual spraying(IRS) in domestic environments (Manhiça-Mozambique)

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Abstract

DDTs and their metabolites were determined in 88 breast-milk samples obtained before and during indoor Residual spraying-IRS with DDT (2002 vs 2006) and 91 straw and branches constituting the wall materials of dwellings collected during and a year after IRS (2006 vs 2007) in Manhica, Mozambique.
A significant increase in 4,4'-DDT and its main metabolite, 4,4'-DDE, was observed between

the 2002 breast milk (median values 2.4 and 0.9 ng/ml, respectively) and the 2006 (7.3 and 2.6 ng/ml, respectively, p <0.001 and 0.019, respectively). However, the observed increases were not uniform and in some individuals high values (5100 ng/g lipid) were determined. Significant differences were found between the concentrations of DDT and related compounds in breast milk according to parity, with higher concentrations in primiparae than multiparae women.
Total DDT levels in straw from 2007 were significantly higher than from 2006.In the 2006 samples, the concentrations of total DDT ranged between 0.3 ng/g and 5600 ng/g. The predominant isomer was 4,4´-DDT (median = 47 ng/g). The straw sampled in 2007 were from sprayed dwellings except in two cases, the concentrations of total DDT ranged between 0.8 ng/g and 26000 ng/g. The predominant isomer was 4,4´-DDT (median=130 ng/g) followed by 4,4´-DDD (median=23 ng/g) and 4,4´-DDE (median= 20 ng/g).
Key words: DDT; IRS; Breast milk; Straw

TU 296
Monitoring of polychlorinated biphenyls (PCBs) in human milk samples - comparative study within the seven years period
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Polychlorinated biphenyls (PCBs), substances with great chemical stability, physical and chemical inertness, which fulfil PBT criteria (persistence, bioaccumulation potential and toxicity), are still present in our environment, even if their production was banned by Stockholm Convention. Human biomonitoring data represents the most reliable estimate of human exposure to POPs/PCBs resulting from recent and historical sources.
Aim of this study was to investigate concentrations of seven PCB congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180) in colostrums collected in 2009 from 22 healthy mothers living in and around the city of Novi Sad, Vojvodina. Results obtained in 2009 were compared with the results obtained in the same area in two previous sampling campaigns in 2003 and 2006 within seven years study.
Samples were taken from healthy mothers whose colostrums were stored into specially prepared glass containers. They were frozen at -20°C until analyzed. Deproteinisation of samples was performed with Carrez I and II solutions. Milk fats were extracted using acetone and n-hexane and cleaned by column chromatography using Al2O3. PCBs were eluted from the column using n-hexane and eluates were analyzed by GC-ECD (VARIAN CP-3380). PCB congeners (Ehrenstorfer, Germany) used as standards were min 99.9% purity. Quality control testing was further certified with reference material ERM-BB446 (ERM, Belgium). Recoveries of developed method were 80 - 102 %. Limit of detection (LOD) was 0.5 ng/g.
Congeners 28, 52 and 101 were below LOD in all 22 samples. Only one colostrum sample was not contaminated with any of seven examined PCB congeners. Mean values of PCB 118, 138, 153 and 180 were 1.0, 1.1, 1.3 and 0.8 ng/g whole milk, respectively. Measured concentration range was from 0.6 to 3.4 ng/g. Total PCBs value was calculated by assuming that the measured seven PCB congeners mean value (3 ng/g whole 3rd day milk) is 30% of total PCBs. Milk fat content was 1.6 - 5.3 %.
After a decreasing trend of assumed total PCBs between sampling campaigns in 2003 and 2006 (10.25 to 3.53 ng/g whole milk), increased value in 2009 (10 ng/g whole milk) indicates the continuous input of PCBs in human body through the food consumption and air breathing, from the environment probably contaminated by PCBs.

TU 297
Crossing regulatory borders - the German concept of a harmonised assessment of PBT substances
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Substances which are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) are subjected to very stringent regulation in the various EU substance regulations.
Any substance of anthropogenic origin that is likely to reach the environment and that resists degradation, accumulates in biota or within terrestrial or aquatic food webs and shows severe toxic effects, is expected to have a much higher impact on the environment than non-PBT substances. If a substance fulfills the PBT- or vPvB-criteria, many regulations aim to ban it from further use, to prevent exposure of the environment.
Across all regulations (industrial chemicals under REACH, Plant protection products under Regulation 1107/2009/EC, biocides on the basis of Directive 98/8/EC and human and veterinary pharmaceuticals by means of EMEA/CHMP/SWP/4447/00 (Medicinal Products for human use and CVMP/VICH/592/98 (veterinary pharmaceuticals), the actual trigger values for PBT assessment are identical, but data requirements as well as assessment "traditions"and regulatory consequences differ greatly. The latter vary from a complete ban or exposure minimisation to no consequences at all (e.g. human pharmaceuticals).
The German Federal Environment Agency - the UBA - aims at harmonising the PBT-assessment across the different substance regulations. The incentive is that the result of a PBT-assessment (i.e. the identification of a substance as PBT or non-PBT) should be consistent, if sufficient data is available, regardless of the individual regulation, as PBT-assessment is hazard-based - not risk-based - and various substances are registered under more than one regulation.
Even if at present the regulatory consequences of the identification of PBT-substances remain different across the various regulations, the procedure of PBT-assessment can - and should - be harmonised.

TU 298
PBT Assessment using the revised Annex XIII of REACH - comparison with other EU frameworks
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The European Regulation EC/1907/2006 (REACH) requires an assessment of PBT (Persistence, Bioaccumulative, Toxic) or vPvB (very Persistent, very Bioaccumulative) properties for all chemical substances produced or imported in amounts exceeding 10 tonnes per year, according to criteria in Annex XIII. However, not all substances on the EU market are screened against these criteria under REACH. For human and veterinary medicinal products, biocides, plant protection products, food and feed additives, etc., other EU legislation is in place regulating marketing and use. Besides this, compounds may also be screened for PBT properties within the OSPAR, Stockholm and IMO Ballast water Management conventions or the UNECE protocol on persistent organic pollutants (POPs), which all have their own suite of PBT or POP criteria. There is thus no uniform PBT/vPvB assessment in Europe, because various legislations use no, limited or dissimilar PBT assessments.
In this study, the PBT/vPvB assessment under REACH is compared to PBT or POP assessments within other frameworks. Attention is paid to how a substance is identified as a PBT/vPvB/POP substance, and what legislative steps, such as authorization or restriction, are taken following the PBT/vPvB/POP assignment. In addition to the different PBT or POP criteria of the various frameworks, there are also differences in the description of the criteria and if a weight of evidence approach is possible. Some EU frameworks still refer to the former Technical Guidance Documents (TGD), which preceded Annex XIII of REACH. Although differences between the TGD and REACH Annex XIII are small, this causes dissimilarities among the frameworks. The risk management follow-up of a PBT or vPvB identification, e.g. authorization or restriction, depends on the legal framework and specific conditions at which a substance is used, and whether a socio-economic analysis is included.
Irrespective of the framework in which a substance is used, individual European Member States may propose a substance for PBT or vPvB identification under REACH. However, only when a use of a substance is identified which is not covered by its regular framework, authorization of that use under REACH is possible. Differences among the EU-based legal frameworks create a challenge to harmonize the assessment of PBT/vPvB substances.

TU 299
Predicting environmental behaviour of chloroxanthene and chlorothioxanthene congeners on the basis of quantitative structure - property relationships (QSPR)
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Xanthene is a heterocyclic chemical compound (containing oxygen heteroatom), widely utilized in the industrial production of fluorescent dyes. It is also used as a drug, inhibitor or contrast for cancer cells detection. Thioxanthene, a sulphur analogue of xanthene, is a component of several antipsychotic drugs (e.g. Chlorprothixene, Clopenthixol). Considering their broad application, significant amount of both compounds may occur in the natural environment and undergo chlorination processes leading to the formation of Cl- substituted congeners.
Analysing structural similarities of xanthene and thioxantene to other polycyclic molecules, they are situated in the same space as dibenzo-p-dioxin and anthracene, which are persistent, bioaccumulative and toxic (PBT) environmental pollutants of prior concern. Hence, xanthene and thioxanthene are prone to exhibit equally hazardous properties. Unfortunately, the availability of experimental data on the key physical-chemical parameters of chloroxanthene/chlorothioxanthene congeners is lacking. Therefore, little is known about an actual overall persistence (POV) and long-range transport potential (LRTP) of these compounds.
The presented research investigation aims at filling the gaps in existing scientific data as well as at estimating the environmental fate of 679 chloroxanthene and chlorothioxanthene congeners. We utilized previously developed QSPR MLR models to predict the values of log KOW (n-octanol/water) and log KOA (n-octanol/air) partition coefficients for all studied compounds. For each molecule, the relevant quantum mechanical descriptors were calculated at the level of the semi-empirical PM6 method. The half-lives in air, water, and soil for particular chloroxanthene/chlorothioxanthene congeners were predicted via the freely available US EPA tool 'The PBT Profiler'. Subsequently, we introduced these results into multimedia mass balance model 'The OECD POV and LRTP Screening Tool' for final POV and LRTP estimation.
The results were visualised as suggested by Klasmeier et al. (2006). Additionally, the differences between environmental fate of oxygen (xanthene) and sulphur (thioxanthene) analogues were investigated. Our study revealed that some of the investigated congeners show POP-like POV/LRTP or POV characteristics. As such, further studies that would verify the necessity to include studied molecules in the international lists of high-priority environmental pollutants are recommended.

TU 300
Persistency assessment of poorly water-soluble substances
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Under REACH a PBT/vPvB assessment is required for substances that are manufactured or imported in amounts of > 10 tons per year. The first step of persistency assessments is a screening like the tests on ready and inherent biodegradation provide. Often substances fail the criterion for ready biodegradability or an unequivocal assessment is not possible because the substance is not bioavailable for the degrading bacteria due to its low water solubility. Careful consideration of the physic-chemical properties of a substance is therefore necessary to choose the appropriate test design. As the biodegradation of a substance strongly depends on its mass transfer and the bioavailability for the degrading bacteria, there are several technical issues which need to be addressed while testing the biodegradability of poorly water soluble substances. Due to the historic background of the guidelines for testing ready biodegradability the test concentration is relatively high (2 - 100 mg/L) and not reflecting environmental concentrations. To improve the bioavailability of poorly water soluble substances in tests for ready biodegradability the pretreatment of the substance as well as the application technique and the agitation during testing needs therefore special consideration. Four techniques for the application of poorly water-soluble substances are commonly used and described in the ISO standard 10634 (1995) and recommended by the REACH guidance. These methods are direct addition, ultrasonic dispersion, adsorption onto an inert support material and the use of non-biodegradable emulsifying agents. So far no single method is recommended and routinely a combination of approaches is used for application. Based on the substance properties the application method has to be chosen carefully e.g. the use of the wrong inert support material can result in too strong adsorption and decreased bioavailability. Careful investigation of the application method prior to the test start can enhance the degradation distinctly. These investigations involve a relatively small extra effort and often more expensive simulation studies can be avoided. The influence of different application techniques on the bioavailability and biodegradation of poorly water-soluble substances was investigated and the results will be presented.

TU 301
Using Molecular Dimensions to evaluate bioaccumulation potential
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In the REACH Regulation (but also under other directives/regulations), PBT and vPvB as-

sessment is compulsory for all chemicals produced or imported above 10 t/year. Although the main regulatory criterion for B or vB attribution is the aquatic bioconcentration factor (BCF) (REACh Annex XIII), an evaluation of all available information is necessary and screening criteria, as log Kow, can be used. So, when direct comparison with criterion lay down in REACh Annex XIII is not possible, other indicators are to be considered in a weight of evidence approach. Among these, the molecular size and molecular weight (MW) may indicate a low bioaccumulation potential. Several descriptors can reflect molecular size but at present, the most reliable is the "average maximum diameter"(Dmax aver) developed by Mekenyan and Dimitrov . These authors showed that a chemical may be considered as not bioaccumulative if Dmax aver > 1.7 nm and MW > 1100 g/mol because very bulky molecules pass the cell membranes with difficulty . To calculate Dmax aver, conformational analysis is necessary and OASIS is the reference software to obtain energetically stable conformers representing conformational space of the molecules. The objective of this work was to find alternative or additional equivalent tools. We have based our work on the review of Brooke and Cronin , and we critically examined different modeling softwares related to molecular dimensions. By this way, we led to the combination of two softwares: Spartan was used for modeling calculations whereas the BBX descriptor from Mol2Mol gave a surrogate value for Dmax aver. This new procedure was tested on a set of 18 molecules reflecting a wide structural diversity and results were compared with data obtained with OASIS. In this communication we will present the results obtained and discuss the scope and limitation of our method in the context of PBT assessment.

TU 302

Accounting for photo degradation in the PBT assessment of chemicals

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In the current REACH guidelines substances are considered persistent if their half-life in marine water is greater than 60 days. Current estimations of half-lives in the aquatic environmental under REAH regulation are mainly done using recommended modeling tools (i.e. EUSES), in which photo degradation is not explicitly considered. REACH guidelines do not specify how "half-life in water"is to be deduced from laboratory measurements of direct photolysis according to official methods (e.g. OECD or EPA test guidelines). However, photolysis in surface waters has been identified as the main degradation pathway for some chemicals / pollutants, in particular those resistant to biotic degradation processes. The overall objective of this work was to investigate how direct photolysis of chemicals in the aquatic media can be accounted for in the current PBT assessment methodologies. In addition, an investigation on how to establish a generic procedure for extrapolating photolysis rates under laboratory conditions to field conditions was performed. The methodology followed was based on the revision of currently used laboratory test methods for direct photolysis, peer-reviewed scientific literature, and main modeling applications currently employed for the calculation of the persistency of a chemical in the aquatic environment. Data still under evaluation although a preliminary assessment point to light attenuation, pH of the water media (in particular for chemicals with acid-base properties) and dissolved organic matter (DOM) concentration being among the most important environmental variables affecting the direct photolysis rates. In addition, indirect photolysis has been shown to play an important role too. A standard procedure for extrapolating laboratory measurements of direct photolysis to half-lives in natural water is proposed. The developed extrapolation method has been incorporated into the multimedia fate model SimpleBox and the effect of incorporating photo degradation on the calculated persistence of some example chemicals in water has been analyzed.

TU 303

Analytical improvements: simultaneous pressurarized liquid extraction, silica gel, fluorosil, celite, alumina, and carbopack cleanup techniques for fish tissues analysis

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Pressurarized liquid extraction (PLE) was combined with multiple cleanup techniques including silica gel, fluorosil, celite, alumina, and carbopack into a single step. This simultaneous analytical extraction/cleanup technique was compared with a traditional packed column approach described by EPA method 1613. EPA method 1613 describes the analysis of polychlorinated dioxins and furans (PCDD/Fs) and polychlorobyphenyls (PCBs) in fish tissues involving multiple cleanup sorbents. Homogenized fish tissue (5 g) with anhydrous sodium sulfate was packed within an extraction cell directly over different combinations of commercially available adsorbents. Simultaneous pressurized liquid extraction and cleanup was performed with dichloromethane and hexane (1:1 v/v) using an Accelerated Solvent Extractor (ASE 350, Dionex). Analysis was performed with HRGC/ECNI-MS and HRGC/HRMS utilizing isotope dilution technique. Cleanup efficiencies for individual and combinations of sorbents within the extraction cell were compared to conventional packed column techniques and gel permeation chromatography. Different ratios of sorbents were also examined. Cleanup efficiencies were examined using target analytes percent recoveries, UV-absorbance and GC/EI-MS full scan spectra. This simultaneous PLE/cleanup technique reduces the intrinsic costs associated with sample preparation (time, labor and supplies) over conventional series approach by as much as 80% percent. The average recoveries of PCDD/F and PCBs in fish tissue (5 g) using PLE/silica gel/fluorosil/celite was 98 ± 8%. Combined cleanup technique allows the extraction of whole zebra fish of ~ 1g without any homogenization step. The recovery from in-fish spiking, on-fish spiking and over-fish spiking of analytes were 86 ± 7%, 83 ± 6%, and 77 ± 7%, respectively.

TU 304

Implementation of alternative fuels in cement plants: a cost-benefit analysis between CO2 emissions and PBT risks

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Cement industry is an industrial sector with very high-energy requirements. The use of alternative fuels such as sewage sludge or refuse-derived fuels (RDF) seems to be an important pathway to solve different problems. On one hand, the emission of greenhouse gasses (CO2) is reduced and a feasible solution is given to the waste management problem, while on the other hand, the partial substitution of traditional fuel may generate a change in the cement plant pollutant emissions and, consequently of the human health risks. Among the pollutants emitted by cement plants, PCDD/Fs and heavy metals cause especial concern because they are persistent, bioaccumulable and toxic (PBTs).

In recent years, many survey campaigns have been done around two different cement plants with different fuel consumption situations. The objective was to monitor the levels of PBT substances in different environmental compartments in order to evaluate the potential effect of the use of alternative fuels on the emissions. Levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and metal were determined in different environmental monitors (soil, vegetation and air) before and after the implementation of alternative fuels. The alternative fuels evaluated were sewage sludge and RDF, respectively. The results were used to perform a cost-benefit analysis. Economical benefit of reduction of CO2 emission was compared with the changes in human health risks due to exposure to metals and PCDD/Fs. In general, terms, the results of cost-benefit analysis in the two cement plants under study showed that the benefits of reduction of CO2 emission were greater than the cost of human health risk due to the exposure to metals and PCDD/Fs. This indicates that the exposure to PBT for residents around cement plants implementing alternative fuels does not pose significant risks for human health.

RA09 - Risk assessment in the marine environment and regulation

TU 310

Crude oil genotoxicity and cytotoxicity after short-term exposure of mussels

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Significantly increased levels of micronuclei, nuclear buds and fragmented-apoptotic cells have been found in bivalves inhabiting the Būtingð- oil terminal area in the Baltic Sea, (Baršienð- et al., 2006a), zones close to the Russian oil platform D-6 (Baršienð- et al., 2008), in oysters and fish caged in Haven oil spill zones even 10 years after the oil spill (Bolognesi et al., 2006a). High levels of micronuclei and other nuclear abnormalities has been found in fish and mussels after 3-week exposure to crude oil processed from the Statfjord B platform in the North Sea (Baršienð- et al., 2006b; Bolognesi et al., 2006b; Baršienð- and Andreikð-naitð-, 2007). The study presents data on genotoxicity and cytotoxicity of the crude oil in gills of mussels Mytilus edulis, exposed to 0.5 ppm of the oil for 1-, 2-, 4- and 8-days. Induction of micronuclei (MN) and nuclear buds (NB) were assessed as the crude oil genotoxicity endpoints, induction of fragmented-apoptotic (FA) and bi-nucleated (BN) cells indicated cytotoxicity potential of the oil. Time-related elevation of MN incidences was detected in all experimental groups compared to control group of mussels. The elevation of MN frequency increased progressively with increasing duration of exposure, from 1.9 fold increase after 1 day exposure to 2.4 fold after 8 day exposure. There was no significant increase of nuclear buds in all experimental groups. Significant induction of fragmented-apoptotic cells (P=0.0115) was detected in mussel gills after 4-day exposure and bi-nucleated cells after 8-day treatment (P=0.0232). The data will be discussed in relation to long-term oil exposures, to genotoxicity and cytotoxicity responses after caging of mussels in oil platform zones, also will be pointed on genetic risk provoked by oil pollution in marine ecosystems.

TU 311

Genotoxicity and cytotoxicity effects in mussels after short-term treatment with different concentrations of pyrene, fluoranthene and their mixtures with benzo(a)pyrene

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Micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells were investigated in 16 experimental groups of mussels, which in the semi-static laboratory system were exposed for 48 hours to 4 µg/l, 12 µg/l, 36 µg/l of pyrene (Py), to 4 µg/l, 12 µg/l, 36 µg/l of fluoranthene (F), to 8 µg/l and 24 µg/l of Py and F mixtures and also to Py and F model mixtures with benzo(a)pyrene (BaP), as well as to 12 µg/l and 28 µg/l model mixtures of Py, F and B(a)P. 4 _g/l of BaP concentration was used in all model mixtures. Control group and control group with PAH solvent acetone of mussels maintained in filtered seawater. Pre-exposure group of mussels was dissected after 4-day acclimation of mussels in unfiltered seawater. Exceptionally high levels of MN (11.43"ð) and NB (14.14 "ð) were detected in mussels treated with mixture of fluoranthene and benzo(a)pyrene at 16 _g/l (F12+BaP4) and after exposure to fluoranthene 12 _g/l (MN - 10.57"ð; NB - 8.71"ð). The highest level of bi-nucleated cells (19.91"ð) was observed in Py4+BaP4 group, fragmented-apoptotic cells (9.50"ð) - in F4+Py4 mussel group. In nine of 16 studied groups, Mann-Whitney U-test revealed significant differences in frequency of bi-nucleated cells between control and exposed groups. Significant elevation of the other responses via control was observed in 4 or 5 groups. Positive relationships between induction of MN and NB, MN and BN, MN and FA, as well as between total genotoxicity (MN+NB) and cytotoxicity (FA+BN) were found by using the Pearson's correlation. Assessment of the BaP influence in model mixtures consisting of 4 _g/l and 12 _g/l of pyrene or fluoranthene and plus BaP of 4 _g/l disclosed significant effects of BaP in mussels treated with mixtures containing low (4 _g/l) concentration of fluoranthene or high (12 _g/l) concentration of pyrene. In the mixture of 12 _g/l of fluoranthene, any effects of BaP were defined (Unpaired T-test).

TU 312

Biochemical responses of mussels Mytillus galloprovincialis to petrochemical contamination along the North Iberian Peninsula Coast

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In order to assess the biochemical responses to petrochemical contamination, mussels, Mytilus galloprovincialis were collected in April, June and October 2010 in seven localities along the North Iberian Peninsula Coast (NIPC). Four localities were selected in the NW Coast of Portugal (Vila Chã, São Bartolomeu do Mar, Viana do Castelo and Carreço) and three localities in the Basque Coast (Arriluze, Gorliz, Mundaka). The sites were chosen according to the level and sources of petrochemical contamination. Three biochemical biomarkers were selected: acetylcholinesterase (AChE) activity was quantified in foot to assess mussels' neurotransmission levels, glutathione S-transferases (GST) activity and the levels of lipid peroxidation (LPO) were analyzed in gills as biomarkers of biotransformation and oxidative cell damage, respectively. All the biomarkers variations were studied in relation to abiotic factors variation using multivariate statistics. The levels of AChE activity were significantly higher in April than in June and October in all the studied localities, except in Arriluze where low activity values were found all along the study period. In mussels collected in the NW Coast of Portugal, no significant differences were found in the levels of GST activity among localities. In general, GST activity was higher in October than in April and June. In the Basque Coast, significant differences were recorded in the levels of GST activity both among localities and sampling times. The levels of LPO were significantly higher in June and October than in April in mussels collected from the NW Coast of Portugal, whereas in the Basque Coast, LPO values were significantly higher in April than in June and

October, except in Gorliz where significantly higher levels were recorded in October. Since the NIPC is a high risk area for marine oil and other chemical spills due to intense marine traphic, this is a most important baseline study allowing the assessment of impacts in future spills. This study was supported by the Portuguese Foundation for the Science and Technology and FEDER funds through the project RAMOCS (ERA-AMPERA/0001/2007; EU AMPERA ERA-NET, ERAC-CT2005-016165), and by the Government of the Basque Country through a post-doc grant to L. Garmendia (ref. BFI09.244) and K-EGOKITZEN project.

TU 313

Exposure assessment of active ingredients and byproducts in antifouling paints and ballast water treatment systems

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Antifouling paints and ballast water treatment installations may be sources of direct inputs of contaminants into the marine environment. Against the background of the requirements of the EU Biocidal Products Directive (BPD), the IMO Antifouling Convention of 2001, and the IMO Ballastwater Convention of 2004 there is a need for reliable modelling tools for the prediction of exposure and risks of new antifoulants and pollutants from ballast water treatment systems. In 1999 the first version of MAMPEC was released. The study was sponsored by the Antifouling Working Group of the European Paint Makers Association (CEPE/CEFIC) and the European Commission (DG XI) The model has since been improved with regular updates of the model. The latest release of MAMPEC version 3.0 is compatible with Windows Vista/ 7 and includes some important new features.

The model predicts concentrations of antifoulants in generalised 'typical' marine environments (open sea, shipping lane, estuary, commercial harbour, yachting marina, open harbour). The user can specify: emission factors (e.g., leaching rates, shipping intensities, residence times, ship hull underwater surface areas), compound-related properties and processes (e.g., K_d, K_{ow}, K_{oc}, volatilisation, speciation, hydrolysis, photolysis, biodegradation), and properties and hydrodynamics related to the specific environment (e.g. currents, tides, salinity, DOC, suspended matter load, port dimensions). MAMPEC includes options for advanced photolysis modelling, and incorporation of wind-driven hydrodynamic exchange and other non-tidal exchange processes important for areas without tidal action, or inland freshwater environments. Included are also the service-life emission scenarios developed by OECD and adopted by EU as the standard environmental emission scenarios, to be used for evaluation of the biocides under the Biocidal Products Directive. The model has been validated for a number of compounds and is today recognized by regulatory authorities in EU, USA, Japan, and other OECD countries. MAMPEC is currently also used in authorisations by GESAMP and IMO for the assessment of active ingredients in ballast water. The model is freely available from the software support site http://delftsoftware.wldelft.nl. The poster presentation will focus on available exposure models, areas of uncertainty, new developments, and research needs.

TU 314

Risk Assessment of copper in antifouling use

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In 1998, the Biocidal Products Directive (BPD) required the chemicals industry to initiate steps to address the risk assessment of chemicals in biocidal applications, including antifouling use. Described is the process of risk assessment of copper, the models used, the assumptions made, and preliminary conclusions of the industry assessment.

TU 315

Individual and mixture toxicity of antifouling biocides using a novel multispecies slime assay

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Antifouling biocides are used in paints to prevent the growth of fouling organisms on submerged structures such as ship hulls. However, many of today's antifouling coatings fail to inhibit settling and growth of microalgal slime, a complex multispecies biofilm which is often dominated by diatoms. Here we present a simple, robust and highly realistic assay for evaluating the efficacy and ecotoxicological properties of antifouling biocides and formulated paints. To reflect the high diversity found in microbial fouling communities, we developed a settling and growth assay that uses microbial communities from natural biofilms as start material. In short, an inoculum is prepared from the biofilms using a scrape, shake, and sieve technique. Biofilms are then allowed to form during a phase of 72 hours of controlled exposure to a concentration-series of biocides. The established slime biofilms can then be analysed by various techniques, here we used biomass (estimated as chl a content). By this approach we include the whole multitude of indigenous, often non-cultivatable species that are the actual foulers. The assay hence integrates the different sensitivities of the various species as well as their ecological interactions during the settling phase. Eight common antifouling biocides were selected for initial testing. All were toxic to the settling of slime communities in a concentration-dependent manner, with EC50 values ranging from 0.6 nM to 4.4 µM. In addition, 2- to 6-component mixtures were tested with EC50 values falling into the span of individual toxicities, indicating the absence of strong synergisms. In fact, mixture toxicities were often concentration-additive, but antagonistic effects were also observed. Implications for risk assessment of antifouling biocides and paints will be discussed. The study is part of the Swedish Marine Paint research programme funded by MISTRA (www.marinepaint.se).

TU 316

Acute toxicity estimation of desalination byproducts at Chuja island desalination plant

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Desalination of seawater has become an important and growing industry due to the present water shortage in the world. However, the desalination may result in environmental impacts, manly derived by the discharge into adjacent coastal waters of brine and additives produced during the desalination processes (e.g., biocides and membrane cleaning chemicals). To measurement of environmental impact by desalination, we approached environmental impact assessment (EIA) procedure proposed by Hoepner (1999) for the desalination plant of Chuja Island in South Korea. We conducted a series of marine bioassays using three phytoplankton, two zooplankton, and one fish species for source water, discharged brine waters and chemical additives. There was no significant toxicity on brine but high toxicity was found at the chemical additives as chlorine and membrane cleaning chemical. However, Chuja plant used little chemicals to treat source waters and produced freshwater. In terms of the habitat susceptibility it is located in relatively insensitive habitat, open rocky shore with gravel bottom. Based on these results, even the environmental impacts by Chuja desalination plant were not significant currently, monitoring strategies have to be established and conducted to estimate long-term effects from desalination in marine ecosystem. This study will be an example for EIA processes of desalination in Korea. This research was funded by Ministry of Land, Transport, and Maritime Affairs, Korea.

TU 317

Disinfection by-product formation by ballast water management systems

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Ship's ballast water is a vector for the spread of alien aquatic species, which have lead to substantial ecological and economical damage in the past. In an effort to resolve this problem the International Maritime Organization (IMO) passed a convention for the management of ship's ballast water in 2004, which is expected to come into force within the next two years. This convention requires ship's ballast water to be treated in order to eliminate alien aquatic species. Any ballast water management system using active substances needs IMO approval. Human health and environmental risk assessment is an important part of the approval procedure requiring identification of active substances and relevant chemicals and submission of specified datasets on their physical, chemical and toxicological properties. Identification of the active substances is straight forward since these are by definition the substances responsible for disinfection. The more critical point is to identify all relevant chemicals, predominantly disinfection by-products (DBPs), formed unintentionally and to assess their risk to human health and the environment. We analyzed the available data on systems submitted to IMO with regard to the formed chemicals focusing on three disinfection principles, namely electrolysis, ozone and UV. Our goal was to provide a basis for future risk assessments by identifying relationships between employed disinfection methods and predominantly detected DBPs as well as relationships between water quality or system performance parameters and DBP formation. We came to the conclusion that selection of DBPs for analysis is based on drinking water conditions and recommend selecting an extended set of DBPs using a system (i.e. treatment method) and hazard based approach.

TU 318

The distribution of the salinity and biogenic elements in the surface microlayer of the Baltic Sea

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Marine surface films are encountered at many places of the world's oceans, particularly in marginal seas and coastal regions. Since the late 1960s, much progress has been made in understanding the role of surface films on mechanisms and mass transfer across the ocean/atmosphere interface. The peculiarities of the distribution of the salinity and nutrients in the surface microlayer and the comparison of such with the distribution in the layers below in the region of deep-bottoms of the Baltic Sea (Landsort, Gotland, Bornholm, and Arkona) were investigated. The investigations were carried out during expedition in August 1982. Air -sea interaction experiments are then described. The sampling in the SML was performed by the trap method. It was shown that surface microlayer (SML) strongly concentrates biogenic substances: ions of inorganic nitrogen and phosphorous compounds. Their concentrations in SML were approximately tenfold higher than in the below surface layer's of the Baltic Sea. The salinity of SML in different parts of the Baltic Sea warred in the range of 0.043 - 0.646 "ð. The dynamics of the values of the salinity and nutrients ions, and their variability in distribution according their concentrations gradients in the different parts of the Baltic Sea has been also shown. The coefficients of enrichment of SML with investigated parameters have been evaluated quantitatively, and the selective range of the hydrochemical determinants has been established. On the basis of the presented data it may be stated that surface microlayer of the Baltic Sea seems to be a significant player in the atmosphere - sea interaction processes.

TU 319

New physiological biomarkers for express indication of aquatic ecosystems state on the base of adaptive capacities assessment of bivalves using standard test-stimuli

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In the present work the results of the experimental study directed on working out of a new cardiac activity and valve movements biomarkers are obtained. A methodological basis of a given research is the estimation of adaptive capacities of test organisms by means of standard test stimuli in the range of species tolerance. Experimental rapid change of salinity of sea water on 50% was used as one of stimuli. On an example of mollusks Mytilus galloprovincialis Lam. of the Black Sea studies of organisms' adaptive capacity in normal state and after toxic influence of 500 mkg/l Cu^{2+} were conducted on laboratory base of Karadag Natural Reserve of the National Academy of Sciences of Ukraine. Distinctive feature of the present study from our previous works performed on bivalves of the Baltic Sea was research of mussels' responses on hypo- and/or hypersalinity stimuli using noninvasive methods of monitoring not only of heart rate (HR), but valve gaping (VG) also. Thus, to study the peculiarities of mussel's responses to test stimuli from their physiological state we used acute dose of Cu^{2+}, which caused experimentally induced temporary decrease in adaptive capacities of tested mollusks.HR and VG were selected as integral parameters of mussel's health status. 14 mussels simultaneously were exposed to Cu^{2+} and their HRs and VGs were investigated during long-term experiments. Effect of Cu^{2+} on mussel's adaptive capacities was examined by test-stimulus after the toxic treatment and compared with cardiac and valve responses patterns before exposure. Analysis of studied parameters after exposure showed the significant differences in recovery time after standard salinity test followed by Cu^{2+} exposure. Increase in discrepancies in individual responses after toxic treatment was also observed.

The results allowed us to propose the following new biomarkers:

- Evoked by standard test-stimuli value of ΔHR dispersion, in %;
- Time of organism recovery after test-stimuli, measured as restoration of individual background specific patterns in HR and in VG, in hrs.

The tendency for rapid recovery period for HR and VG to their initial values, during salinity tests can characterize better physiological status of tested organisms as having better adaptive capacities to restore their normal physiological state after test-treatments. The latter can give us an

opportunity to assess ecosystem health status for studied sites.
The work was supported by RFBR grant N 08-04-92424 BONUS_a.

TU 320

Embryotoxic and dioxin-like potential of Baltic Sea sediments and extracts as determined using the fish embryo test with Danio rerio and a RTL-W1 cell-based EROD induction assay

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The Baltic Sea, one of the largest brackish water regions in the world, is considered to be an especially sensitive and endangered marine ecosystem. The average residence time of Baltic Sea water ranges between 25 and 50 years, thus increasing the accumulation of pollutants.
For a deeper insight into the ecotoxicological effects of pollutants in sediments, both chemical and biological testing is important. The ecotoxicological risk assessment of native sediment samples using biological tests is affected by the bioavailability of persistent organic pollutants in the water phase. Direct sediment contact tests represent the bioavailability in ecosystems for different species, but extracts are essential for many biological tests and also for chemical analytics.
Baltic Sea sediments were investigated for their ecotoxicological impact on the development of zebrafish (*Danio rerio*) embryos. Preliminary tests adapted the whole-sediment toxicity test to samples with salinities above fresh water level. Using the fish embryo test system, whole sediment samples, ASE extracts and mild methanol/water extracts of all sampling sites were then assayed for deleterious effects. Further investigations of the extracts were conducted with the EROD induction assay using RTL-W1 cells for detection of dioxin-like activity. Target analytes were also determined analytically.
A no-effect salt concentration of 6.6 “ð in the testing medium (equals 28 “ð for the original sample) could be derived, and thus allowed to perform the whole-sediment fish embryo toxicity test with brackish Baltic Sea sediments. Results from the embryo toxicity test in 96 well plates with sediment extracts from ASE and shaking extractions with methanol/water were compared to data obtained testing native marine sediments. Findings revealed the bioavailable hazardous potential of sediments as well as the actual risk for fish embryo development. Finally, results on EROD inducing potential indicated putative dioxin-like activity at the investigated sampling sites. The data show an ecotoxicological burden for several Baltic Sea sediments, that is likely accessible for sediment-dwelling organisms.

TU 321

Large and medium scale spatial variability in biomarker responses in herring (Clupea harengus membras), a commercially and ecologically important fish species in the Baltic Sea

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The BEAST project (“Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health”, 2009-2011) is the most extensive international research project in the field of biological effects of pollutants carried out in the Baltic Sea region so far. As part of the sampling programme of BEAST, herring (Clupea harengus membras), a key species of the Baltic Sea ecosystem and one of the most important commercial species in the area, was collected from different sea areas (Gulf of Bothnia, G. of Finland, G. of Riga, G. of Gdansk, and Belt Sea) for the measurement of selected biomarkers (lysosomal membrane stability, acetylcholinesterase activity, glutathione S-transferase activity and catalase activity) as well as recording of histopathological changes. The results obtained showed marked between and within sea area variability, the former indicating differences in natural levels of certain parameters caused by the different abiotic regimes (mainly salinity and temperature conditions) in different parts of the Baltic Sea, while the latter being probably related to the prevailing local contaminant situations in each study area. The results imply that the causes of large and medium scale variability in biological effects needs to be carefully considered when interpreting and comparing the results attained from large and variable sea areas such as the Baltic Sea.

TU 322

Assessment of ecosystem health in the Baltic Sea using the embryonic development of Monoporeia affinis as a bio-indicator

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The current work is a part of the Bonus project “BEAST”that targeted at developing integrated measures of chemical pollution and tools needed to detect and understand human-induced pressure on the Baltic Sea ecosystem. The project aims at establishing links between chemical pollution and biological effects within individuals, and higher organizational level. A possible candidate for evaluating chemical - biological effects at population level is analyzing the embryonic development of the Baltic key species amphipod Monoporeia affinis. The present work evaluates embryo malformations of Monoporeia affinis as a bio-indicator for regional assessments in the Baltic Sea. A total of 11 independent regional studies from polluted recipients are synthesized in a meta-analysis to provide quantitative and statistically defensible summary of the relationship across studies. Results show that embryo malformations meet several criteria for a pollution bio-indicator. It is shown that embryo malformations generally arise in the studied recipients, despite various types of emissions sources, suggesting that the variable works as a general indicator of pollutant effects in the Baltic Sea. Results show that the proportion of malformations increases with decreasing distance from point source, suggesting that there is a strong relationship between the studied emission sources and the malformation rate. The synthesis also indicates that embryo malformation is a sensitive variable that can detect pollution from point sources as far away as 20-31 km. It is concluded that the variable provides an unequivocal input which could facilitate decision-making and development of remedial action plans.

TU 323

Is pollution changing the composition of estuarine zooplankton communities in the NW coast of Portugal?

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Zooplankton is considered critical for pelagic ecosystem functioning, since it connects producers to several fish species that depend all life or in the juvenile phase from this food source. Pollution can have negative effects on zooplankton communities (biodiversity, structure and/or functioning), with negative consequences for the functioning of the pelagic ecosystem. The aim of this study was to compare the effects of pollution on structure and dynamics of zooplankton communities taking advantage of two adjacent estuaries in the NW coast of Portugal with different levels of pollution (the estuaries of Minho and Lima Rivers). Zooplankton samples were collected monthly during the high tide for 1 year (2010-2011), using nets with different meshes (0.063-0.500 mm) equipped with a flow meter. Additionally, abiotic parameters were determined, indicative of water quality and primary production (chlorophyll *a*). The diversity of both estuarine communities was compared using diversity indices and analyzed in relation to variation of abiotic parameters using multivariate statistics. The preliminary results indicate significant differences in main taxonomic groups between the two estuaries and suggest a main role of pollution on these differences. This work was financially supported by the Portuguese Foundation for the Science and Technology (FCT), FEDER funds, European Social Fund and MCTES national funds through the project RAMOCS (ERA-AMPERA/0001/2007; EU AMPERA ERA-NET, ERAC-CT2005-016165) and a post-doctoral grant to Luis R. Vieira (FCT: SFRH/BPD/47407/2008).

TU 324

Implementing the tissue residue approach for a key Antarctic species - Antarctic krill (Euphausia superba)

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Persistent organic pollutants (POPs) are well known to accumulate in polar food webs; yet there is little ecotoxicological data available for environmental risk assessment of POPs in these extreme ecosystems. The premise of the tissue residue approach (TRA) is that xenobiotics cause toxic effects at similar tissue residues across species and geographical ranges, regardless of differences in external factors such as ambient temperature. Empirical data are however, fundamental for validation of the TRA for polar species. p,p’-DDE has been identified as a priority POP accumulating in Antarctic krill, which play a central role in the Southern Ocean ecosystem. This study evaluated the toxicological sensitivity of Antarctic krill to p,p’-DDE exposure on a body residue/effect basis. Baseline toxicity (‘narcosis’), which is a commonly applied endpoint in the TRA, was used as indicator of sublethal toxicity. Several different exposure scenarios were tested: i) 96 h aqueous exposure of adult krill, ii) 1, 3 and 9 d aqueous exposure of larval krill and iii) 10 d dietary exposure of adult krill. For all exposure scenarios, krill immobility was induced at the low end of the body residue range (4 - 16 mmol/kg lipid weight (l.w.)) known to cause sublethal narcosis in temperate species (e.g. small fish, crustaceans). Internal effect concentrations (IECs) were relatively constant across tests (3.3 - 6.2 mmol/kg l.w.), although the degree of narcosis varied between tests. Adult krill reached partial immobility during 96 h aqueous exposure (IEC50partial immobility = 3.9 mmol/kg l.w.), whereas complete immobility and mortality were observed in the 10 d dietary exposure tests at similar internal concentrations (IEC50complete immobility = 3.3 mmol/kg l.w.). Complete immobilisation was observed in 20 % of larval krill after only 24 h exposure (IEC20complete immobility = 3.6 mmol/kg l.w.). The apparent variability in toxicological sensitivity between different life stages may be partly explained by differences in p,p’-DDE uptake rates. The observed differences in the narcotic response of adult krill between 4 and 10 d exposure indicate however, the possibility of delayed effects of p,p’-DDE exposure in Antarctic krill. Overall, the findings for Antarctic krill are in support of the TRA indicating that effective body residues for baseline toxicity in this polar species are comparable to those measured for temperate species.

RA11 - Risk assessment of chemicals within REACH integrating alternative methods and non testing strategies

TU 330

Opportunities and limitations of using alternative methods and non testing strategies in REACH registration dossiers

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By 1 December 2010, substances manufactured or imported at or above 1000 Tonnes/Year (T/Y) as well as Carcinogenic Mutagenic or Reprotoxic substances category 1 and 2 manufactured or imported ≥ 1 T/Y, or substances classified as dangerous for the aquatic environment with R50/53 and manufactured or imported ≥ 100 T/Y had to be registered under REACH.
The lack of data on the hazardous properties of chemicals was the driving force behind the development of a new chemicals policy in the EU nevertheless one of the objectives of REACH is to promote alternative methods for the assessment of hazards of substances both to reduce animal testing and to reduce the costs. The Consultancy for Environmental & Human Toxicology & Risk Assessment (CEHTRA) helped the industry sector to comply with their regulatory obligations providing input on more than one hundred REACH substance dossiers including several chemical classes such as organic, inorganic substances, mono and multi constituents.
The aims of this poster are to illustrate cases where non testing techniques have been used for filling data gaps to perform the environmental risk assessment for these substances in compliance with REACH. Both the tonnage and the objectives of information requirements have been taken into account. In this study, existing data coverage, reasoning for waiving tests, adequacy of possible alternative methods (QSAR modeling, in vitro and read-across), evaluation of existing data as part of a weight-of-evidence approach and experimental testing strategies are addressed. The final objective is to identify the most reliable approaches which have gained regulatory credibility and the limitations in using non-testing strategies under REACH, based on our experience, after the first registration dossier submission’s deadline.

TU 331

Lessons from ECETOC TRA based exposure scenarios

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ECETOC TRA integrated tool has been the corner stone of exposure scenario modeling and risk characterisation in the first phase of REACH. The tool incorporates three principal methods to calculate Predicted Environmental Concentrations (PECs) and Risk Characterisation Ratios (RCR):
1- The Environmental Release Category (ERC) method developed for Tier I REACH scenarios,

and Specific ERCs (SpERCs)
2- In Tier 2; the OECD use models and the A&B tables from the Technical Guidance Document (2003).
3- Measured data approach in tier 3
Based on specific case studies, we discuss in this communication "work-arounds"which we used to provide a greater level of environmental relevance in our Chemical Safety Reports.
In the first part, we will share the statistical analysis of CEHTRA's REACH dossiers submitted in 2010 (proportion the different tiered levels of Environmental Risk Assessments, related time spend on them,[3DOTS]). In the second part, technical difficulties for a proper risk assessment will be summarized as well as the proposed solutions. Last, several technical adaptations of the existing tool will be presented.

TU 332
A step change towards risk assessment in the 21st Century? Practical experiences with REACH.
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Chemical Regulation and the means by which data is generated for the purposes of risk assessment is undergoing a tremendous shift. There is a strong impetus in Europe in particular to move towards non animal approaches to address data gaps for specific endpoints either in lieu of testing or as part of weight of evidence approach within Integrated Testing Strategies (ITS). An Exposure assessment considering workers and/or consumers is another pertinent factor to consider in the derivation of a robust risk assessment. The recent EU chemicals legislation REACH, in particular provides considerable flexibility in the application of non-testing approaches such as (Q)SARs, chemical categories and read-across for data gap filling.
There have been a number of efforts aimed at developing technical guidance, tools, and techniques for non-testing and tiered exposure approaches. Despite these efforts, there remains limited practical insight about how these approaches can be applied in the assessment of substances. Here, we provide an overview of the available approaches and how we have practically utilised them to address our REACH requirements.

TU 333
The Klimisch Scoring System: a system in desperate need of modernization
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The evaluation of data quality under REACH and the HPV program is a key component for conducting robust hazard and risk assessments. The Klimisch scoring system is an approach used to assess the reliability of data, particularly toxicological and ecotoxicological data. The intention of the system is to harmonize data evaluation worldwide. However, recent studies have recognized the deficiencies in the Klimisch scoring system highlighting that interpretation of Klimisch criteria by evaluators is subjective, and semi-quantitative at best. Without data quality harmonization, an assessment can lead to different reliability codes which can affect the robustness of the risk assessment. In this presentation we describe a tool we have developed to provide a more consistent and logical evaluation framework to the Klimisch system while maintaining the same fundamental reliability codes. This tool has been tested and validated on a training set of studies by regulatory scientists. A second validation exercise further increased the accuracy and consistency of assigning reliability codes to studies compared to the original Klimisch approach. We pose some fundamental questions about the pertinence of the Klimisch system and whether a complete revision (while ultimately retaining the same 4 reliability codes) would be worthwhile.

TU 334
Solvent abuse in ecotoxicology testing
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Attempts have been made to standardize aquatic ecotoxicity testing for the purposes of hazard assessment in norms and Guidelines (e.g. OECD 200 series). Generally, and with some exceptions, these documents describe methods applied to "ideal"substances without specific properties which lead to the need to modify the test design (e.g. adsorbing, volatile, reactive and poorly soluble chemicals). For these cases guidance is provided in supplementary documents such as OECD No. 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures. In the specific case of poorly soluble substances (for the sake of argument defined here as <1 mg/L in the medium used), OECD No 23 has the following to say on the use of solvents: "because of the potential for interaction with the test substance resulting in an altered response in the test, their use should be restricted to situations where no other acceptable method of media preparation is available". Nevertheless, multiple testing facilities across the world are still performing flow-through studies using solvents as standard and because they usually manage to maintain the test concentrations throughout the study, they are generally being considered as "high end"valid studies for risk assessment purposes.
Alas, multiple cases have come to light which demonstrate that many of these studies are critically flawed. Notably, concentrations are maintained with high precision at levels in some cases greater than the measured water solubility limit of the test substance as measured in an OECD 105 solubility test (in pure water). Interesting, as OECD No 23 points out "It is unlikely that a solvent concentration of 100 mg/l will significantly alter the maximum dissolved concentration of the test substance which can be achieved in the medium". Yet, further investigation on several substances has revealed that the OECD 105 measured solubility may be one or more orders of magnitude greater than the actual solubility in the test medium used in aquatic toxicity test. Effectsfrom these flow-through tests with solvent have been observed above, at or close to the pure water solubility limit and these are systematically attributed to toxicity. In reality it would seem that the organisms are succumbing to a slow suffocation by undissolved droplets of test substance maintained in emulsion throughout the study, termed here "the vinaigrette effect". How then, to separate genuine toxicity from the vinaigrette effect?

TU 335
A QSAR-based compound prioritization for lab-testing for chemical safety assessment
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The practical implementation of non-testing strategies such as Quantitative Structure-Activity Relationships (QSARs) is still an area of development. To this end, there is a need to solve issues on how to practically integrate QSARs model predictions in risk assessment with the long-term goal to increase the use of non-testing information for regulatory decisions while meeting the main challenges of quantifying and reducing uncertainty. We discuss to what extent different strategies of prioritization for testing chemical compounds result in various strengths of background information for chemical safety assessment under REACH. An application of a strategy, based on predicted data from QSAR models, to select a set of compounds on the ECHA preregistration list for further testing is provided and discussed. We propose to design a strategy of testing based on criteria related to the strength and spread of background information, the costs and the purpose of testing and with respect to the specific circumstances in the application which is given by the available background information.

TU 336
Biotransformation of chemicals: linking octanol-water partitioning to Michaelis constants to search for general mechanisms
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The accumulation of xenobiotics in organisms is a key factor in the assessment of chemical risk and depends on transport and transformation processes. While rates of exchange with air, water, and food can be predicted fairly accurately from properties of chemical substances and biological species, biotransformation rates (k_m) are difficult to obtain. This is one of the main limiting factors in modeling accumulation, whose extent significantly depends on the degree to which biotransformation occurs.
As most enzymes follow first-order Michaelis Menten kinetics, biotransformation rates for many substances can be approximated as:
$k_m\ (d^{-1}) = V_{max}\ (\mu M \cdot d^{-1}) / K_m\ (\mu M)$
where K_m (Michaelis constant) indicates a measure of the affinity of the substrate for an enzyme and V_{max} is the maximum reaction rate.
Though biotransformation rates apply to a specific combination of a chemical and a species, some general patterns are noted. For instance, accumulation of metabolisable compounds appears to be a factor of about 50 lower than that of persistent equivalents. This suggests that the underlying mechanisms may be (somewhat) more universal than usually thought. Perhaps, biotransformation potential of xenobiotic as well as biotic compounds has evolved according to similar principles. So far, such general mechanisms have hardly been investigated.
A preliminary approach to study the possible underlying mechanisms of xenobiotic and endobiotic metabolism can be the use of the Hansch relationship expressed in the following form:
$-\text{Log}\ K_m = a \cdot \text{Log}\ K_{ow} + b$
Here, K_m and K_{ow} represent the equilibrium constants for respectively the binding with an enzyme and the partitioning between octanol and water of the chemical. This linear relationship linking lipophilicity and metabolism was verified in studies on P450 drug metabolism.
In the present work, this equation was applied to enzymes involved in the metabolism (mainly in mammals) of endobiotics or xenobiotics, for example fatty acids, alcohols, and aldehydes. The $\text{Log}\ K_{ow}$-$\text{Log}\ K_m$ relationships were studied in order to understand the meaning of the regression coefficients obtained for various groups of compounds.
The final aim of this work is the prediction of K_m for different chemicals, based on lipophilicity and other fundamental physicochemical parameters (e.g. molecular mass, charge, etc.), thus providing a possible tool to quantify k_m for a better assessment of bioaccumulation.

TU 337
Study on the toxicity of perfluorinated compounds to aquatic organisms
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Because of their global distribution, environmental persistence and potential risk to human beings and ecosystems, poly- and perfluorinated compounds (PFCs) are of particular concern for research and regulatory communities. However, insufficient toxicity data are available to accurately assess their possible environmental hazard. Therefore, the toxicity of a number of structurally different PFCs for daphinds, algae, fish and lettuce was determined. It was found that the adverse effects of PFCs decreased with increasing fluorinated carbon chain length (nC).Smaller daphnid species tested were found to be more sensitive to these PFCs than larger daphnids. Toxicity patterns across species were found to be similar, offering possibilities for read across prediction of toxicity of either untested PFCs or untested aquatic organisms.

TU 338
Predicting respiratory sensitization of chemicals by accounting for their soft-hard electrophilicity
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In order for a chemical or its metabolite to induce respiratory sensitization, it must be able to bind covalently to proteins in a similar fashion to the formation of protein-hapten adducts that lead to skin sensitization. This hypothesis is supported by the high number of respiratory sensitizers that test positive in the local lymph node assay (LLNA) for skin. The main purpose of the present work is to discriminate the reactivity factors that result in skin and respiratory sensitization. Hard-soft electrophilicity of protein reactive sites in chemicals has been used as a means to categorize their interaction with nucleophiles of different hardnesses. Given the diversity of proteins in skin (Cys-, Lys-, and others), no specificity of electrophiles is anticipated with respect to skin sensitization. On the other hand, due to predominancy of Lys-nuclephiles in the lung, one would expect electrophiles with harder-electrophilic sites to cause respiratory sensitization. The reactivity module is combined with a simulator of lung metabolism. The specificity of lung metabolism as compared with liver/skin, such as upgrading oxidative reaction and downgrading reactions of reduction, alcohol/aldehyde dehydrogenases, etc. could modulate the effect of reactivity. The lung metabolism simulator produces metabolites of parent chemicals which are further filtered by the reactivity model. Development of an explicit nasal deposition is outside the scope of this work. Instead, we have focused on specific ranges of critical physicochemical parameters providing bioavailability information to the site of action. It is envisaged that the metabolism and reactivity components will be combined into an integrated model, using the OASIS software platform, to predict skin and/or respiratory sensitization.

TU 339
Hazard characterization and classification tool for complex inorganic materials
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Ores, concentrates, metal intermediates, slags and alloys are complex metal-bearing materials. These complex material streams from the metals sector are often characterized by a known but variable composition (variable depending on source and process). There is a need to reduce animal testing and to avoid costly standard laboratory tests on these material streams required by REACH and CLP while ensuring a science based hazard assessment. This poster presents MECLAS (www.meclas.eu), a hazard characterization and classification tool for complex inorganic materials. The ecotoxicological and toxicological hazards of such materials are estimated based on the hazard and the (if available, bio-available) concentration of the individual constituent species and/or minerals in the material. The background model is based on the CLP mixture rules and is estimating acute toxicity, skin corrosion/irritation, serious eye damage/irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity (for single and repeated exposure) and acute aquatic acute and long-term ecotoxicity. Knowledge on hazards of the various metal species ((self)classifications, (eco)toxicity reference values) were collected and stored in a background database. The tool's validity is based on an unambiguous conservative algorithm from EU CLP Guidance: summation/additivity formula to determine classification and, back-calculation to related hazard criteria. The applicability domain of the tool includes all complex inorganic containing materials. An example will finally demonstrate how elemental chemical analysis, mineralogical speciation analysis and bio-availability tests (transformation dissolution tests for environment, bio-elution tests for human health and surface correction) on source samples are inputted to the tool in a tiered way and how the hazard profile can be estimated for all endpoints mentioned above. In this way, it is demonstrated how complex inorganic materials can be assessed under CLP, animal testing can be reduced and costs can be saved.

TU 340

INES-plugin for refined environmental risk assessment of chemicals under REACh Regulation: improvement of the ECETOC TRA tool

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For REACh registration purposes, various tools have been made available to assess the risk of chemical substances for the environment. Among these tools, ECETOC TRA (latest version released in June 2010) is based upon a Microsoft Excel® format, as a core of 9 linked spreadsheets. It allows Environmental Risk Assessments of chemicals to be performed making use of Exposure Scenarios, following the recommendations reported in Chapter R16 of the REACh guidance (Environmental Exposure Estimation, May 2010). Environmental Release Categories (ERCs) and Specific ERCs (spERCs) are implemented in the interface of ECETOC TRA. The current version of the tool can use up to 24 scenarios per substance; this means that regional concentrations for each environmental compartment are calculated considering the sum of 24 use scenarios as a maximum. As a comparison, EUSES allows a maximum of 20 scenarios following production. This represents a serious limitation in the use of the tool for certain classes of substances: for instance, substances which are used in a sizeable number of applications (e.g. as solvents and fragrance products), cannot be properly risk-assessed using the available tools. The worst-case assessment of the Environmental risks performed with conventional tools may be too conservative, and may thus lead to recommend too costly Risk Management Measures (RMMs). For such substances, a second round of assessment, including less strict RMMs may be performed. CEHTRA developed INES-plugin (Increase Number of Exposure Scenarios-plugin), an internal macro function to allow hundreds of scenarios be taken into consideration (i.e. far more than 24 scenarios). This upgrade allowed to model in a more appropriate way regional concentrations for such substances. In the current version, the full description of uses can be extended to a maximum of 1920 use descriptions, outperforming in terms of refinement all other environmental risk assessment tools.
CEHTRA had the chance to carry out numerous substance assessments in the perspective of the REACh registration using the upgraded tool. For a number of other assessments related to substances, the uses of which being poorly described by a limited number of use scenarios, might benefit from an increased finesse inherent in the sophistication of the tool used to perform them. The poster compares and contrasts assessments performed by the CEHTRA-modified tool with assessments performed using available tools.

TU 341

Development of an ecotoxicological database on hazardous chemicals in accordance to REACH Regulation: the DESC example

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A software system was designed and developed for managing information regarding chemicals produced and/or imported in the UE market, in accordance to a REACH (EC Regulation No 1907/2006) goal to organize the available information on substances chemicals and to make them easily accessible to the public. To achieve this objective three operative steps were performed: requirement analysis, conceptual analysis and logical project.
The requirement analysis consisted of drafting an initial document (requirement document) shared and commonly agreed with the stakeholder, represented by the Italian Ministry of Environment, in which the system goals were defined. The document describes what the software system should do and what are constraints in its implementation. Firstly it contains a description of needs of the system functions and the way to achieve the strategic objectives previously set. Secondly it defines a glossary describing all the ecotoxicological technical terms used in the system. Finally the document outlines the requirements and describes the services. The main function of the software was that to archive information related to hazardous chemicals, subdividing into five classes: 'General Information', 'Classifications', 'Overview', 'Chemical-physical properties ' and 'Ecotoxicological properties and environmental distribution". The software was equipped with a default set of features for consultation and data query that allows users to analyze the entire dataset of information.
The conceptual analysis consisted of the construction of conceptual models representing the system requirements and was implemented by means of the UML (Unified Modeling Language), a standardized general-purpose modeling language for software engineering: three different graphs were developed to take in account the data structure and functionality of the system and to model the dynamic aspects of system behaviour over time.
The conceptual analysis evidenced that the best solution to create a software able to manage huge a amount of data on chemicals was that to implement a database. For this purpose an Entity-Relationship Model (ER) was chosen. Finally the logical project was realized by developing a client/server system.
The database and the website were implemented by using respectively Microsoft SQL Server Express 2005 and ASP.NET 2.0 technology and are currently available on the internet at http://www.dsa.minambiente.it/SITODESC/.

TU 342

MOPAC@home - an online database for small organic compounds

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Molecular descriptors derived from 3D structures, in particular quantum chemistry descriptors, are frequently reported as a useful representation of chemical compounds, that can significantly improve the quality of QSAR or QSPR models to predict physico-chemical and biological properties, as well as toxicity of chemicals.
Unfortunately, the results of these studies are often not traceable and thereby not assessable, as the 3D structures of the molecules, that were used to derive the models, are not always accessible. Another obstacle, that makes it difficult to work with 3D structures is, that computing a global minimum energy conformation can be very time expensive, especially for large and therefore flexible molecules.
We introduce an online database http://www.cadaster.eu/mopac that optimizes molecular conformations with the semi-empirical AM1 algorithm from the MOPAC7 package. For each molecule in the database not only one structure is available, but a whole set of them, derived from different initial conformations.
The database supports stereochemistry and provides several ways to access the conformations. Molecules can be examined by an interactive on-line interface, a batch of molecules can be uploaded and a web service is available.
There are two main features for accessing the results of the AM1 optimization:
1) Users can search the database to determine conformations with global minimum energy
2) User can retrieve optimized local minimum energy conformation starting from a user provided conformation.
Furthermore users can upload and add molecules to our database as well as also their specific conformations. This mechanism allows updating the global minimum conformations, if better ones are uploaded.
To enable this service, we developed a robust pipeline that generates initial conformers, optimizes them with MOPAC7 and calculates for each conformer a set of quantum chemistry descriptors (HUMO-LUMO energies, partial charges, dipole momentum, etc.). The structural optimization is done by a BOINC server, which distributes the work units to volunteers supporting the project.
Both the structures and the provided quantum chemistry descriptors can be an important contribution to advance environmental toxicity prediction and drug discovery. Furthermore, the optimized conformations produced by this database could be used as a reference for QSAR/QSPR models, which depend on 3D descriptors.

TU 343

ChemProp - chemical properties estimation software system

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The software system ChemProp comprises models to estimate compound properties and a database for chemical structures and properties, together with tools to manage compound sets.
The QSAR part addresses models for physico-chemical properties with particular remark to partitioning processes of environmental relevance and degradation, environmental fate, ecotoxicological endpoints, and human toxicology. It mainly employs models based on the topological matrix and thus does not require quantum chemistry. Particular remark is given to the applicability domain and model uncertainty.
The integrated database allows for structure and substructure searching, and addresses tautomerism and stereomerism. For effective data handling, it provides access to data in external resources in combination with the structure search facilities.
The software system is an essential part of the outcome from the EU Integrated Project OSIRIS (contract No. 037017), and is linked to the OSIRIS webtool. Respective financial support is acknowledged. In addition, it has been funded by the EU project 2-FUN (contract No. 036976)

TU 344

Life Project ANTARES - Alternative Non-Testing methods Assessed for REACH Substances

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The EU Regulation REACH which entered into force in 2007 in the EU represents today the most advanced system in the World to register, evaluate and authorize Chemical Substances. According to latest estimates some fourty or fifty thousand substances will be processed in 3 phases, starting with high production volumes (from 1000 t) and the most concerning substances. The deadline of the 1. phase has been 30 11 2010.
To correctly evaluate the impact on the environment and human health Industry will pay a high cost (billions of euros), and millions of animals might be sacrified to produce the necessary toxicity data. To limit these problems, REACH legislation promotes the use of non-testing methods, including Read Across and quantitative structure-activity relationship (QSAR). Read Across bases the evaluation of the unknown compound on the values of similar chemicals, while QSAR uses some chemical descriptors to evaluate the toxicity. Experience on the validity, predictivity and feasibility of these tools is still under discussion. ANTARES wants to effectively address the problem to check how to use the non-testing methods, for which endpoints and in which way.
In Action 1 ANTARES foresees on overview on current common methods to evaluate their practical availability, costs, number of animals, etc. to identify higher requirements for the alternative methods. For this Federchimica took actions to provide an overview of laboratories which can provide the current tests.
Results: Numerous contacts were launched with public and private laboratory structures in Italy. Most of the collaborating companies offer phys-chem testing, 7 laboratories offer toxicological and/or ecotoxicological testing. 12 Italian laboratories have indicated GLP certification. A well-known GLP certified European laboratory also collaborates in the project.
5 Italian companies offer tests for at least 50 % of Reach tox endpoints, 4 of them can conduct equal/more than 50 % of Reach ecotox endpoint testing. Some laboratory indicated in vitro/alternative testing capability.The non-Italian laboratory covers more than 90 % of all requirements, one Italian laboratory has same capabilities.The monitoring of the Italian laboratory situation for Reach testing will continuo to update the situation for GLP certification on tox and ecotox

testing activities/capabilities. The next registration deadline 2013 will involve many substances which need new studies compared to the 2010 registered substances.

TU 345

New developments in the OpenTox project

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The goal of the EC-funded FP7 project OpenTox (www.opentox.org) is to develop an open source-based predictive toxicology framework that provides a unified access to toxicological data and models. OpenTox provides tools for the integration of data, for the generation and validation of in silico models for toxic effects, libraries for the development and integration of modelling algorithms, and scientifically sound validation routines. OpenTox supports the development of applications for non-computational specialists in addition to interfaces for risk assessors, toxicological experts and model and algorithm developers.

OpenTox is relevant for the implementation of the REACH legislation as it allows risk assessors to access experimental data, (Quantitative) Structure-Activity Relationship ((Q)SAR) models and toxicological information from a unified interface that adheres to international regulatory requirements including OECD Guidelines for validation and reporting, and emerging guidelines from theEuropean Chemical Agency (EChA) and the European Joint Research Centre (JRC). The OpenTox framework is being populated initially with data and models for chronic, genotoxic and carcinogenic effects. These are the endpoints where computational methods promise the greatest initial potential reduction in animal testing required under REACH.

The OpenTox approach moves beyond existing attempts to solve individual research issues within this area, by providing a flexible, extensible, and user friendly framework that integrates existing solutions as well as providing easy access to new developments. For maximum transparency OpenTox is being organised as an open source community project. This allows a critical evaluation of the implemented algorithms, ensures a widespread dissemination and should attract external developers. Facilities for the inclusion of confidential in-house data and for accessing and integrating commercial prediction systems are also included.

TU 346

QSAR and QSPR models for emerging pollutants: WP3 activities within the FP7 European Project CADASTER

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The EU-REACH regulation encourages the use of alternative in vitro and in silico methods in order to minimize animal testing, costs and time. Among these, quantitative structure-activity relationships (QSARs) represent a useful tool to predict unknown activities/properties for existing or even not yet synthesized chemicals. The development and validation of QSAR models for four classes of emerging pollutants (brominated flame retardants, fragrances, perfluorinated compounds and (benzo)triazoles is the central topic of Work Package 3 (WP3) within the FP7 European project CADASTER (CAse studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment). The final goal of the project is to exemplify the integration of information, models and strategies for carrying out hazard and risk assessments for large numbers of substances, organized in the four representative chemical classes. The aim of this poster is to summarize the WP3 activities within CADASTER project and the QSAR/QSPR models developed so far for the four classes of compounds under investigation. This modeling activity involved different project partners in universities and research institutes across Europe (University of Insubria, Linnaeus University, IVL Swedish Environmental Research Institute, Ideaconsult Ltd. and Helmholtz Zentrum München), and was realized by different modeling approaches.

For each class, ad hoc QSARs were developed for all the available experimental data (i.e. physico-chemical properties, environmental and mammalian toxicity) in order to characterize environmental behavior and activity profile of the chemicals. In agreement with the OECD principles for the validation of QSARs for regulatory purposes, all the proposed models were checked for their robustness, external predictivity and applicability domain.

QSAR predictions, together with structural analysis (e.g. similarity analysis and multivariate ranking methods), were used for the identification of priority compounds (also among the ECHA pre-registration list) to optimize the experimental testing to be performed in WP2.

TU 347

Physico-chemical property prediction of emerging pollutants: PFC and (B)TAZ for environmental distribution

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Perfluorinated compounds (PFCs) are a family of chemicals with a long carbon chain which is predominantly substituted by fluorine. They are used in different materials as non-adhesives, waterproof fabrics, fire-fighting foams, etc. (Benzo)triazoles (B/TAZs) are another class of chemicals with multi-nitrogenated aromatic ring system. They are widely used in industrial processes, deicing agents (1H-BTAZs), pharmaceuticals and pesticides. These chemicals are considered as "emerging pollutants"as they are broadly distributed in the environment because of their extensive use and are considered to be hazardous as they cause adverse effects to humans and other non-target species. Their high concern as pollutants, lack of data and crucial Authorisation under REACH legislation urges for a need to maximize the information inherited in the existing data. Internally robust and externally validated QSPR models were developed for the endpoints, as also recommended under the REACH regulation, to predict large set of unknown properties for existing or not yet synthesized chemicals.

For PFCs, three QSPR models each on Water Solubility (WS), Vapor pressure (VP) and Critical Micelle Concentration (CMC) were developed and structural applicability domain (AD) study was verified. 174 (78.7%) compounds were found within the AD of all three models. The multivariate analysis by Principal Component Analysis (PCA) was performed for the prediction of these chemicals. In addition, Bioconcentration Factors (BCF) values were collected for most common PFCs and the relationship between the end-points was studied. The increasing trend of BCFs is in opposite direction to that of WS and CMC and the trend is found different for carboxylates and sulfonates.

For B/TAZs, four QSPR models each on WS, VP, KOW (Octanol/Water partition) and Melting Point (MP) were developed. 351 compounds were studied for structural AD study and out of which 66 are found within the AD of all four models for which at least one experimental data was available. These compounds were studied using PCA in a multivariate plot to understand their leaching and volatility behavior. Comparison with soil sorption partition coefficient (KOC) was performed by using predictions from earlier published models. More soluble, more volatile and more sorbed chemicals are highlighted. The 1H-B/TAZs were found to be among the more soluble and less sorbed compounds.

TU 348

QSAR prediction of aquatic and mammalian toxicity of triazoles and benzo-triazoles

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Triazoles and benzo-triazoles (TAZ/BTAZ) are potentially hazardous chemicals that adversely affect humans and other non-target species, and are on the list of substances of very high concern (SVHC) in the European regulation of chemicals REACH.

TAZ/BTAZ are synthetic molecules used in various industrial processes (to obtain pharmaceuticals and agricultural products), and have a wide application as anti-corrosives, cleansing agents for textiles, flame retardants, photographic emulsions, etc[3DOTS]Furthermore they are abundantly used as components of liquid deicing agents for aircraft and airport runways. Because of their wide use they have been found distributed throughout the environment, mainly in water compartments. The amount of experimental data available for these molecules is insufficient for a comprehensive characterization of their environmental and toxicological profile and they have been included among the four classes of chemicals studied in the European FP7 Project CADASTER (CAse studies on the Development and Application of in Silico Techniques for Environmental hazard and Risk assessment).

In this study we investigated and modeled by QSAR different endpoints of interest to define the potential aquatic toxicological profile of hundreds of TAZ/BTAZ, and the possible correlations among their aquatic and mammalian toxicity. The studied end-points were: LC_{50} in *Onchorhynchus Mykiss*, EC_{50} in *Daphnia Magna*, and EC_{50} in algae. Data for mammalian acute toxicity in rat (LD_{50} oral exposure) were also investigated and modeled. Different theoretical molecular descriptors were calculated by different proprietary and freely available online software (DRAGON, Hyperchem, and the CADASTER online platform for the calculation of molecular descriptors - www.cadaster.eu). The endpoints of interest were modeled by multiple linear regression (MLR), and the Genetic Algorithm was used to select the relevant molecular descriptors by the MLR-Ordinary Least Squares (OLS) method. The best models were validated for their predictive performance using leave-one-out, bootstrap and the scrambling of the responses. External validation was also performed depending on the dimension of the studied experimental datasets. The reliability of the predictions was always evaluated in order to verify the chemical applicability domain of the models.

TU 349

Prediction of uptake and elimination rates for polar organic compounds in aquatic species

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Ecotoxicological models are important tools to conduct environmental risk assessment. Ideally, the model should be based on mechanistic understanding of the processes, and parameters should be obtained by properties of the compound and species. Only this allows extrapolation to other species and chemicals.

Uptake and elimination are fundamental processes in most compartmental models. Uptake and elimination rates are commonly considered to be a function of organism mass, water/air flow through gills/lungs, and diffusion through water and lipid layers. The diffusion of organic chemicals through the lipophilic membrane depends mainly on the octanol water partition coefficient (Kow).

However, for more polar compounds (logKow<3) octanol is not a sufficient surrogate of the membrane, because other characteristics such as e.g. hydrogen bonds become more important. Descriptors accounting for polar characteristics have been included in many QSAR approaches predicting the absorption of pharmaceuticals. These QSARs focus on absorption through membrane layers, hence absorption through mammalian intestine, blood brain barrier and skin by passive diffusion.

In addition to passive diffusion, carrier mediated transport can become relevant, if the xenobiotics can use transport systems of endobiotic compounds. Uptake of pharmaceuticals is often a mixture of passive diffusion and carrier mediated transport.

The objective of this study is to improve the prediction of uptake and elimination rates of polar compounds on organism level with a focus on aquatic organisms. The approach consists of improving the prediction of 1) passive diffusion and 2) carrier mediated transport. Prediction of passive diffusion may be improved by incorporating descriptors accounting for polar characteristics and molecular volume. The influence of carrier mediated transport will be examined by investigating kinetics and affinities of selected biotics and their transport proteins and extrapolating the findings to structural similar xenobiotics of relevant environmental concern. The estimation of rates for passive diffusion and carrier mediated transport will be based on datasets of compounds with a logKow < 3, i.e. uptake of selected pesticides and industrial chemicals into aquatic organisms, and absorption of pharmaceuticals through mammalian cell lines.

TU 350

How useful are QSARs within a regulatory framework? Validation and applicability domain assessment of QSARs related to PBT endpoints.

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The regulatory use of Quantitative Structure Activity Relationship (QSAR) models is expected to increase under chemical management systems such as REACH. In silico model predictions are a time & cost-effective alternative for animal testing and benefit animal welfare. In 2004 the OECD responded to the need for guidance on QSAR application by developing acceptance criteria for the validation and regulatory use of QSARs, which were also adopted in the REACH guidance. Of particular interest are the criteria about the external validation to determine the true predictive power of a model and about the applicability domain (AD) since predictions are most reliable if they fit the model's AD. Six models predicting PBT (Persistency, Bioaccumulation, Toxicity) endpoints were selected for external validation and AD assessment. The evaluated P models were BIOWIN and MultiCase; the B models were BCFWIN and CAESAR; and the T models were ECOSAR and MultiCase. Validation set chemicals (79 substances) data were selected from Risk Assessment Reports of the European Chemicals Bureau and others. The AD assessment of the models was done using structure similarity methods of Ambit Discovery. In the validation of P models, a combination of BIOWIN 3 and 5 models performed the best: precision ranged between 80-92%, predictive value between 67-96%, false negative and positive ratios were 8% and 20%, respectively. Highest values for precision (92%) and predictive value (96%) were obtained for not ready biodegradable chemicals. In the validation of the B models, it was concluded that both CAESAR as BCFWIN did not pass the criteria for regulatory use (false negative ratios >50%). The validation of the T models showed that the chronic ECOSAR model for fish can serve as first screening tool in risk assessments. The acute ECOSAR and MultiCase models did not pass the regulatory criteria. With respect to the AD assessment, The used structural similarity methods were not capable to completely capture the mechanistic basis of the models. These findings indicate the need to develop a global similarity test to determine whether the structural features in a validation chemical are covered in the training set of a QSAR. Wider application and acceptance of QSARs for regulatory use will require further model development and more thorough validation and AD assessment. The large amount of high quality experimental data that becomes available within REACH can be used to achieve these requirements.

TU 351
Comparison of approaches to define Applicability Domain for the application of QSAR models
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QSAR validation is a critical phase to analyse performance of the models and interpret their mechanistic relevance. One of the five OECD principles for QSAR validation requires implementing a defined domain of applicability for a given QSAR model. This allows a better understanding of how reliable the model predictions are within a given chemical space. In other words, the predictions that fall within the model domain of applicability are reliable and the ones outside refer to extrapolations.
There are several approaches already developed till date to define applicability domain for QSAR models, based on structural aspects or mechanistic understanding, however most of them offer several advantages accompanied with some major limitations. Moreover these approaches cannot make any decisions on their own unless an expert judgement is involved. The idea of this poster presentation is to provide a brief overview of such commonly used approaches to define the applicability domain, addressing their basis of interpretation, algorithm implemented and the reliability they offer in terms of their approach. We made an effort to compare the features of these approaches on simulated and real QSAR datasets to provide a better scenario of existing situation and to address the current drawbacks that need to be considered in the future.

TU 352
The use of (Q)SARs as part of a larger registration strategy for REACH
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The European Union REACH regulations require the submission of detailed information on the physico-chemical, toxicological and ecotoxicological endpoints of chemicals. For the last few years, WRc has been heavily involved in REACH, aiding companies and consortia to develop registration strategies, commission laboratory testing, collate and evaluate data, fill data gaps and prepare dossiers for registration.
One of the methods employed to fill such data gaps is (Quantitative) Structural Activity Relationship ((Q)SAR) modelling. (Q)SAR modelling requires the identification of chemicals of structural similarity to the chemical of interest. Data on the toxicity of these structurally similar chemicals is then used to predict the likely toxicity of the chemical of interest. These results can then be used for a number of purposes:
- To guide experimental testing strategies;
- To provide mechanistic data to support chemical grouping;
- To improve the evaluation of existing data as part of a weight of evidence approach; and
- To potentially provide a full replacement for toxicity testing.
However, it is not simply enough to generate a prediction using (Q)SAR models. Consideration must be given to the robustness, reliability and appropriateness of these predictions. To this end, the Organization for Economic Cooperation (OECD) has derived guidance on the use of (Q) SARs, which state that (Q)SARs must include the following characteristics:
- A defined endpoint, i.e. the endpoint should be something that can be measured experimentally such as an LC50 or an EC50.
- An unambiguous algorithm, this is to ensure transparency in the description of the model algorithm. However, the OECD has acknowledged that this may not be possible in commercially-developed models.
- A defined domain of applicability, i.e. the limitations of the model should be clear.
- Appropriate measures of goodness-of-fit, robustness and predictivity.
- If possible, a mechanistic interpretation.
This poster presentation will demonstrate several examples where (Q)SARs have been employed as part of a larger strategy to aid clients with REACH registration. It will include examples where we have considered the application of these predictions to be appropriate, and examples where the use of (Q)SARs were not considered to be appropriate for the chemical and/or endpoint of interest, and the reasoning behind such opinions. In the interests of client confidentiality, specific chemical names will not be included.

TU 353
Exposure assessment under REACH: the backward approach
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Chemical safety assessments under REACH require the lead registrants to gather detailed information on the uses in their supply chain in order to demonstrate safe use for the environment and human health. This has shown to be a rather difficult and elaborate procedure which often results in little options for refinement. In order for the lead registrant to be compliant with the REACH requirements, a practical solution is to apply a backward approach for the environmental exposure calculations.
The backward approach aims at defining the maximum allowable daily discharge to the environment and is partly an iterative procedure. The steps determining the iteration involve the selection of the most sensitive environmental compartment, the application of risk management measures (RMM), and an assumption regarding the regional exposure estimate. Due to the lack of refinement data, many exposure scenarios require the application of a number of generic RMMs to guard safe use, and these measures not only influence the calculations but also affect the legal obligations for downstream users. Practical experience has shown that this backward method in many cases offers a good solution to move forward when communication in the supply chain ceases or a generic approach is more appropriate (e.g. when numerous members take part in a SIEF or consortium).

TU 354
Development of methodologies for risk trade-off analysis toward optimum chemical substance management
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Chemical substance management in Japan is shifting from regulation and management based on the degree of harmful effects toward management based on risk assessment. Management based on risk assessment involves seeking low-risk alternative substances and making maximum use of the benefits of chemical substances, as well as seeking to keep risks within an acceptable range. However, if appropriate risk management does not take place, risks other than the initial ones faced when selecting an alternative substance could emerge, thereby countering the risk reduction effect (i.e. a risk trade-off emerges). Nevertheless, with regard to current risk assessment technology, the exposure information required to evaluate many chemical substances is inadequate, and quantitative comparison of risks among different chemical substances is currently difficult. Given the above, this project aims to develop risk trade-off analysis techniques that businesses can utilize for highly precise quantitative analysis of chemical substance risks. The new techniques will also enable businesses to compare and consider the various common indicators of each risk as well as select appropriate alternative substances. In this presentation, we will introduce the conceptual framework of the project along with a case study on brominated flame retardants (BFRs).

RA13 - The future of ecotoxicological risk assessment - biological traits, ecological vulnerability, improved SSDs, indirect ecological effects

TU 358
Time-dependent SSDs
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In ecotoxicology, endpoints are generally estimated at the end of some pre-determined exposure period (eg. 4-day fish survival, 21-day Daphnia magna reproduction, etc). The analyses focus solely on the response as a function of concentration. However the response is generally a function of both time and concentration and ignoring the time dependency can lead to severe bias in environmental risk assessment (Baas et al., 2010, Heckmann et al., 2010). For aquatic organisms, it is usually possible to test its response at intermediate points in time. For effects on mortality or reproduction, it is part of the standard protocol, however these data are usually not used in subsequent data analyses.
The species sensitivity distributions (SSDs) has become a key instrument in water quality guidelines. While the technique is regarded by most as a significant improvement on the use of safety factor, it is not without its problems and limitations. One of the most severe shortcomings is its reliance on the largely discredited NOEC. As an alternative to the conventional NOEC-based analyses, Fox (2010) has recently described a model-based Bayesian method for the estimation of no effect concentrations (NEC) and hazardous concentrations (HC). We extend this approach by adding the time dimension into the models. Using time-series data sets, we investigate the changes in NEC over time, and their impact on the HC value and uncertainty.
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TU 359
One SSD model, three HCp estimators: but which is better?
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The species sensitivity distribution (SSD) model is firmly embedded in the regulatory arena as a method to derive the so-called 'predicted no-effect concentration' for a defined species assemblage exposed to a toxic stressor.
The REACH technical guidance document (TGD) (ECHA, Guidance on Information Requirements and Chemical Safety Assessment), states that the log-normal SSD "is a pragmatic choice", an assumption that has become commonplace in the ecotoxicological risk assessment community. The best way to fit a log-normal SSD for purposes of hazard assessment, on the other hand, is confusing. The sought-after quantity which intermediate ('Level 2' within the REACH TGD) risk assessments are based upon is the hazardous concentration to 5% of the defined species assemblage (the HC5).
A standard approach is to estimate a median of the HC5 from the sampling distribution. This estimator has well understood statistical properties by construction. However, two alternative estimators - also based on a log-normal SSD - frequent the risk assessment literature. These estimators are constructed by least squares estimation of the ordered logarithmically transformed

toxicity data modelled onto the corresponding plotting positions (cf. quantile plots). Standard hypothesis testing and diagnostics of the linear regression are inappropriate without further constraints (cf. generalized least squares).
We consider evaluating which estimator, subject to the log-normality assumption, exhibits the best performance. The problem reduces to a fundamental problem of how to measure the performance of an estimator. This can be done by (1) 'discrepancy' between the estimator and 'true' value, or (2) 'discrepancy' between the true potentially affected fraction of species and the intended level.
Evaluation of different 'standard' criteria (variance, bias, etc.) under the perspective of (1) indicates that the median estimator performs better for all reasonable samples sizes. For (2), the results concur on important scales of discrepancy. However, this performance is highly sensitive to the chosen criterion/scale and sample size. We conclude that the median estimator is preferable and that controversy could be overcome by a risk assessor reporting probabilistic distributions for risk managers in a Bayesian framework in addition to summary statistics; the median estimator is known to be a special case of this.

TU 360

Acute toxicity of organic chemicals to Gammarus pulex correlates with sensitivity of Daphnia magna across most modes of action

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We investigate the sensitivity of the freshwater crustacean amphipod Gammarus pulex towards organic xenobiotic compounds in comparison to the sensitivity of the crustacean cladoceran Daphnia magna. Also we studied the influence of the chemical's mode of action on the relationship between the sensitivity of G. pulex and that of D. magna. We tested the acute toxicity of twelve compounds (Malathion, Aldicarb, Carbofuran, 2,4-Dichloroaniline, 2,4-Dichlorophenol, 1,2,3-Trichlorobenzene, 4,6-Dinitro-o-cresol, 2,4,5-Trichlorophenol, Ethylacrylate, 4-Nitrobenzyl-chloride, Sea-nine, Imidacloprid) with different modes of action and physicochemical properties towards the freshwater amphipod G. pulex in laboratory experiments. Additional toxicity data was collected from literature and databases (data pairs for 44 chemicals in total). The chemicals were assigned to seven mode of action groups. The relationship between the sensitivity of G. pulex (48h-LC50s and 96h-LC50s) and that of D. magna (48h-EC50s) was investigated using regression analysis and correlation plots.
G. pulex is two to three orders of magnitude more sensitive towards neonicotinoids than D. magna. For organophosphates we find that D. magna is more sensitive than G. pulex by approximately a factor of six. None of the other mode of action groups exhibited a significant difference between the sensitivity of D. magna and that of G. pulex, however chemicals with the same mode of action grouped together in the same area of the correlation plot.
For the whole dataset, with the neonicotinoids included, 68% and 93% of all G. pulex toxicity data are within one and two orders of magnitude of the D. magna data, respectively. Without the neonicotinoids 75% of all G. pulex toxicity data are within one order of magnitude of the D. magna data and 100% within two orders of magnitude.
The regressions with all data and with all data minus neonicotinoids were both significant linear relationships with slopes around one. When neonicotinoids are excluded the regression resembles the 1:1 line very closely. As the slope is around one and the intercept around zero we conclude that G. pulex is generally equally sensitive towards organic xenobiotics as D. magna. Such a simple prediction rule would predict sensitivity of G. pulex based on that of D. magna within one order of magnitude for 75% of all compounds and within two orders of magnitude for 100% of the compounds in our dataset, if neonicotinoids are excluded.

TU 361

Trait-based sensitivity assessment of specific toxicants: getting deeper into specific traits

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Assessing the sensitivity of different species to chemicals is one of the key point to predict the effects of toxic compounds in the environment. Trait-based predicting methods proved to be extremely efficient in order to assess the sensitivity of macroinvertebrates toward compounds with non specific toxicity (narcotics). Nevertheless predicting the sensitivity of organisms toward compounds with specific toxicity is much more complex, since it depends on the mode of action of the chemical.
We tried to predict the sensitivity of several freshwater macroinvertebrates toward two classes of plant protection products: organophosphates and pyrethroids. We built two databases: one comprehend sensitivity data (retrieved, evaluated and selected from the EPA ECOTOX database) and another one which collects biological traits. Aside from the "traditional"traits usually considered in ecological analysis (i.e. body size, respiration technique, feeding habits, etc.), we use multivariate analysis to relate the sensitivity of organisms to some characteristic (physiological, biochemical, etc.) which may be involved in the process of intoxication. We investigated for example whether the complexity of nervous system is relevant to assess the effects provoked by neurotoxicants like organophosphates and pyrethroids.
Results show that biological traits could be used to predict sensitivity to toxic substances, although more studies are needed to provide sound predictive methods. One key point to achieve a complete mechanistic understanding of the process is the choice of traits, whose role in the discrimination of sensitivity should be clearly interpretable, and not only statistically significant. A relevant drawback is the lack of information on the traits with the necessary taxonomic detail.

TU 362

Main factors driving population dynamics of a pollution indicator species

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The marine deposit-feeding polychaete *Capitella capitata* is a classical re-colonizing species frequently used as an indicator of habitat disturbance (natural and anthropogenic). It is now recognized that *Capitella Capitata* is in fact a complex of sibling species of which some species are very opportunistic whereas others are both less mobile and less tolerant to disturbance. Some of these sibling species have been extensively used for both laboratory experiments and field surveys, though in the field different sibling species are indistinguishable based on morphological characteristics alone. With a combination of various types of laboratory experiments and model simulations the present study aims to pinpoint the most important factors driving the dynamics of field populations of *Capitella* spp., in order to focus further research, monitoring and management of disturbed areas. The importance of larval dispersal ability and settling behaviour was explored through both a matrix metapopulation study and targeted settling experiments. With increased focus on incorporating more ecological relevance into ecotoxicological studies, we designed experiments to mimic and explore the boom-bust dynamics of *Capitella teleta*. Our results emphasize the influence of dispersal ability and settling behaviour on the population dynamics of classical re-colonizing polychaetes and highlight the importance of understanding the principal factors driving re-colonization of disturbed sediments. Not surprisingly, food availability seems to be the main driver for both larval dispersal and settling behaviour as well as the main determinant of density dependence in *Capitella* spp., and is more important for settling in organically contaminated areas than is the presence of sediment associated contaminants at ecologically relevant concentrations. The take home message from this study is that in order to extrapolate to field populations for risk assessment and management purposes, it is necessary to choose representative model species for which we understand and can incorporate key aspects of their ecology and biology. Such knowledge can help to focus testing and research on endpoints that are more relevant for effects on populations.

TU 363

Ecotoxicological research in terms of sensitivity distinctions of lotic insect larvae with Imidacloprid

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Abstract
The contamination of running or stagnant waters with harmful substances, in particular insecticides, can be very dangerous for macroinvertebrates. Despite the fact that aquatic insects represent the most important and vulnerable group of organisms inhabiting streams and rivers, there are only a few species that have been used routinely in bioassay studies. Against this background the sensitivity of lotic Ephemeroptera, Trichoptera and Plecoptera larvae was examined. Due to the fact that the neonicotinoid Imidacloprid is one of the most used insecticides worldwide it was selected as the test substance.
The acute toxicity of Imidacloprid was determined on different larvae species and larval stages. The test period was 96 h in M4 medium. The test was conducted with 5 different concentrations with 5 replicates each and 10 replicates for the control. After acclimatisation of the larvae in M4 medium, they were transferred to 100 ml beakers. Depending on their size, 1-5 larvae were inserted in each beaker. Each beaker was aerated to ensure the oxygen saturation of the medium.
The first results show a high sensitivity of the larvae towards Imidacloprid. The EC50 after 96 h for Epeorus assimlis is 5,06 µg/l, Habrophlebia lauta 31,18 µg/l, Cloeon dipterum 43,33 µg/l, Baetis rhodani 5,21 µg/l and Hydropsyche spec. 23,07 µg/l. This indicates that the various species differ in their sensitivity towards Imidacloprid. The different larval stages within on species seem to react distinct towards Imidacloprid.
All the tested species are more sensitive compared to Daphnia magna (239,07 mg/l after 48 h). Chironomus tentans shows an EC50 of 5,75 µg/l (Stoughton et al. 2008) and C. riparius 12,94 µg/l after 96 h (Pestana et al. 2009). Nevertheless Chironomus spec. are sediment dwellers and therefore are well suited organisms for sediment bioassays but less suited for other types of assays. These first outcomes show the importance of using not standard organisms for a better assessment of the situation in the field. It is expected that limnic macroinvertebrates, present in the water at the time of application, will react more sensitive to pesticides than standard organisms in the laboratory do. For example Imidacloprid can be found at concentrations of 1-14 µg/l in surface water (Jemec et al. 2007). Compared to Chironomus, the larvae of Ephemeroptera, Trichoptera and Plecoptera are better suited to reflect the stream and river ecosystem.

TU 364

Resistance to chemical contamination in Daphnia longispina: consequences on the phenotypic response to predation risk.

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Natural populations historically exposed to low levels of chemical contamination may evolve resistance to that particular contamination. Theories on adaptive changes predict that the acquisition of such resistance may involve fitness costs, which can be considered in terms of energetic and resource allocation.
On the other hand, aquatic species rely on plastic traits to defend themselves against natural stressors like predators. The induction of these antipredator defences is mediated by water-borne chemical cues. These induced defences also have associated costs which can include decreased tolerance to future environmental change such as contamination
Because both acquisition of resistance to contaminants and phenotypic plasticity can involve fitness costs , the present work intended to study if an increased resistance to copper on the cladoceran Daphnia longispina O.F. Müller is associated with loss of plasticity in terms of the response to fish predation rik . To attain this objective, four cloned lineages of D. longispina, exhibiting different sensitivities to lethal levels of copper, were exposed to three levels of predator-release kairomones from Gambusia affinis: 0.25, 0.5, and 1 fish/l, plus a control (solely ASTM). Life cycle parameters (time to release and number of neonates at first brood, total number of neonates per female, growth, and intrinsic growth rate), feeding inhibition, and respiratory rate, were monitored for each cloned lineage. A similar pattern of response, for all the measured endpoints, was observed for the two lineages most sensitive to copper. And, this pattern of response was different from that of the two resistant lineages. However, though differences in the monitored endpoints were observed between the sensitive and resistant D. longispina lineages, a significant association between responses to kairomones and resistance to lethal levels of copper was not observed.

TU 365

Trait-based approaches for the assessment of agricultural impact on invertebrate communities of Swedish streams

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We used a data set of benthic invertebrates from 200 streams in south Sweden to test the use of trait-based approaches for the assessment of multiple stressors originating from agricultural practices in the catchment (and assumed pesticide impact). Agricultural land use for the streams varied from 4-95% of the catchment, thus providing a data set reflecting a gradient from largely unimpacted, forested catchments to those with an almost exclusively agricultural land use. Moreover, field-level information on crops and expected use of pesticides were used to estimate pesticide impact on these streams. Multivariate methods were used to identify gradients in community composition and to test for correlations of observed community changes with proxies for the impact of agricultural land use on stream integrity. Traits that corresponded with these environmental gradients will be discussed. We test the power of a suite of biological metrics, including the trait-based SPEAR and taxonomy-based EPT and pollution metrics, to quantify the integrated

impact of agricultural activities on stream invertebrates. An attempt is made to distinguish the impact of pesticides from those caused by habitat degradation, using a suite of habitat descriptors extracted from field protocols. Lastly, we identify important landscape elements (from GIS) that contribute to the vulnerability of streams.

TU 366

Susceptibility of different life stages of Folsomia candida (Collembola) to hydrophobic organic compounds

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Hydrophobic organic compounds (HOCs) represent important component of soil contamination and poses a great threat to ecosystem function and human health. Since soil ecosystems are often exposed to organic pollutants, it becomes important to evaluate the risk of these chemicals to soil organisms and populations. For that purpose, several standard toxicity tests with soil organisms are already established. The collembolan Folsomia candida is a common soil invertebrate, used in standard ISO (11267) and OECD (232) toxicity test guidelines. Prevalently chosen endpoints in these tests are the EC50 concentrations for reproduction (50 % reduction in reproduction) for 10-12 day old animals. Subsequent analysis like microarray gen expression profiles are often based on these calculated EC50. However it remains unclear which life stage is the most sensitive. In the development of soil invertebrates the physiology and the morphology changes rapidly. Standard test can't reveal if the toxic effects applies to eggs, young hatchlings or adults. In this study 2-5 day old eggs and two different age classes (2-5 days and 26-28 days) of Folsomia candida were exposed to three different hydrophobic organic compounds (phenanthrene, 1,2,4,5-tetrachlorobenzene and 2,3,5,6-tetrachloroaniline). In earlier standard tests these compounds showed different effective concentrations based on their logKow. Phenanthrene and 1,2,4,5-tetrachlorobenzene both showed baseline toxicity whereas for 2,3,5,6-Tetrachloroaniline evidence grows to have an uncoupling effect. Natural standard LUFA 2.2 soil was used as substrate. Survival, reproduction and hatching success were evaluated after 28 or 35 days, respectively. The endpoint concentrations were calculated with a log-logistic model. Over test period total soil concentration were extracted from subsamples and were measured by HPLC. The obtained results showed high differences among life stages and among compounds when compared within the test and with results from standard toxicity tests We conclude that for future ecological risk assessment the life stage analysis should be taken into account.

TU 367

Selected biological traits reveal soil invertebrate exposition to trace metal contamination

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For decades, human pressures have threatened the ecological integrity of soils. The draft Soil Framework Directive (COM(2006) 232) has listed the main eight soil degradation processes to which soils in the EU are confronted, of which soil biodiversity loss and contamination. These two aspects have been successfully monitored in stream ecosystems using invertebrate biological traits. In this context, we aimed at developing a similar approach for soil monitoring.
In the present work, we aimed at elucidating soil macro-invertebrate response to trace metal (TM) contamination, in term of composition and functional role. Soil macro-invertebrates can be exposed to trace metal either by ingestion or by contact; they can also avoid to be exposed by escaping. We proposed to evaluate these three ways using a trait-based approach. We assumed that the type of exoskeleton, the diet of invertebrates and the wing morphology are related to the way invertebrates are exposed to pollutants. So we hypothesized that the proportion of (i) soft-body, (ii) geophageous and (iii) wingless invertebrates decrease as trace metal concentration increase in soil. Geophageous invertebrates are also key in terms of soil aggregation and organic matter turn over. We selected five plots located in the 1500-ha-wide agricultural plain (Pierrelaye-Bessancourt, France). This plain has been contaminated by irrigation with raw wastewater of Paris city for 100 years. As a result, the contaminated soils display strong and heterogeneous accumulation of organic matter, metal pollutants and dissolved salts in surface horizons. In this agricultural plain, high values of soil metal contents have been recorded, e.g. up to 1500 or 15 mg kg-1 dw soil for Zn or Cd, respectively
The present work demonstrate that functional trait approach allow to discriminate three different responses at soil contamination increase. The negative linear relationship of soft-body invertebrates to TM concentration reveals an additive effect of contamination. This functional trait is very sensitive since difference was observed between communites in uncontaminated and weakly contaminated plots. The response of geophageous or wingless invertebrates reveals a threshold effect, but at different soil contamination level. The lack of geophageous invertebrates can imply drastic losses in soil functioning, ie soil aggregation, porosity or organic matter turn over.

TU 368

Potential application of traits-based bioassessment approaches for use in the study of multiple stressor impacts on freshwater ecosystems in the Athabasca oil sands region (Canada)

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The Mackenzie River Basin headwaters (Canada), including the Athabasca and Peace River watersheds, is currently facing significant environmental challenges from a range of human activities, including, but not limited to: bitumen mining of surface and subsurface oil sands deposits, currently operating and future hydro-electric schemes, agriculture and small-scale urbanization. The major stressors to freshwater communities in the area are thus hydro-carbon concentrations, changes in pH values, and reducing water availability. As commonly observed at the global scale, the co-occurrence of these major stressors is common in the area and it constitutes a great management challenge. Biological monitoring systems has not been widely implemented in the oil sands region (with the exception of the Regional Aquatics Monitoring Program), although there is a desire to expand their use within the region. One potential future application of biomonitoring data collection is their use in the development of specific diagnostic indicators of environmental stress. The single stressors diagnostic development is particularly needed where, as in the oil sands region, co-occurring, and potentially co-acting stressors, have to be ranked in terms of their ecological importance with the goal of identifying drivers of any observed ecological change. It has been recently recognized that the linkage of trait responses to stressor gradients can enhance causal assessment and thus support the development of new generation diagnostic tools. A review of available traits-based approaches for developing single stressors diagnostic was undertaken and it gave useful indication on potential pathways to develop comparable indicator of oil sands related stressors i.e. hydro-carbon contamination. The review has been focused on identifying: 1) available biomonitoring data in the region; 2) natural sources of variability and major anthropogenic stressors affecting biological communities; 3) available trait information and 4) traits-based approach for bioassessment with focus on oil sands region. Based on this review, final consideration on potential to develop traits-based diagnostics to tease apart multiple stressors affecting the biological communities in the Mackenzie River Basin headwaters were drawn.

TU 369

Effects of tebuconazole on the structure and metabolism of stream microbial communities

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Streams are often subjected to diffuse pollution, especially in extensive vineyard areas where the use of plaguicides is frequent. The main objective of this study was to determine the effects of Tebuconazole (fungicide, TBZ) in the structure and metabolism of algal and bacterial communities forming biofilms. Biofilms colonized in reference (Saint Joseph, SJO) and polluted (Saint Ennemond, SEN) stream sites from la Morcille stream (Beaujolais region, Eastern France) were exposed to two different TBZ dosages (2 microgTBZ/L; 20 microgTBZ/L plus controls). Biofilm structural (algal and bacterial abundances and community composition) and functional descriptors (primary production, respiration, metabolic diversity) were surveyed during a 22-days experiment by using artificial stream channels. We observed a decrease in the proportion of live/dead bacteria in biofilms from the two study sites (SJO and SEN). However, decreases in bacterial respiration and photosynthetic efficiency were only consistent in biofilms from the reference site (SJO), but not in those from the polluted site (SEN). The Biolog EcoPlates analysis determined that moderate dosages of TBZ (2mg/L) may homogenize the metabolic diversity between reference and polluted communities. TBZ inputs in streams may have consequences in ecosystem processes, such as the organic matter decomposition, since it decreases bacterial activity. But also, TBZ decreases the photosynthetic efficiency, and therefore, the potential primary production in the stream environment. Our results confirm that the TBZ (fungicide) has indirect effects on algal and bacterial communities, especially in those communities that has not been previously exposed to this toxicant.

TU 370

A new ecotoxicological module for water quality assessment and monitoring

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The European Waterframework Directive (2000/60/EC) aims at achieving a good ecological and chemical status of water bodies in Europe until 2015. Whereas the ecological status assessment builds on different moduls, such as hydromorphology, macroinvertebrates, aquatic macrophytes/algae and fish community assessment, the chemical status is defined as compliance to environmental quality standards set up for selected priority and dangerous substances, which are being reviewed regularly. We propose a new ecotoxicological module bridging between the biological and the chemical approach. Based on exisiting data for macroinvertebrates for the biological module, the SPEAR-index (SPecies At Risk) provides an initial screening tool. In case the SPEAR-Index indicates problematic values at a site, a thorough ecotoxicological assessment should be performed in a second step. In this step we propose GamTox, the new multi-metric and multi-level ecotoxicity test for application both in the laboratory and in situ. GamTox can be performed as short-term acute test, as long-term chronic test and in continuous online biomonitoring with the automated Multispecies Freshwater Biomonitor®. Different parameters can be recorded in the animals, such as survival, behaviour (locomotion, ventilation, feeding, precopula), reproduction and mode-of-action related biomarkers, such as MTs (metals) and AChE (neurotoxins). The international Gammarus working group is currently validating and standardizing GamTox.

TU 371

Ecological vulnerability and degradation of chernozems in Republic of Moldova

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Moldova is characterized by very complex of soil cover. Main soils are chernozems, 70% of the land surface. Land reform in Moldova has not created conditions for increasing soil fertility, sustainable land use, increasing agricultural production, exerting therefore negatively impact the country's economy. In this context Moldova risks to lose the greatest wealth of country - chernozems fertility. Soil quality and vulnerability of most agricultural land is poor and a part of land - critical. The most serious forms of soil degradation are erosion, dehumification, and impoverishment of nutritive elements, salinisation, secondary compacting, and active land sliding. These forms and processes decrease soil production or destroy completely the soil cover. The ecological damages are also significant: depletion of agricultural fields, biological degradation, surface water pollution with nutrients and organic substances. Erosion is the main factor of soil degradation. According to soil surveys, soil eroded area has increased during 40 years from 594 000 ha in1965 to 878 000 ha in present, increasing annually by 7100 ha. Depending on the degree of erosion eroded soil fertility decreases in the following: weakly eroded - by 20%, moderately eroded - 20-40%, strongly eroded - 40-60%, very strongly eroded - 60-80%. The greater the degree of soil erosion, the greater is their vulnerability to this factor. During the 100 years of exploitation of soil in agriculture the humus content decreased by 35-45%. Losses depend on the speed of humus mineralization (dehumification) and erosion processes. Soil humus balance is negative - minus 0.7 t / ha. The total annual agricultural land lost 2.4 million tons of humus. Natural conditions in Moldova ranks among the primary tasks of irrigation, especially for the South, which is the most vulnerable to climate conditions, where the immersion factor is 0.5 to 0.6, and droughts are repeated at a frequency of three years. Currently, irrigation of agricultural land is carried out primarily by local sources of water (inland rivers, lakes, ponds) that is characterized by high degree of mineralization, alkaline and chemically adverse reaction. As a result, there is secondary soil salinisation and solonețizarea, which contributes to increased ecological vulnerability. The existing land use has led to compaction of the arable layer. Recently plowed chernozem layer of structure is characterized by massive structural elements strongly compacted.

TU 372

Do pesticides affect trends in microarthropod communities according to food preferences as indirect effect?

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In agroecosystems the use of plant protection products is common practice. During the applica-

tion a fraction of these products reaches the soil, making the soil community exposed to different pesticides. These products have been developed to kill the target organisms (fungi, plants or insects), but may give adverse effects also on non-target species. The effects can be direct on the species, or indirect, due to the effect of the pesticide on another organism (e.g. prey or predator). An example is the effect of a fungicide on fungi eaten by fungivorous animals.
These possible indirect effects were studied in the field, by investigating a microarthropod community exposed to plant protection products in a vineyard in Northern Italy. Three sampling points were chosen, in a gradient of contamination: one inside the field, and two 4 m and 10 m away from the last plant row. The community was sampled in a sampling scheme drawn according to the insecticide applications. In particular, to evaluate indirect effects, three samples were examined: two after the application of herbicides and fungicides but before application of the insecticide chlorpyirifos, and the third after chlorpyrifos application. All the information about the active ingredients used was obtained and the exposure of the community was assessed with traditional ecotoxicological tools. The sampled organisms were identified at least to the order level, but for some taxa to the family level, or a division into major groups was made. Information on the diet was obtained from the literature to divide the different taxa into groups according to their food preferences. Results were related to the control 10 m away from the field. The hypothesis was that before the use of chlorpyrifos, there could be indirect effects on the abundance of organisms due to the elimination of food sources, while after chlorpyrifos application the direct effect of the insecticide should become visible.
Different trends were observed in the community, according to the food preferences of the taxa: bacteriophages, for example, showed a decrease between the two dates before chlorpyrifos application, while the numbers of fungivorous, phytophagous and detritivorous organisms were almost constant. Coprophagous, necrophagous and secondary consumers showed a constant decrease during the entire productive season. The ratio between indirect and direct effects was highlighted, demonstrating that direct effects overshadow the indirect ones.

TU 373
SPEAR application on a small-scale scenario
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Despite numerous studies have dealt with the topic, the real extent of the effect caused by pesticides loads in freshwater communities is still largely unknown.
One of the main problem is to distinguish between natural and anthropogenic environmental factors, as well as between different anthropogenic factors. Species at risk (SPEARpesticides) index uses biological traits of organisms to determine whether organisms are vulnerable or not to pesticides exposure. The SPEARpesticides index have already been successfully validated over large-scale areas in different biogeographical regions of Europe using both species-level or family-level data on stream invertebrates.
Here we propose a different application of the index on a smaller, detailed scale, in order to verify the reliability of the SPEARpesticides index in site-specific risk assessment.
We consider two different mountain streams in Trentino-Alto Adige region, northern Italy. They are located only a few kilometres apart and present similar characteristics (i.e. hydrology, hydrochemistry, etc). However, one of them is considerably prone to pesticide exposure, due to intensive apple orchard cultivation in the watershed, while the other presents almost no human impact, and could be used as reference site. The exposure scenario due to runoff events was modelled for the entire productive season of 2010. Macrobenthos community was sampled monthly in both streams and taxa abundance was counted.
The SPEARpesticides index was used to analyze differences between the community composition of the two rivers, in order to verify if those differences were due to pesticides exposure. SPEARpesticides indicates a clear shift of the community composition over time in the impacted river. The comparison with the reference site shows clearly that this alteration is not driven by natural factors and it is likely to be caused by exposure to pesticides. The last sampling, performed more or less one month after the last treatment, shows a consistent recovery of the community of the impacted river. The fast resilience of the community can be probably linked to high input of organisms from non-polluted areas that are present upstream.

TU 374
Indirect effects of Carbendazim in freshwaters. A laboratory study using a simple detritus based food web.
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An ecologically grounded understanding of food webs (considering the conceptual framework of density- and trait-mediated indirect effects) and of species interactions in the system of interest is essential for understanding the ecological effects of contaminants on population dynamics, community structure and vital ecosystem functions. Few studies exist on indirect effects concerning facilitator species or trophic cascades within detritus based food webs (i.e. shredder-collector interactions). Detritus processing is vital for river ecosystems with detritivores and the microbial community contributing for the decomposition of coarse particulate organic matter into fine particulated matter and for recycling of nutrients that can be transported downstream and used by other organisms. This means that monitoring of the ecological quality of river ecosystems should incorporate assessment of toxic effects on processing and consumption of detritus.
This work contributes with an example of how indirect effects of fungicides on leaf litter decomposition process can be measured and to what extent microbial communities, shredders; collectors and their interactions might be affected.
A gradient of Carbendazim concentrations (0; 5µg/L and 50 µg/L) was used to show differential microbial conditioning of alder leaf litter over a 14 day conditioning period. Effects of Carbenzim on microbial community structure (Fungi and Bacteria) were assessed with denaturant gradient gel electrophoresis (DGGE), a polymerase chain reaction (PCR)-based fingerprinting technique. A microbial functional endpoint was also assessed by assessing the oxygen consumption of the leaf microbial communities. Afterwards, leaf discs conditioned in the different fungicide concentrations were used in multispecies systems with the shredder Sericostoma vittatum (Trichoptera) and the collector Chironomus riparius (Diptera) to assess changes in leaf degradation rates through effects on invertebrate feeding and consequent changes in collectors' growth due to differences in food availability and quality. Results are discussed in terms of how carbendazim contamination can affect detritus food webs by disrupting ecological interactions and have significant effects on aquatic macroinvertebrate communities and ecosystem function.

TU 375
Ecological vulnerability of macroinvertebrates, comparing sensitivity to vulnerability for chlorpyrifos
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The ecological vulnerability of a population (or higher level) expresses some intrinsic characteristics which are likely to influence the response of the considered population or system to a given stressor. The Ecological Vulnerability Analysis previous developed at Alterra, uses species traits of wildlife species to estimate likelihood of exposure to a contaminant, internal regulation mechanisms, and population recovery mechanisms. Specific for chlorpyrifos, the data on wildlife species showed that vulnerability for chlorpyrifos was mostly determined by exposure through habitat. Further, r-strategic species were more vulnerable and K-strategic species were less vulnerable to chlorpyrifos. However, for the set of wildlife species used in the original analysis there were no toxicological data available to include in the analysis. But for aquatic macroinvertebrates toxicological sensitivity data for chlorpyrifos are more readily available. It is therefore interesting to compare the sensitivity data to the vulnerability data, and compare different ways to include the sensitivity data in the vulnerability assessment. This is analyzed for the pesticide Chlorpyrifos. Vulnerability results for the aquatic macroinvertebrates will be discussed in light of the results of the vulnerability of wildlife species.

TU 376
Preliminary ecotoxicological assessment of the vulnerability of groundwater ecosystems to plant protection products
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The present EU groundwater legislation has often been disputed for only considering groundwater as a source of drinking water and not as an ecosystem with an intrinsic value and vulnerability. In the present study, preliminary groundwater threshold values were calculated for all plant protection products (PPPs) currently included in Annex I to Directive 91/414/EEC. Due to the very low availability of toxicity data for true groundwater organisms, data for surface water organisms from taxonomic groups known to be well represented in groundwater were used as surrogates. Three different approaches were used: (1) 'first-tier' (*Daphnia magna* and *Vibrio fischeri*), (2) species sensitivity distributions, and (3) the case-based model PERPEST. For the majority of the PPPs, the trigger value of 0.1 µg/L appears to be sufficiently protective. However, it may not fully protect groundwater life from side-effects of several insecticides. Implications for the environmental risk assessment of groundwater and recommendations for future research are discussed.

TU 377
Influence of a municipal solid waste landfill in the surrounding environment: toxicological risk and odour nuisance effects
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The environmental impact produced by municipal solid wastes has received special social and environmental attention in recent decades. Communities concern is driven mainly by possible toxic emissions causing human health concern, but also for general environment degradation due to induced heavy traffic load, landscape appearance and odour. Landfills are a strong environmental stressor. Waste treatment plants are now large, complex realities where different biological processes take place under controlled conditions. Still the results are, mainly due to the large amounts of waste material treated, that areas in the proximity of landfills are vulnerable not only to emissions, to possible toxic compounds, but also to nuisance such as odour pollution. All these factors have a dramatic impact in the local environment producing environmental quality degradation.
Most of the human health problems come from the landfill gas (LFG), from its non-methanic volatile organic compounds (NMOCs). The most practiced control technologies for landfill gas are flares but several hazardous air pollutants (HAP) are present in LFG, some of these being carcinogen for humans.
In MSW, as well as in LFG several odorants are present and both emissions during landfill operations and LFG uncontrolled emissions are responsible for environmental odour pollution.
In this work we present an integrated risk assessment for carcinogenic emissions and odour pollution study, to describe environmental quality in the landfill proximity. The study is based on sampling campaigns to acquire emission values both for carcinogenic compounds (dioxins and related compounds, PAHs, benzene and vinyl chloride) and odour.
This risk assessment and odour emissions were based on the current management of the facility. In particular all concentration values in the emissions from the landfill were measured and used in a simulation model (AEROMOD) to estimate maximum concentrations and depositions in correspondence to five sensitive receptors. The impact of leachate contamination was not been evaluated as the landfill is situated in a non aquifer with no drinking water supplies or surface water receptors (UK EA, 2004).
Results for the different scenarios and cancer and non cancer effects always showed risk estimates which were orders of magnitude below those accepted from the main international agencies (WHO, US EPA) and those due to background contamination. Odour might be significant for a limited downwind area.

TU 378
Another interpretation of classical multiple comparison procedures used in ecotoxicology
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Even if the misuse of p-values to define toxicity thresholds from No Observed Effect Concentration (NOEC) is now recognized - both statistically and biologically -, this old habit still remains firmly fixed in the ordinary run of things. The purpose of this work is to convince NOEC advocates to change their mind. With the estimation of x% Effective Concentrations (ECx) or No Effect Concentrations (NEC), some well-known alternatives based on dose-response curve modelling could have been enough attractive, but here we propose another alternative: an innovative interpretation of classical multiple comparison tests. Our approach consists in calculating simultaneous 95% confidence intervals (CI) for mean ratio-to-control associated to a Dunnett-type procedure. Our "selling"propositions are: the easiness of implementation through turnkey R packages, the solid statistical bases of the approach, the independence towards any model assumption underlying the dose-response curve, and the establishment of toxicity thresholds as the x%

Effective Concentration (ECx) values with their uncertainty intervals whatever the x, even 0. Our talk will detail the complete approach (from a theoretical and a practical point of view), from raw data until the calculation of 95% CI on ECx, with x equal to 0, 5 and 10. Four data sets, concerning different type of toxic compounds and collected during standard 21-day Daphnia magna bioassays, are analysed. To support our demonstration, 95% CI on ECx calculated with our approach are compared to those estimated with a classical fit of the 3-parameter log-logistic model. Both methods are in good agreement.
Of course, some cons could be argued against our approach like rather large intervals, inevitably bounded by concentrations tested in the experimental design, or intervals on EC0 always left bounded by 0. Nevertheless, the most sceptical potential users will grant us that our approach is an easy and statistically convenient way to overstep the misuse of p-values which are certainly not dedicated to establishing thresholds, values of which strongly depending on the sample size without to be associated with their uncertainty.

TU 379

Defining relevant ecological endpoint in the context of integrated environmental assessment of chemicals

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The recent developments of ecotoxicological risk assessment are oriented toward an increased attention on approaches characterized by growing "ecological realism", with the objective of a better assessment of the possible effects on ecosystem health.
The traditional approaches, focused more on chemicals than on the site-specific environment, tend to be adapted and modified with more focus on the structure and functions of communities and ecosystems subject to complex and combined stress factors.
In the present European regulation, ecotoxicological risk assessment, refers mainly to production and use of chemicals, with a few attention to other contexts, such as strategic environmental assessment of plans and policies or life cycle assessment of products and good.
Therefore, several issues are still critical to perform a comprehensive assessment of chemicals: extrapolation of results from laboratory to field; spatial and temporal distribution of risk ; relevant archetypes and emission scenarios (in a life cycle perspectives that requires to include raw material, production, use phase and end of life); assessment of ecological vulnerability from population to community and ecosystems; role of integrated indices to consider impact along the trophic chain; role of ecological indirect effects on ecosystem functioning; role of statistical tools (such as Species Sensitivity Distribution) with respect to the role of key species; framework and methods for assessing uncertainty and indirect ecological effects of chemicals.
A critical review of endpoint adopted in the above mentioned tools and methodologies will be presented, highlighting the relevance of multidisciplinary approach to define new paradigm in the dialogue among ecotoxicology, ecology and other disciplines involved in the integrated assessment.

TU 380

Assessing the effects of ammonia on aquatic communities in the field

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Ammonia can act as both a toxicant to animals and a nutrient for plants. We used recently developed tools for assessing the degree to which aquatic communities are impacted by their environment, relative to unstressed reference conditions, to assess the effects of ammonia on different components of aquatic ecosystems in the UK. The approach uses a dataset of spatially matched chemical and ecological data. Quantile regression is then used to analyse the datasets, which accounts for other stressors (such as chemical toxicants or habitat degradation) that may have caused impacts on the communities. Thresholds for the effects of ammonia on fish, invertebrates, macrophytes and algae are derived for different forms of ammonia exposure (total ammonia or unionised ammonia, expressed as average or 90th percentile concentrations). The results are compared against the quality standards for ammonia that are applied in UK freshwaters.

TU 381

High functional redundancy in a herbicide-exposed planktonic community

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A mechanistic food web model was used to predict functional effects of the herbicide linuron from microcosm data. On a functional group level, our analysis showed that a reduction in carbon uptake rates of sensitive primary producers caused a density decrease of the latter with increasing linuron concentrations. In response, heterotrophs started feeding on more abundant tolerant primary producers which allowed them to maintain feeding rates at control levels - indicating functional redundancy within the planktonic community. On an ecosystem level, total gross primary production (GPP) was up to hundred times lower in the linuron-treated microcosms than in the controls. However, this could be explained by herbicide effects on macrophytes - the main contributor to total GPP. In contrast, linuron did not change planktonic GPP more than a factor 2 and did not alter bacterial production. We conclude that the planktonic community exhibited high functional redundancy when facing herbicide stress.

TU 382

Unexpected Malachite Green sediments and fish contamination originated from a wood panel factory

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Malachite Green (MG) is an extensively used biocide in the aquaculture industry world-wide. It is highly effective against important protozoal and fungal infections. Basically, it works as an ectoparasiticide: it has also been used to control skin flukes and gill flukes. Aquaculture industries have been using malachite green extensively as a topical treatment by bath or flush methods without paying any attention to the fact that topically applied therapeutants might also be absorbed systemically and produce significant internal effects.
Malachite green has become a highly controversial compound due to the risks it poses to the consumers of treated fish including its effects on the immune system, reproductive system and its genotoxic and carcinogenic hazard. Though the use of this dye has been banned in several countries it is still being used in many parts of the world due to its low cost, ready availability and efficacy. There is concern about the fate of MG and its reduced form, leucomalachite green, in aquatic and terrestrial ecosystems since they occur as contaminants and are potential human health hazards.
Some fish samples (trout) exported by a large fish farm in Friuli (Northern Italy), controlled by food quality control officers, showed a substantial amount of MG, not enough for killing the fishes but largely exceeding the food safety limits. Mass balance of MG in the fish farm, however, indicated that the pollution source should have been search in a different site, located quite upstream the fish farm.
Going backwards, after a labor intensive and deep investigation, the scientists found that sediments each time were more and more polluted by MG. Finally, the MG main source, was located upstream a factory using MG as dye for manufacturing custom-colored wood panels. The regulatory intervention of the authority forced the factory to eliminate MG in the industrial processes. The sediment data provided the scientific basis for river recovery.

TU 383

A comparison between natural and alternative sea water piling materials merging ecological and ecotoxicological issues

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Wood is extensively used for maritime works, above all mooring piles, docks, bulkheads and navigation channel marks ("briccole"), especially in estuarine and marine coastlines. In Venice lagoon (Italy), it has been evidenced a great acceleration in the decay of traditional natural piling materials mainly due to the wood borers in addition to the water mechanical and bacterial action. In the lagoon it has been estimated that along the shipping channels there are about 22,000 wooden piles constituting the briccole and around 10,000 are present for boats mooring. Each year, a large number of them must be replaced. Local authorities in order to keep navigation safety and increase environmental protection identified a set of potentially ready-to-use piling materials concerning allocthonous wood, traditional wood with specific add-ins or treatments and others that are completely synthetic. The aim of this study is to fill the gaps in the knowledge about the lagoon distribution and ecology of the main species of wood boring invertebrates through in situ and ex situ activities, defining a preliminary model of habitat suitability. Simultaneously, ecotoxicological analysis have been carried on leachates generated from the proposed materials considering various exposure scenarios. The purpose is to ascertain if their toxicity was significantly different from that of historically used timbers. A battery of toxicity tests composed of sensitive saltwater species was taken into account (i.e. *Vibrio fischeri*, *Phaeodactylum tricornutum*, *Crassostrea gigas*, *Amphibalanus amphitrite* and *Tigriopus fulvus*). Species-specific toxicity scores and a final index were generated on the basis of some literature suggestions to facilitate toxicity data interpretation. The merge of data from ecological and ecotoxicological surveys, bearing in mind the hints of local and national authorities (i.e. cultural heritage protection), will promote the identification of the most suitable piling materials for the proper application.

TU 384

Cellular changes in microalgae exposed to effluent from pulp and paper mill

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Pulp and paper mill effluents, especially those from processes using molecular chlorine, which produce organochlorine compounds derived from lignin and resin acids which have been pointed out as very toxic to many organisms such as fish, algae and microcrustaceans. The aim of this study was to characterize the possible changes in the ultrastructure of unicellular microalgae - Scedesmus subspicatus - using transmission electron microscopy (TEM). The microalgae were grown in different concentrations of effluent (0-6-25 and 50%, with 0% as control) for 72 h under constant aeration, at 25 oC and under continuous illumination (1500 lux). Samples containing algae were fixed with glutarandeído (2.5%) and washed with phosphate buffer - pH = 7.1. Post-fixation and washes, the samples were treated with osmium tetroxide, diluted with 1% sodium phosphate buffer 0.1 M for 3 hours and then washed with phosphate buffer - pH = 7.1. Dehydration was performed with acetone (30-100%) and infiltration with Spurr resin. Algae cells exposed to the effluent showed significant changes in the plasma membrane and cell wall, chloroplast morphology and increased concentration of the starch stored. Additionally, morphological cell alterations were observed such as the elongation in a given direction. The observed changes are more intense in samples containing higher concentrations of effluent.

TU 385

Toxic effects of effluent from pulp and paper mill on photosynthetic capacity of microalgae

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Several studies have demonstrated toxic effects of effluent from pulp and paper mill for microalgae. These effluents contain many substances with toxic potential for these organisms, for example, chlorophenols, resin acids, lignin derivatives, among others. The aim of this study was to assess the photosynthetic capacity of microalgae Scedesmus subspicatus in medium containing different concentrations of effluent from the production of bleached pulp through conventional process. It was evaluated the rate of algal growth, the kinetics of oxygen production, changes in concentration of chlorophyll a and b, quantum yield and maximum fluorescence intensity, rate of quenching photochemical and non-photochemical quenching. The algal growth rate was evaluated during 72 h under continuous illumination (1500 lux) at a temperature of 25 oC and constant air flow. Photosynthetic parameters were measured using a PAN fluorometer, in samples collected after 48 and 72 h of exposure to the effluent (0, 6, 12, 25, 50 and 100%, 0% used as control). The toxic effects were indicated by the reduction of algal growth rate correlated to increasing effluent concentration. The alteration of photosynthetic capacity was shown by the reduced maximal fluorescence intensity, slower production of oxygen as well as lower values of oxygen concentration in the stationary phase. We also observed a reduction in the concentration of chlorophyll depending on the effluent concentration used. The results indicated significant changes in the photosynthetic system of microalgae, especially in their PR system.

TU 386

Intraspecific competition delays recovery of population structure

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Ecotoxicological field studies have shown that total abundance and biomass often recover shortly after pulsed toxicant stress. In contrast, population structure showed comparatively long-term alterations before reaching pre-treatment conditions. We investigated two mechanisms that may explain the prolonged recovery of population structure: latent toxicant effects on life-history

traits on the individual level and competition on the population level. To test these hypotheses we exposed populations of Daphnia magna to a pulse of the pyrethroid Fenvalerate. For several generations the populations were kept at two different degrees of competition: strong competition at carrying capacity and reduced competition main- tained by simulated predation. After disturbance due to Fenvalerate exposure, biomass recovered after 14-17 days. In contrast, size structure characterised by a lack of large and dominance of small organisms recovered after 43 days in populations with strong competition. Size structure recovered twice faster in populations with reduced competition. We explain this as follows: due to toxicant induced mortality, food availability and consequently birth rate increased and populations were dominated by small individuals. In populations without predation, these cohorts grew and eventually exerted high intraspecific competition that (i) stopped further growth of juveniles and (ii) increased mortality of adults. These demographic processes were mainly responsible for the prolonged recovery of size structure. In con- trast, for populations with predation, the regular harvest of individuals reduced competition. Juveniles developed continuously, allowing a fast recovery of size structure in these dynamic populations. In risk assessment the duration for populations to recover from (toxicant) stress, is crucial for the determination of ecological acceptable effects. We conclude that competition needs to be considered in order to understand and predict recovery of size structure.

TU 387

The secret in the sauce: the effects of varying environmental conditions on the toxicity of mine effluent constituents to coldwater fish

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Due to method standardization, current regulations may not afford protection against adverse effects of mine developments under non-standard pH, temperature, and hardness conditions of arctic waters. These effects may be due to liberated minerals, processing by-products, and service community wastes. Canadian mines are controlled under the Fisheries Act, regulating "Any substance that is[3DOTS]deleterious to fish or fish habitat or to the use by man of fish that frequent that water". These regulations limit effluent parameters based on toxicological evidence produced via assay methods published by Environment Canada. For coldwater fish, rainbow trout is used as a surrogate and employs conditions optimal to that species. In contrast, locations of concern exhibit temperatures 10°C colder than standard test conditions, hardness 2-10 times lower than dechlorinated water, and variable pH due to contamination and natural geology.
The study aims to characterize the variability of effluent toxicity across a range of species and conditions while assessing whether the current standard test is protective enough to cover this variation by comparison of standard and 'amended standard' (varied conditions) assays. Eighteen contaminants (NaCl, NaNO2, NaNO3, Na2SO4, KCl, CaCl2, Cd, Cr, As, Cu, Zn, Mg, Ni, Al, MoO4, Se, NH4OH, NaClO, and toluene) were tested using five species of fish native to Northern Canada (arctic charr, lake trout, lake whitefish, round whitefish, and arctic grayling) across a range of pH (5.5, 6, 6.5, 7, 7.5, and 8), temperature (5,10, and 15ð-ðC), and hardness (30, 60, and 120ppm) conditions and their observed effects modeled as 24,48,72, and 96-hour LC50 values.
By comparing these results to each other and to those of the standard rainbow trout test, we have assessed the effect of pH, temperature, and water hardness on contaminant toxicity and time-to-toxic effect within and between species while characterizing the ecological protection afforded by current regulations. The data suggests that, in some instances, the standard has failed to capture the potential toxicity exhibited in non-standard species and non-standard, real-world conditions. Should this variability of non-standard conditions exceed the resiliency of the standard test to act as a surrogate, the mandate of the Fisheries Act to provide adequate protection to fish, fish habitat, and human use of fish will not be met. This potential risk is guiding the project's second phase of research.

TU 388

Asellus aquaticus as a sensitive test organism

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Toxicological test with Agrochemical showed that Asellus is one of the most sensitive species. Acute toxicity on Asellus aquaticus compared to effect on Daphnia magna, Chironomus riparius, Hyalella azteca, Lumbriculus variegatus and Gammarus pulex will be presented.

TU 389

The case of Phaeodactylum tricornutum in wastewater toxicity monitoring

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Marine phytoplankton is highly productive in coastal ecosystems where it generally occurs the major exposure and uptake of contaminants associated with anthropogenic pollution discharges. Phytoplankton presents very high surface-to-volume ratio and may respond rapidly to suspended contaminants. Therefore, some phytoplanktonic species are frequently used as pollution bioindicators, providing information about potential impacts on the supported marine-coastal food webs. The diatom Phaeodactylum tricornutum is commonly used in toxicity testing according to the standardised protocol UNI EN ISO 10253:2006. Unfortunately, it seems that a complete inventory of the tested substances and matrices has not been compiled yet. The purpose of this study has been to review scientific literature about P. tricornutum and to specifically assess its sensitivity and reliability towards wastewater samples. More than a hundred wastewater samples were taken into considerations. They originated from domestic, industrial and mixed domestic-industrial sources and were treated according to two main wastewater treatment technologies (Activated Sludge Sequencing Batch Reactor and Ultra-Filtration Membrane Biological Reactor). Results showed the existence of two main effect scenarios: biostimulation and toxicity. The main question was about how to weigh biostimulation compared to toxicity and if it was possible to merge both of them in a wastewater toxicity score to rank samples and provide some management tools. Referenced and improved approaches were taken into consideration.

TU 390

Assessing effects of Ultraviolet B radiation on Physa fontinalis using biochemical biomarkers

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Biomarkers are widely used in order to detect stress in organisms as early warning tools after exposure to different environmental conditions. In addition, biochemical biomarkers can help to understand stressors mode of action. The aim of this study is to evaluate the effects of UVB radiation in different biomarkers of the aquatic snail Physa fontinalis. Incident UV-B levels are increasing due to ozone depletion and can penetrate to significant depths in aquatic environments having a major impact on organisms both directly and indirectly, inducing among other effects DNA damage, mortality, and mutagenesis. 96 hours acute tests were conducted using different shell lengths of the organism to different exposure times of UV-B showing that survival was significantly affected. Biomarkers activity (e.g. AChE and GST) was determined and correlated with the observed mortality.
This study shows the importance of including UV-B exposure and the inherent effects in environmental risk assessment procedures. UV-B induces changes in aquatic organisms as in their role in the environment, and therefore these effects can be transposed to ecosystems, possibly jeopardizing their services.

TU 391

Physiological measurements in Cerastoderma edule (Bivalvia: Cardidae) at contaminated sites at the northwest Portuguese coast

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Ria de Aveiro is a highly productive coastal lagoon in Portugal, and it receives a diverse input of contaminants derived from industrial and urban effluents, port and shipyards activities and agriculture runoffs. The purpose of this investigation was to evaluate the physiological parameters, as rates of oxygen consumption and clearance, survival in air exposure and energy reserves with cellular energy allocation (CEA), of the common cockle *Cerastodema edule* collected at six stations along Ria de Aveiro. Animals (20-30 mm) were collected in July-10 during the low tide and acclimatized at laboratory in artificial seawater, one hour before the assays. Oxygen consumption was determined by simple static respirometry, using cockles (n=10) held for one hour in 50mL gastight syringes filled with artificial seawater and one animal per syringe. To measure clearance rate cockles were individually placed in 200 mL chambers (n=5 per station) filled with Neutral Red solution (1.5 mg.L-1) prepared in seawater. This method was based on the rate of absorption of Neutral Red by animals, that was recorded at 15min-intervals for 45 minutes at 530nm. To analyze the capacity of air survival, animals (n=21 in triplicate) were kept in 1L glass jar at 20ºC and 100% humidity during eight days. Deaths by means of extended shell gape were recorded every day. Finally, the CEA in *C. edule* tissues (n=10) was measured based on the energy reserves available and the energy consumed during the assay. The results of this study intended highlight the importance of physiological parameters analyzes as good indicator of animal fitness and its evaluation could be used as biomarker.

TU 392

Effects of copper on the life cycle traits of Physa fontinalis

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Copper is an essential metal to several organisms but like other metallic elements can cause toxicity when in excess. Copper can enter into the aquatic system through industrial effluents, agriculture runoffs and domestic wastewaters. This study aimed to assess the survival, egg hatching, growth and reproduction of the freshwater pulmonate snail Physa fontinalis after exposure to copper sulfate in laboratory conditions. A 96h survival test using adult and juvenile snails with different shell lengths showed increasing toxicity as the shell length decreases. Significantly low copper values (~1µg/L) appear to adversely affect the hatching and survival of egg masses as well as the reproduction and growth of the aquatic snail suggesting that P.fontinalis is more sensitive compared to other aquatic snails (e.g. Lymnaea luteola L, 96h EC50 ~ 28µg/L) or even other aquatic organisms usually used as model organisms in ecotoxicological approaches.

CS02 - Environmental disasters & oil spills

WE 002

Do oil droplets matter? Oil spill effects on North East Arctic cod larvae

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Oil exploration and production in the Atlantic moves northwards towards spawning and nursery areas of fish species that sustain some of the world's largest fisheries. Models are therefore needed that can simulate the effects of oil dispersions on early life stages of fish. In this study, we calibrated a newly developed individual based model (IBM) to experimental data to infer effects of the water soluble fraction (wsf) and of an oil dispersion (wsf and droplets) on two key endpoints of North East Arctic cod (Gadus morhua) larvae: feeding rate and survival probability. The model was calibrated on data from two experiments that had identical chemical composition and concentration of the wsf (ΣPAH = 0.03 to 30 µg L-1) and only differed in the presence/absence of oil droplets. Both exposure types (wsf and dispersion) decreased feeding rate (control: 0.4 d-1) and survival probability (control: 0.96). Decreases occurred in a concentration-dependent fashion and did not significantly differ between exposure types, with EC50s of 2 (feeding) and 7 µg/L ΣPAH (survival probability). Additionally, the fit of the IBM model to growth and survival data was not significantly different between the two exposure types. Our results thus indicate that oil droplets did not significantly contribute to oil toxicity towards ingestion and survival of cod larvae. More complex models that consider oil droplet toxicity in addition to the toxicity of the wsf are therefore concluded not to be more accurate than simpler models that neglect oil droplet toxicity.

WE 003

Do oil dispersants make spilled oil more toxic to fish?

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The recent Deepwater Horizon disaster was the world's largest unintentional oil spill. It was unprecedented in its duration, volume spilled, and the technology applied for control and clean-up. Among these unique features was the continuous and wide-spread application of oil dispersant, at the surface and at the discharge, 1500 m deep, generating public concern about dispersant toxicity and the effects of dispersion on oil toxicity. Recent USEPA reports claim little difference in acute toxicity to marine fish and invertebrate species among commonly available dispersants and between dispersed and non-dispersed Louisiana Sweet Crude. The EPA reports were technically correct: the toxicity of waterborne hydrocarbons does not vary with chemical dispersion. How-

ever, the agency did not tell the entire story, omitting any consideration of loading. Our research on the chronic toxicity of dispersed oil to fish embryos demonstrates that toxicity expressed as oil loading increases by a factor of 10 to 1000 times with dispersion, primarily because 10 to 1000 times more oil enters the water column. From a practical perspective, the risk of oil toxicity to fish increases an equivalent amount because the action of dispersant is on the exposure component of the risk equation, not on the potency of the toxic components of oil. Wise decisions on the application of dispersants requires that all contributors to risk be considered including toxicity, exposure, receptor and the modifying effects of the receiving environment.

WE 004

Marine environmental impact of oil spill on vertebrate: gene expression changes in marine medaka, Oryzias javanicus

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The accidents like an oil spill give the enormous effects on marine and coastal ecosystem as well as individual organism. In December 2007, an oil spill in marine crane Hebei Spirit polluted the mid west coast and it spread very fast throughout the whole west coastal regions. In this study we aimed to discover the marker genes which expressions were changed by exposure to ecotoxicants or pollutants using the marine medaka (Oryzias javanicus) microarray and to develop as biomarkers for the detection of environmental pollution like oil spills. Also, we investigated the effects of organic pollutant on transcriptional changes after fishes were exposed to bisphenol A, polychlorinated biphenyl, 17beta-estradiol as well as WAFs (water accommodated fractions) from crude oil and compared the profiles. These results suggested that transcriptional changes of genes could be good biomarkers to detect stress levels in O. javanicus and provided the general translation about effect of oil contamination on marine organism. And the compact microarray chip development including new biomarkers could be very helpful in detecting of ecotoxicological stress responses.

WE 005

Temporal and geographical trend in the genotoxic effect of the extracts of accidentally oil-spilled marine sediments on the blood cells of striped beakperch (Oplegnathus fasciatus)

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To investigate the genotoxic effect of marine sediment on aquatic organism, sediment samples were collected from 13 sites along the coastal line of Taean Country, Korea after the Hebei Spirit Oil Spill had accidentally occurred. Striped beakperch (Oplegnathus fasciatus) blood cells were exposed to sediment extracts and DNA single strand breakage in the blood cell was examined using the comet assay. Thirteen days after the spill, Levels of DNA damage in the blood cells to sediment extracts were 3.86 in mean tail moment. The highest DNA damage of 13.75 was found at Sinduri, one of heavily oiled sites. One and half year after the spill, DNA damage decreased at most sites analyzed with mean tail moment of 1.58. From this decreased DNA damage, this study area was supposed to be very quickly recovered from oil spill just within one and half year. The samples showed a significant ($p<0.001$) correlation between concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediments and DNA single strand breakage in the blood cell during both spatial and temporal sampling periods. Therefore, the comet assay could be a successful tool in assessing genotoxicity of PAHs in marine sediment affected by oil spill.

WE 006

Soil and groundwater contamination analysis and assessment to identify the responsibility of a chemical company in an environmental disaster

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In an area in the North of Italy, nearby a chemical plant site, high levels of organic and inorganic pollutants in groundwater have been detected, determining a potential health and environmental hazard. This work followed a public authority investigation; we carried out an analysis and evaluation of a large set of pollutants in soil and groundwater in order to identify which amount of environmental contamination is chargeable with that factory. A large survey of almost 600 samplings (soil and water) has been planned and carried out in an area close to the facilities, monitoring organic and inorganic compounds (i.e. arsenic, cadmium, mercury, nickel, benzo(a) pyrene, benzene, toluene, hexachlorobenzene, polychlorinated byphenyls); geostatistical modelling allowed to determinate the spatial distribution of pollutants in groundwater system.
The research has demonstrated the cause-effect relationship between the industrial activities and the kind of pollution detected in the soil and groundwater.

WE 007

Risk assessment of residual concentrations derived from a blow out of a crude oil well in Italy: a tool to achieve the complete release of the impacted areas after years of bioremediation activities

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In 1994 the site located in Piedmont, Northern Italy, was the scene of a large inland crude oil spill following an oil well blow-out. The event resulted in approximately 15,000 m3 of light crude oil being released overland contaminating both soil and groundwater.
Land use in the area is agricultural with the majority used for the production of rice. The area, just beneath a thin loamy superficial layer is underlined by 60 m of fluvioglacial materials constituting a high permeability/high yield phreatic. Groundwater levels at the site fluctuate by 6 m seasonally with higher levels during the summer period due to surface recharge from agricultural irrigation practices. Lenses of non-aqueous-phase-liquids (LNAPLs) were detected floating on the water table ever since the blow-out.
Since the regulators and farmers requested that the impacted soil be remediated without altering its agricultural and biological properties, bioremediation was selected as the preferred approach. Landfarming and biopile were used for surface soils, bioventing for contaminated vadose zone soils, bioslurping for LNAPL floating on the water table and natural attenuation for dissolved-phase hydrocarbons. This integrated approach lasted 5 years and lead to the reclamation of the original impacted area. The regulators allowed the usual agricultural activities to be resumed all over the impacted area.
A risk assessment was performed using the software RBCA Tool kit 1.3b (2000). The unsatured soils and groundwater were selected as the secondary source of contamination. The selected receptors were on site workers (farmers) and off-site residents;the migration pathways were outdoor vapour inhalation and dermal contact and ingestion of surface soil. The groundwater was considered as an environmental receptor. Two exposure scenarios were assumed based on the cycle of cultivation of rice: Scenario 1 comprising activities during which the ricefields are dry and the water table is around 11 m below ground; Scenario 2 comprising activities during which the ricefield are flooded and the water table is around 6 m below ground. A study was also performed on mais and rice samples to assess the effects of the residual concentrations on the quality of the crops and the possible consequences to humans of their consumption.
The risk assessment allowed the complete release of the areas. A monitoring period for the groundwater and a campaign of outdoor air measurements are being planned with the regulators.

WE 008

Environmental factors affecting the toxicity of dispersed oil for herring embryos (Clupea sp.)

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When oil spills occur, chemical dispersants can be used to break up oil slicks in an effort to mitigate the negative impacts on organisms. However, the dispersed oil in the water column may expose pelagic and benthic organisms to the toxic constituents of the oil. Environmental factors, such as salinity and temperature, may effect the solubility and therefore, the concentration of polycyclic aromatic hydrocarbons (PAH) being delivered into the water column. To best treat oil spills, managers need to know how dispersed oil may affect the early life stages (embryos and larvae) of coastal fish species at different environmental conditions. Therefore, the toxicity of Water Accommodated Fractions (WAF) and Chemically Enhanced Water Accommodated Fractions (CEWAF) of Alaska North Slope (ANS), Medium Grade South American (MESA) and Arabian light crude oils were tested during the embryonic development of Pacific herring (Clupea pallasi) from two different populations. Toxicities of Arabian light crude oil CEWAF and WAF at 7.5"ð, 15"ð and 30"ð were tested during the embryonic development of Atlantic herring (Clupea harengus) from a spring spawning population; and at 7°C, 10°C, and 15°C from a fall spawning population. Toxicity of test solutions was assessed based on percent survival, percent developing normally, and prevalence and severity of morphological abnormalities. The most commonly observed morphological abnormalities were those associated with PAH exposure: spinal curvature, pericardial and yolk sac edemas, cranio-facial malformations and skin lesions. Abnormalities were produced by both WAF and CEWAF of all oils tested and were observed in both Pacific and Atlantic herring. Exposure to colder water increased spinal curvatures and cranio-facial malformations while warmer water increased yolk sac edemas and skin lesions at lower concentrations. Embryos exposed to crude oil also suffered from increased mortality, reduced heart rates, and slightly reduced lengths compared to control embryos. This study suggests that managers of oil spills considering dispersant use can minimize toxicity to coastal fish by incorporating information on salinity and temperature of the receiving environment.

EC03 - Biodegradation and bioremediation of organic pollutants

WE 010

Enhanced biodegradation screening tests: Degradation potential natural environmental media

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To improve the environmental relevance of persistency assessments without moving up to the level of simulation tests, several potential enhancements to the ready biodegradation tests are given by the REACH guidance. Substances that degrade in these enhanced biodegradation screening tests are not considered as ready biodegradable but the results are used in persistency assessments. The inoculum used for ready biodegradability tests is derived from sewage treatment plants, e.g. activated sludge, sewage or secondary effluent, whereas for the enhanced screening tests only the use of natural environmental media as source of inoculum is allowed. As the biodegradation potential and environmental realism depends on a representative diversity of bacteria in the inoculum test strategies that can maximize the diversity and adaptation of the micro-organisms were developed. The test duration can be prolonged up to 60 days, the test volume and with it the probability of introducing competent bacteria can be increased or the biomass concentration can be increased. Furthermore low-level pre-adaption and semi-continuous assessments are possible. So far only little experience is available on the use of these approaches and the degradation potential of natural environmental media like marine or fresh water. As the inoculum can be derived from different sources and environments the degradation potential may vary distinctly. The degradation potential of different inocula derived from different environmental sources in the context of persistency assessment of chemicals was compared and the results will be presented.

WE 011

Investigation of the role of adaptation in the biodegradation of chemicals in the impact zone of an untreated discharge using OECD 314E

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In many parts of the world, particularly in developing regions, untreated wastewater is routinely emitted directly into surface water bodies. This scenario is typically associated with high levels of suspended solids, biochemical oxygen demand (BOD), nitrite and unionised ammonia in receiving waters, resulting in significant ecological impairment. The receiving water will treat the untreated discharge through a mixture of physical, chemical and most importantly biological "self-purification"processes. This scenario presents a number of difficulties for conventional risk assessments for 'down-the-drain' chemicals. Most fundamentally, in the absence of removal by secondary sewage treatment, the predicted environmental concentration (PEC) will often exceed the predicted no-effect concentration (PNEC). However, since the ecosystem in the receiving environment will already be significantly impacted by other constituents present in raw wastewater, an assessment of the biodegradation during "self-purification"is required.
A guideline now exists for simulating this scenario in a laboratory, OECD 314(E) 'Biodegradation in an untreated - surface water mixing zone'. The guideline has been developed to determine the final extent and rates (primary and ultimate) of a chemical upon its release in an untreated

discharge scenario. It also allows a comparison of the relative rate of removal of the test chemical in relation to the key water quality parameters which can be used to define the impacted zone in the receiving water. These guidelines are relatively new (2008) and little data have been published on the fate of chemicals in studies of this nature.
This guideline was used to investigate the fate of 14C radio-labelled test chemicals incubated with 1 part wastewater to 2 parts surface water, using a batch system. As well as looking at the "non-adapted"situation, a two phase test design was also investigated. Here, a preadaptation period of 14 days was used during which the water quality parameters were monitored, before renewal of a portion of the test medium spiked with the 14C labelled test material. This allowed the determination of the influence of a pre-exposure cycle on biodegradation rates of the test chemical and also the nitrification cycle. These results are discussed in relation to risk assessment in the impact zone.

WE 013

Assessment of persistence of chlorinated paraffins using OECD biodegradability tests (ready and enhanced)

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Tetradecanes with varying degree of chlorination have been synthesized to assess their persistence. Tetradecanes with 41.3, and 45.5% chlorine reached >60% biodegradation at day 28 in Closed Bottle tests. The tetradecanes with a higher degree of chlorination are not readily biodegradable. In the prolonged Closed Bottle tests biodegradation percentages of chlorinated tetradecanes were of >60 at day 56 (chlorinated tetradecane (50.0%)) or close to 60 (chlorinated tetradecane (55.0%)). The chlorinated tetradecane (60.2%) reached a biodegradation percentage of 40 at day 84. Chlorinated tetradecane (41.3, 45.5 and 50.0% Cl) should therefore not be considered as persistent. The biodegradability of chlorinated tetradecanes is retarded by increasing degree of chlorination resulting in partial degradation of tetradecanes with higher degrees of chlorination.

WE 014

Degradation of the artificial sweeteners acesulfame, cyclamate, saccharin, and sucralose in soils

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Artificial sweeteners are consumed in substantial quantities as sugar substitutes and were previously shown to be ubiquitously present in the aquatic environment. Sweeteners may also end up in soils through various pathways. Wastewater-polluted surface water may be used for irrigation of agricultural fields. In certain countries, digested sewage sludge is applied as fertilizer in agriculture. Furthermore, untreated wastewater may seep into subsoils through leaky sewers. The sweetener saccharin is also registered as additive in piglet feed and the compound may get into agricultural soils via application of manure. Finally, saccharin is a major soil metabolite of certain sulfonylurea herbicides. We studied the degradation of four artificial sweeteners in six different soils. The soils were selected to cover a wide range of properties, in particular pH (4.0-7.5), organic carbon (1.2-18%), and texture. Batch incubation experiments were conducted at 20°C and 34-60% of the maximum water holding capacity. Cyclamate, saccharin, acesulfame, and sucralose were degraded with half-lives of 0.4-6 d, 3-12 d, 3-49 d, and 8-124 d, respectively. A first-order kinetic model fitted the concentration data reasonably well. The fast degradation of cyclamate and saccharin in soils is consistent with the extensive elimination observed in wastewater treatment plants (WWTPs, ≈ 90 and ≈ 99%, respectively). Somewhat less expected was the fairly rapid degradation of acesulfame and sucralose in soils, which is in contrast to their persistence in WWTPs. These differences may be rationalized by the presence of different microbial communities in activated sludge and soil. Furthermore, residence times in activated sludge of typically a few hours are obviously much shorter than contact times in soils.

WE 015

Bacterial degradation of 1H-benzotriazole

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The complexing agent 1H-benzotriazole (BT) is a widely-used corrosion inhibitor in cooling and heating fluids, dishwashing detergents, and aircraft de-icing fluids. BT, as a high production volume chemical, is among the compounds with the highest detected concentrations in wastewater effluents and surface waters. Due to its apparent persistence against biodegradation, it is ubiquitously present in the aquatic environment. This is even more alarming since the compound might have ecotoxic effects. Generally, BT was considered non-biodegradable by bacteria under oxic and anoxic conditions.
In this study, we investigated the presence of an intrinsic biodegradation potential for BT in two environmentally relevant compartments: sewage sludge and deep aquifer sediments. An aerobic sewage-sludge-derived mixed culture coupled BT degradation with bacterial growth. During biodegradation of BT, N-methylaniline and further transformation products with absorption maxima at 367 and 550 nm were formed. This is the first report on bacterial growth with BT at mesophilic temperatures and underlines that sewage sludge is a habitat of the respective microorganisms. The anaerobic intrinsic biodegradation potential for BT was studied in deep aquifer sediments eventually contaminated by heat transfer fluids leaking from borehole heat exchangers. Concentrations of BT and its derivative tolyltriazole stayed constant over a period of 200 days indicating that no intrinsic biodegradation potential was detectable under the various redox conditions investigated.

WE 016

The isolation and characterisation of bacteria capable of degrading the strobilurin fungicide, azoxystrobin

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Azoxystrobin [methyl (E)-2-{2-(6-(2-cyanophenoxy)pyrimidin-4-yloxy)phenyl}-3-methoxyacrylate] was the first of the strobilurin group of fungicides to be released in 1996. It has a broad spectrum of activity and has been approved for use in over 80 different crop types, which equates to greater than 400 crop/disease systems in total.
A major route of azoxystrobin degradation is considered to be due to microbial action, beginning with the demethylation of one of the side chains to produce the metabolite, azoxystrobin acid. However, the extent to which azoxystrobin is completely mineralised to carbon dioxide (CO2), and the identities of organisms that are potentially involved with the degradation process remain unknown.
In this study azoxystrobin-degrading cultures were produced from a sandy loam soil from the UK by a sequential enrichment series. Comparisons were made between the degredative capabilities of soils that had had no previous exposure to azoxystrobin and soil that had been exposed to a 25mg/kg dose of the compound 4 months prior to the start of the study.
Bacteria were isolated and tested for their degradative capabilities. The 16S rRNA from isolates that degraded azoxystrobin to a high level were sequenced. Degrading organisms were then characterised using assays to determine whether the following aspects impact degradation: 1) The media used to grow the colonies 2) The presence of alternative carbon and nitrogen sources.
The results of the sequential enrichments show that in the first enrichment the soil which had previously received azoxystrobin showed a greater capacity to degrade the compound. However, after the first enrichment there was no difference between the two soils.
Two strains capable of degrading azoxystrobin were isolated. Sequencing identified them as Cupriavidus basilensis and Rhodanobacter sp. Characterisation assays showed that solid growth media type had no significant impact on azoxystrobin degradation. Both of the degrading organisms required an additional nitrogen source to be present for degradation to occur. The presence of an alternative carbon source reduced azoxystrobin degradation by approximately 40%.

WE 017

Isolation of Vibrio sp. in marine sediment from the NW coast of Baja California, Mexico and its growth in the presence of DDT

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Studies have shown that bacteria can survive in contaminated environments and contribute to the biotransformation of persistent organic pollutants like DDT in marine sediments. The bacteria possess enzymes such as dioxygenases, that are able to modify the benzene ring of organochlorine compounds; for example, the gene VFA0416 DOPA 4, 5 introduces two oxygen atoms in the benzene ring, displacing the chlorine atom by a hydroxyl. This gene has been found in *Vibrio fisheri* ES114. Because of the importance of DDT, a bacterium was isolated from marine sediment from the NW coast of Baja California, Mexico and identified biochemically and molecularly as belonging to the genus *Vibrio*. Laboratory assays were run in triplicate using minimal salts medium containing DDT and minimal salts medium without DDT at 28°C and 8°C for 96 hours. Bacteria were stained with DAPI and counted under a fluorescence microscope, and their specific growth rates (μ) and duplication times (g) determined. To search for the gene VFA0416 DOPA 4, 5 in *Vibrio* sp., the genomic and plasmid DNA was extracted and amplified by PCR, using Vibrio fisheri ES114 as positive control. Highest specific growth rates and lowest duplication times occurred in both culture media at 28°C because temperature accelerated cell metabolism. Plasmid DNA of Vibrio sp., contained VFA0416 DOPA 4, 5, which is known to degrade DDT. The concentrations in the minimal salts medium did not affect the growth of Vibrio sp. since the specific growth rates were similar to those obtained in the medium lacking this compound.

WE 018

Quantification of Alkane monooxygenase genes to monitor the biodegradation process in environmental field

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Prediction of pollutant biodegradation is essential for the correct management of the process. With the aim to find suitable methods to monitor bioremediation strategies, within the large collaborative project of 16-member consortium, BACSIN (Bacterial Abiotic Stress and Survival Improvement Network 1), we studied environmental samples from an area located in the Czech Republic. Hradcany is a former military airport highly contaminated with petroleum hydrocarbons and under air sparging bioremediation treatment (bioventing) since 1997. Alkanes (saturated hydrocarbons of different sizes and structures) are major components of oil spills, that can be efficiently degraded by several microorganisms. We focused on alkane (Alk) monooxygenases, a family of important enzymes involved in alkane degradation which catalyse the initial oxidation of the alkane substrate to a 1-alkanol in aerobic conditions. Could these genes be good candidates to monitor bacterial catabolic activities in bioremediation strategies? More than 60 homologues of Alk genes with high sequence diversity are known 2. We developed a molecular method based on TaqMan quantitative Real Time PCR (qRT-PCR) to quantify Alk genes of bacterial strains present in the Hradcany area. We analyzed 12 soil samples at different depths (3 from a clean area, 3 from a low contamination area, 3 from a medium and 3 from a high contamination area) and 4 groundwater samples from clean, low, medium and high contamination areas. Alk genes quantification is expressed related to microbial biomass levels in each sample and an increase of gene copy number is clearly detectable in some samples. This molecular method is very accurate and precise and allows identification and quantification of Alk genes during the degradation process in environmental samples e.g. groundwater and soil, contaminated by hydrocarbons. Our molecular approach can be used to assess the progress of bioremediation in a contaminated site.
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WE 019

Microbial ecology approach for evaluating the effects of contaminants on soil and water ecosystems

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Traditional parameters used for the Environmental Risk Assessment (ERA) of environmental contaminants are principally chemiodynamic and physico-chemical properties. Furthermore ecotoxicological effects on organisms in water and soil are assessed with standard acute and chronic ecotoxicologic tests on freshwater, marine and soil organisms.
Microbial communities are valuable indicators of the occurrence of disturbances due to exogenous physico-chemical stressors. The assessment of variations in microbial community structure is of basic importance in order to evaluate the impact of an environmental stressor.
Complex microbial communities may serve as ideal and ecologically relevant toxicity indicators. A number of microbiologically driven processes has been proposed to evaluate the effects of xenobiotics on ecosystems. Proteins, genes, metabolites, or lipids that, when expressed, present a pattern of molecular change in an organism in response to a specific environmental stressor, can be defined as environmental biomarkers.
The number of techniques to study microbial communities has increased exponentially over the last 20 years and the advent of culture-independent methods, such as molecular biological techniques, has changed the view of microbial diversity. Among these techniques epifluorescence microscopy ones, such as direct count of bacterial abundance (DAPI count), vitality (Live/Dead

cell viability assay), and Fluorescence In Situ Hybridization (FISH) have been showing effective tools for studying specific microbial populations in soil and water ecosystems. We show how the applications of these techniques to contaminated soil and water ecosystems allow to highlight the presence of particular bacterial groups involved in chemical degradation.

WE 020

Root exudates from sunflower as a powerful chemoattractant for pollutant-degrading bacteria and its effect in bacterial dispersal

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Bacterial movement through saturated porous media may be restricted by several factors, including increased path lengths (tortuosity), bacterial adhesion to surfaces, and geometrical restrictions due to pores of small diameter and dead ends. We propose bacterial chemotaxis as a mechanism that could enhance bacterial transport through saturated porous media. Chemotactic bacteria can therefore constitute a useful vector for relevant catabolic activities and/or nutrients in bioremediation projects.

We studied the chemotactic response of *Pseudomonas putida G7* towards root exudates of *Helianthus annuus* and its individual components (organic acids and aminoacids) by capillary assays. In well-controlled column systems, we studied the effect of these compounds on bacterial transport in porous media.

Our data suggest that *Helianthus annuus* exudates are a powerful chemoattractant for *Pseudomonas putida G7* and it could be used in bioremediation, improving bacterial dispersal.

WE 021

Chemoeffectors change the swimming pattern of chemotactic bacteria and its transport in porous media

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Bacterial chemotaxis causes movement to higher (attraction) or lower (repellency) compound concentrations. Our working hypothesis was that positive and negative chemoeffectors change the bacterial motility pattern of individual cells and this has a direct effect on deposition in porous materials. We studied the chemotaxis response of a naphthalene-degrading strain (*Pseudomonas putida G7*) to several compounds by capillary assay and its motility behavior by computer-assisted motion analysis. We also assessed the influence of these chemoeffectors on bacterial strain deposition in sand. When bacteria detected a positive chemoeffector, changes of direction of individual cells were less frequent and smooth movement increased. The bacterial deposition on the surface sand was low, thus promoting bacterial transport through sand. However, when bacteria detected a negative chemoeffector, the changes of direction were much more frequent and abrupt. This also had a direct effect in bacterial transport, promoting collision efficiency and bacterial deposition.

Positive or negative chemoeffectors may modulate bacterial transport in terms of pollution to be treated.

For localized and surface contamination in soils and aquifers, negative chemoeffectors would help to confine chemotactic bacteria in the contaminated site. In contrast, treatment of more dispersed areas of contamination and at a certain depth can be benefited with the use of positive chemoeffectors that increase the transport of chemotactic strains.

WE 022

Influence of rhizosphere and root tissues on mineralisation of 14C-hydrocarbons in a pristine pasture soil

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Many plant species enhance the biodegradation of petroleum hydrocarbons, but the specific mechanisms by which this occurs are not clearly understood. Rhizospheres and crushed root tissues from 4 plants were screened in soil spiked with 12C/14C-hydrocarbons (phenanthrene, naphthalene, hexadecane or octacosane) dissolved in toluene to achieve a concentration of 10 mg kgð'1 and activity of approximately 83.3 Bq g-1, respectively. Among the plants tested include Reed Canary Grass (Phalaris arundinacea), Channel Grass (Vallisneria spiralis), Black Raspberry (Rubus occidentalis) and Goat Willow (Salix caprea). The effects of rhizosphere and root tissues addition on the catabolism of 14C-phenanthrene, 14C-naphthalene, 14C-hexadecane or 14C-octacosane to 14CO2 by indigenous microbial communities was assessed in freshly spiked soil slurry. It was found that rhizospheres amendment was as effective as crushed root tissues. Comparison of the degradation potential of the effective root tissues suggested that phytochemicals were the major substances that stimulated the apparent constitutive activities involved in degradation of PAHs. For a better understanding of the plant-microbe interactions involved in rhizosphere-stimulated mineralisation, the hypothesis that phytochemicals stimulate PAHs degradation will be investigated further by examining degradation of phenanthrene and naphthalene in soil amended with α-pinene, p-cymene or a mix of monoterpenes (α-pinene, limonene and p-cymene in 1:1:1 ratio). This study supports the claims that the abilities of various plant species to stimulate mineralisation of organic contaminants in soil are highly variable ranging from negative or no effect to highly stimulatory effects. These findings provide insight into the mechanisms by which plants enhance degradation of PAHs, and may have practical application for remediation of PAH contaminated soils.

WE 023

Biodegradation of a polycyclic aromatic hydrocarbon in a soil co-contaminated with metals

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Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are among mixed pollution of major concerns in the soil environment. Overtime PAHs and metals become less bioavailable. In this present study, the impact of metals (zinc and copper admixture) on phenanthrene biodegradation was investigated over 63 day incubation period. The effects of zinc and copper concentrations (0, 50, 100, 500 and 1000mg/kg) on phenanthrene biodegradation, the effects of 'ageing' on bioavailability of these contaminant mixtures and their impact on bacteria measurement during phenanthrene biodegradation in soil were assessed, using respirometry and bacteria enumeration. The presence of zinc and copper at low concentrations (50 and 100mg/kg) had no significant effect on phenanthrene biodegradation, but phenanthrene biodegradation was significantly reduced at higher Zn and Cu concentrations, 'Ageing' significantly increased phenanthrene catabolism in Zn and Cu-amended soil at low concentrations, but the development of phenanthrene biodegradation was significantly reduced by the presence of Zn and Cu at higher concentrations. Bacterial cell counts decreased significantly as contaminants aged in soil but phenanthrene biodegradation correlated with increase in bacteria enumeration. Biodegradation efforts to remove polycyclic aromatic hydrocarbon in the presence of high concentrations of mixed-metals might be impeded. An insight into longer term ageing of metals and PAHs in the soil is necessary in order to assess the risk of these co-contaminants in soil.

WE 024

Impact of biochar and wood vinegar on the sorption/degradation of glyphosate in fine sandy soil

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Burning of wood in the absence of oxygen produces three end products: gases, solids (biochar) and liguids (wood vinegar). When biochar is returned to soil several positive effects on soil quality can take place, such as reduced leaching of N fertilizers, enhanced plant growth, and altered pesticide behaviour in soils. Wood vinegar is a new innovation in plant protection working against various weeds, harmful insects and molluscs. It consist of over 10 000 chemicals, such as asetic asic, formaldehyde, ethyl-valerate and furfural. In our preliminary studies we observed that BOD addition had positive effects on soil microbial activity. However, nothing is known about the combined influence of biochar and wood vinegar on the soil processes.

The purpose of our study was to explore the effects of biochar and wood vinegar (both originated from Betula sp.) additions on soil organisms and plants. The effects of the two substances on the retention/degradation of nutrients (N, P, K, Ca, Mg and S) and a herbicide (glyphosate) in an arable soil was also investigated. The experiment, conducted in a green house, consisted of 80 flower pots (1,5 l) filled with sandy arable soil. At the initiation of the study four treatments were established, each with 20 replicates: soil mixed with 1) biochar, 2) biochar and wood vinegar, 3) wood vinegar and, 4) control (neither biochar nor wood vinegar). The volume of the added biochar was 25000 kg/ha and that of wood vinegar 5000 kg/ha. English ryegrass (Lolium perenne) seeds were sown on half of the pots in each treatment and after 45 days half of the pots were treated with glyphosate (4 L/ha). Soil samples were taken 3, 48 ja 87 days after initiation of the study for the analysis of microbial activity, biomass of nematodes, nutrient and glyphosate concentrations. Furthermore, after each soil sampling the pots were irrigated with 3 dl of tap water, and the percolates were analysed for nutrients and glyphosate. The biomass and glyphosate concentration of plants were measured 88 days after the initiation of the study.

EC06 - Fate and exposure modelling including scenario analysis

WE 029

Applicator risk assessment of pesticides used in Korean orchard

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This research was carried out to propose the Korean method for estimating the agricultural occupational pesticide exposure level and estimate the risk of pesticide applicators who use high toxicological pesticides in korean orchard. The UK-POEM (UK-Predictive Operator Exposure Model) was proposed as a bench-marking model and analysed its performance properties. To extrapolate the Korean agricultural conditions, application equipment, application method, work rate per day, application volume and spraying time of pesticide was surveyed for Korean 204 orchard farmhouse. This survey indicate that the major application equipments are speed sprayer(64.9%) and motor sprayer(33.9%). When they sprayed the pesticide with a speed sprayer, they worked for more than 4 hours on area of 4 ha per day. In case of using motor sprayer, they worked for more than 4 hours on area of 1 ha. Based on the above survey result, Korean method for estimating the pesticide exposure level of agricultural worker was proposed finally.

The risk of pesticide applicators was calculated as above method. The class II (highly hazardous) pesticides used in korea were 17 products, and 11 products were spray type pesticides. The using information based on the pesticide label and the data searched through survey of actual condition on pesticides were used for calculate the pesticide applicator exposure dose. The risk quotients of these pesticides against the pesticides applicator were calculated as devide pesticide exposure dose by reference dose which were presented by EFSA(European Food Safety Authority), JMPR(Joint FAO Meeting on Pesticide Residues), and US/EPA(United States Environmental Protection Agency). Omethoate showed the highest risk quotient and the values were 338 and 75 when the applicator spray using speed sprayer and motor sprayer respectively. Risk quotients of all class II pesticide were above 1. This result means that the risk potential of these pesticides is very high. These pesticides will be revaluation as Korean re-registration system.

WE 030

Development of environmental fate model for herbicides of paddy fields using grid-catchment integrated multimedia modeling system (G-CIEMS) for all Japan area

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In Japan, monitoring data were actively used for representative values of chemical concentrations in environment in order to estimate risk analysis. Although observed values have higher reliability compare to calculated values by an environmental fate model, observed values have temporally-spatially specific information for limited numbers of chemicals. In order to cover temporally-spatially variable concentration for enormous amount of chemicals, model calculation should be applied for extensive ecological risk analysis.

Pesticides are absolutely necessary for steady supply of crops and efficient management of farmers. However, pesticides should be carefully controlled because of its original purpose of herbicidal, insecticidal, or fungicidal actions. Rice is a most major crop plant in Japan. Since schedules of rice transplanting collect to a specific period in each region, pesticides are also intensively used and run off to rivers. Herbicides are especially need to be regarded because these are thrown in paddy water and that why have high run-off ratio. For improving ecological risk analysis of pesticides, it is important to calculate weekly variation of pesticide concentrations in all rivers in Japan for many kinds of pesticides. To improve prediction accuracy is especially important during high level period that continuing from several days to about two weeks.

We had developed the multimedia environmental fate model G-CIEMS (Grid-Catchment Integrated Multimedia Modeling System) and Japanese GIS data set used for this model. In this study, we developed the method to predict daily variation of concentrations of many herbicides to paddy fields in all area in Japan. For developing the method, we collected, analyzed, and make a database of various kinds of relative information. We calculated daily emission amount of 5 kinds of herbicides for each river segment and air mesh, which data suitable for G-CIEMS model. In order to validate this model, we used national river survey results performed by the Ministry of Environment in Japan. To evaluate the reliability of this model, peak concentration and peak days were compared between predicted variations and observed variations for seven sites. Con-

centration differences between predictions and observations were almost less than one order of magnitude (16 sets / 18 sets). Peak day differences between predictions and observations were almost less than two weeks (16 sets / 18 sets).

WE 031

Realism of common approaches for normalisation of degradation rates - How to derive soil moisture?

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Degradation rates of plant protection products derived from field degradation studies have to be normalised to reference soil moisture and temperature (pF 2.0, 20°C) in order to make half lives comparable. These half lives can then be used for environmental fate modelling as recommended by the FOCUS work group on degradation kinetics (2006) or the EFSA panel (2010). Soil moisture and temperature are usually not (or only occasionally) measured in field degradation studies. Thus, they have to be calculated by hydraulic models like PEARL using the pedo-transfer function of van Genuchten-Mualem. In this project we compared different approaches (Rosetta, Hypres, German guideline, inverse modelling with PEST, best-fit to measured data) for the estimation of the soil hydraulic parameters needed for the van Genuchten-Mualem equation, and we compare the calculated soil parameters with measured values. Based on the different approaches we calculated the soil moisture and temperature for all FOCUS scenarios in PEARL 3.3.3 and derived correction factors needed for the normalisation.

The results show that the estimated soil hydraulic parameters vary strongly and consequently lead to significantly different correction factors for the soil moisture normalisation, resulting in different normalised half lives. The comparison between observed and simulated soil moisture showed partly huge differences and implausible results for simple approaches (e.g. Rosetta texture for soil type sand), but it could also be shown that parameters derived from measured water retention curves gave not the best fit to measured soil moisture. Thus, the most realistic way for normalisation would be to measure the soil moisture on a daily basis during the field experiments. Since such measurements are quite cost and time intensive, a convenient method for a realistic estimation of soil moisture is inverse modelling, e.g. with the software PEST, based on gravimetric water contents available from the residues analysis.

WE 032

Influence of climate data on estimated substance parameters of field accumulation studies

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Realistic degradation (half life DT_{50}) and sorption (Freundlich coefficient K_{om}) parameters of plant protection products may be derived from field experiments using inverse methods. For such evaluations soil and weather data are a necessary input. Ideally, climate data (rainfall, temperature, humidity[3DOTS]) are measured directly on the experimental site. However, often only data are available from weather stations some distance away (often about 10-15 km). In this study the potential error in DT_{50} and K_{om} is investigated due to the use of off-site rainfall data.

Two different error types are considered: (a) cumulative rainfall is different (± 10 and 20%) but the temporal rainfall pattern is identical for on- and off-site stations and (b) cumulative rainfall is identical, but the daily rainfall varies significantly.

Error type (a) is addressed by increasing/decreasing daily rainfall systematically by up to ± 20%. This represents the expected maximum difference in cumulative rainfall over a distance of 10-15 km. Error type (b) is addressed by creating artificial rainfall sets with identical statistical properties (e.g. wet/dry cycles, monthly rain distribution) as the on-site rainfall, but with very different daily rainfalls.

Changing the cumulative rainfall has only small influence on the water content of the soil (relative change < 5%). Larger changes were observed in the pressure head (>10%) and the cumulative percolation. From those hydraulic variables DT_{50} is essentially driven by soil water content. This explains why the DT_{50} is hardly influenced.

On the other hand K_{om} is expected to be more sensitive because the time to reach a certain soil depth is approximately proportional to the quotient of retardation and water flow. Thus an increase in water flow, i.e. rainfall, can be compensated by an increase in retardation, i.e. K_{om}, and vice versa. Differences in daily rainfall have only small influence on both parameters as long as the cumulative rainfall is identical. Only differences in the cumulative rainfall lead to evident changes of K_{om}.

It is concluded that the use of weather data from off-site stations is acceptable as long as differences in cumulative rainfall are sufficiently small. This can be expected for weather stations some distance away but located within the same geographical region. Substantial differences in daily rainfall (as can occur even within small distances) had only minor influence on the substance parameters obtained.

WE 033

The FOOTWAYS Pro online risk assessment platform: first real-world application in a French basin

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FOOTWAYS Pro is an online platform for pesticide exposure and risk assessment connecting a web portal with a high-performance computing cluster fully dedicated to pesticide fate modelling with MACRO and PRZM. The *FOOTWAYS Pro* approach builds partly on the FOOTPRINT methodology, but alleviates the existing limitations of the FOOTPRINT tools with respect to regulatory applications. In *FOOTWAYS Pro*, MACRO and PRZM results are newly and specifically generated in each assessment ("modelling on demand"). This leads to a much larger flexibility in model input and parameterisation compared to the pre-modelling approach in FOOTPRINT.

A *FOOTWAYS Pro* assessment consists of three major steps: 1. Assessment setup by the user through the web portal, 2. Modelling activities on the cluster; 3. Visualisation of outputs on the web portal (maps, CDFs, descriptive statistics and single time series of the different output variables, and the newly developed water quality management indicator PITSA).

A first real-world application of *FOOTWAYS Pro* has been implemented for an 800 km² basin, an area under intensive agricultural use which is also a major drinking water source. Local input data (1 : 250000 soil map, crop statistics at municipality level, local weather time series) were used to set up the agro-environmental scenarios for the study area.

On the web input interface, the user can choose between a number of local cropping practices with different levels of plant protection intensity and different combinations of applied products. It's also possible to specify new pesticide application programmes with a new combination of existing products. With the results provided on the web output interface, the user can i) compare the environmental performance of the various programmes for the different crops, ii) compare the risks to ground- and surface water due to crop protection measures between crops, iii) identify problematic areas and iv) explore the effect of mitigation measures. The study has a pilot character for the implementation of the Sustainable Use Directive and the national ÉCOPHYTO 2018 action plan in France.

WE 034

Fate of bifenthrin applied in pepper field-lysimeter

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To investigate runoff and erosion loss of bifenthrin from the field by rainfall, the influence of slope degree and length, ridge direction and plastic film mulching on runoff rate of bifenthrin from the pepper field were measured. The EC type formulations was applied at the rate of 5.6 g a.i./ha on June 21 2003 and it rained two and four days after the application. The wash-off rate was 2.1% of the applied bifenthrin. The average runoff losses of bifenthrin from a series of pepper grown-lysimeter plots were 0.33% for the mulched contour ridge plots, less than 0.1% for the mulched up-down direction ridge plots and 0.66% for the no-mulched contour ridge plots(control). The runoff concentrations of bifenthrin ranged from less than 0.1 μg L-1 to 0.2 μg L-1 for the mulched contour ridge plots, less than 0.1 μg L-1 for the mulched up-down direction ridge plots and from 0.1 μg L-1 to 0.2 μg L-1 for the controls. The average erosion rate of bifenthrin were 1.1% for the mulched contour ridge plots, 6.9% for the mulched up-down direction ridge plots and 1.2% for the controls. The average erosion rate of plots except the mulched up-down direction ridge plots were 0.39% for 10% slope-plots, 0.54% for 20% slope-plots and 2.51% for 30% slope-plots with different slope lengths. Residues of bifenthrin in soil at nine days after the application ranged from 0.006 mg kg-1 to 0.024 mg kg-1 except the soil under the mulch, and decreased to from 0.007 to 0.012 mg kg-1 at seventy one days after the application.

WE 035

Development of a GIS integrated dynamic surface water model to predict environmental concentrations of pesticides in an alpine stream located in Northern Italy

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The use of pesticides in agricultural areas can determine a chemical loading to surface water, which could be relevant in the exposure of non-target organisms such as macroinvertebrates. In order to evaluate such exposure, predictive approaches are needed together with tools to assess the risk for the aquatic ecosystem. A new dynamic aquatic model (DynaNet), which integrates a previously developed model (Dyna) with GIS tools, was developed and applied in order to calculate pesticide concentrations in water and sediment of an alpine stream located in the Non Valley of Trentino Alto Adige Region (Northern Italy), in which apple orchards are predominant. The environmental scenario was developed dividing the catchment in sub basins and classifying the stream in different links according to the Strahler order. The segmentation was done in a GIS environment and streams were connected by a downstream flow. Loadings data of water and pesticides deriving from treated orchards were obtained using the SoilPlus model, which was parameterized to describe average soil conditions for two pesticides. Pesticide use statistics in the valley were acquired from local authorities. Links were then parameterized using available environmental parameters and water flow rate data at a hourly time step. DynaNet model was then developed to predict fate of pesticides in the water/sediment system using a new adaptive time-step algorithm for the numerical solution of the mass balance differential equations. The model uses the variable volume storage method to calculate water flow variation during the rain events and to perform concentration calculations. Finally, the model was evaluated comparing predicted pesticide concentrations with concentrations measured at a sampling point located near the stream outlet.

WE 036

Site-specific theoretical risk assessment for pesticides: a case study

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Current procedure for the Ecological Risk Assessment are based on the comparison between a Predicted Environmental Concentration (PEC) and a toxicological end-point (L/EC50, PNEC, etc.). In Europe this methodology is usually applied for regulatory purpose on standard scenarios, assumed as representative of different European regions. Site-specific approach are nevertheless encouraged by new regulations (the Water Framework Directive is the first and best known example), in order to reduce the uncertainty factors due to environmental generalization.

In this work we employed classic ERA methods to a small scale scenario. We considered a mountain stream (Novella River) of Trentino-Alto Adige region, in the northern Italy. The watershed of Novella River is extensively used for the cultivation of apple orchards, and only narrow buffer zones are present between the river and the orchards; runoff events are then likely to cause significant loads of pesticides in the river. The PECs of all active ingredients used in the Novella basin were calculated with suitable models. Details are described elsewhere (Trenti et al., this meeting). Theoretical risk assessment approaches, based on the laboratory toxicity data available in the literature, were applied to the individual chemicals and to the mixtures of different composition that may occur in the river during the whole productive season. Risk was assessed using traditional approaches proposed by the European regulation on plant protection products as well as by applying suitable risk indices. Where possible, an SSD curve was built using the actual taxa of the considered community. Zoobenthos community of the river was monitored monthly before, during and after the treatment application period. The results of theoretical risk assessment are compared with changes in the natural community.

WE 037

TOP-RICE model: simulation of leaching to groundwater and exposure in ponds due to pesticide use in paddy rice fields

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The Pesticide Environmental Risk Assessment Project (PERAP) is a cooperation platform between Chinese and Dutch government bodies, research institutes and private sector. The

TOP-RICE model has been developed in the framework of PERAP. It enables the prediction of concentrations leaching to groundwater and of exposure concentrations in surface water (secondary ditches and natural ponds) due to pesticide use in paddy rice fields. The model will be used in pesticide registration in China.
The TOP-RICE model is based on the pesticide fate models PEARL and TOXSWA. PEARL simulates the fate of pesticides and metabolites in soils. With PEARL the concentrations leaching to groundwater can be calculated as well as concentrations in run off from paddy fields. PEARL uses the SWAP model to simulate water flow and heat transport. Processes covered by PEARL are pesticide transport, sorption, transformation, wash-off from plants and volatilization. TOXSWA simulates the exposure concentrations in surface water and sediment of pesticide and metabolites. TOXSWA incorporates processes such as transport, transformation, sorption to sediment and suspended solids and volatilization. Considered sources of contamination of the surface water and sediment are drainage, runoff and spray drift.
To account for a paddy water layer on top of the soil column, PEARL has been modified. PEARL now accounts for the water and mass balance of a paddy water layer and includes degradation processes in the water layer and runoff to pond and ditch. The dynamics of the paddy water layer is driven by the irrigation schedule and the crop cycle. TOXSWA has been extended with new concepts regarding the water and mass balance of a natural pond located in a lower area with no in- or outlet. Both models are coupled such that the runoff from the paddy rice field calculated by PEARL is input for the surface water calculations by TOXSWA.
Two scenarios were developed for pesticide use in paddy rice in China (one close to the Yangtze River and one in south China). These scenarios aim to represent 90th percentile exposure concentrations both for groundwater and surface water.
The TOP-RICE user interface enables the user to calculate environmental concentrations for these scenarios and for user-defined application schemes and substances. A special substance database has been developed that contains all relevant substance properties. This database can be used as a stand alone program or coupled to the TOP-RICE model.

WE 038
Using an integrated watershed model to identify potential measurement sites for passive pesticide sampling
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In the poster we present the concept and first results of a multiple-tiered study carried out in two catchments in Luxembourg. In this study we use an integrated watershed model to identify sites in the river course which are likely to have high risks of pesticide exposure according to the regional agricultural practice. The identified sites are then monitored with a passive sampler campaign in the coming year in order to test the model results. If the results of the passive sampling campaign agree with the modeling, the model outputs are further linked to an ecotoxicological model in order to evaluate the effects of pesticide exposure on the river biology.
For the watershed modeling we selected the widely known SWAT model which has also been applied in several pesticide fate simulation studies before. The model will be applied to the Wark and to the Mamer catchments. Both watersheds have an area of around 85km2 and a comparable share of arable land on the watershed area with 19% and 21% respectively. According to the official agricultural statistics winter cereals (mainly wheat and barley) as well as corn and oilseed rape are the main cultivated crops in the two watersheds. Both watersheds exhibit different geologic and hydrogeologic settings. In the Wark catchment soils are mainly underlain by impermeable bedrocks such as schist and marl. Here lateral subsurface flow and a quick catchment response to rain storms are expected. The Mamer catchment shows more heterogeneous conditions with soils underlain by permeable sandstone in the north and the centre of the catchment and soils underlain by impermeable marls in the south. Here the catchment discharge could be characterized by a mixture of groundwater contribution and lateral subsurface flow. Based on the different hydrologic conditions we expect also differences in the dynamics of pesticide exposure in both rivers.

WE 039
Recommendations for simulation calculations with FOCUS PELMO to predict environmental concentrations of plant protection products and their metabolites in groundwater (PECgw) in Germany
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In the national authorisation procedure in Germany the leaching behaviour of a plant protection product is determined in a stepwise procedure as described in the FOCUS Groundwater report (2009). The recommendations given in this paper are related to tier 1 and tier 2 of the FOCUS 2009 guidance.
The tier 1 assessment in the EU is based on the FOCUS 2009 standard scenarios. In the member state evaluation for Germany, a subset of the standard scenarios with climatic and soil conditions found to be relevant for Germany are taken into account (Hamburg and Kremsmuenster). The soils of the two scenarios cover the pH-ranges of agricultural soils and allow addressing the pH-dependent behaviour of compounds.
Normalised degradation rates may be used from either laboratory or from field dissipation studies. For the parameterisation of the degradation behaviour of an active substance and its metabolites in soil the recommendations of FOCUS with respect to the number of available DT50-values in different soils should be followed.
For the parameterisation of the sorption behaviour of an active substance and its metabolites in soil the recommendations of FOCUS should mainly be considered. With respect to the correlation of sorption to soil properties (pH, OC) additional detailed recommendations are given on the choice of appropriate sorption parameters for leaching assessment.
Furthermore recommendations are given on how to use other modelling parameters e.g., crop rotation, plant uptake factor, formation of metabolites, correlations / multi-correlations of substance parameters to soil properties, and application of statistical methods.
Tier 2 of the leaching assessment consists of more refined modelling approaches. This includes on the one hand providing data on specific processes e.g., surface degradation or non - equilibrium sorption and on the other hand the use of refined scenarios. The latter one is appropriate when the standard tier 1 scenarios are not representative of a specific crop or the relationship between compound and scenario properties.
The approach presented here should contribute to a future harmonisation of exposure assessment and risk management for plant protection products at European level.
In the abstract presented here tier 1 and tier 2 leaching assessments are described. Further recommendations are planned for the tier 3 and tier 4 assessments.

WE 040
Modelling and monitoring of short chain chlorinated paraffins in the Nordic environment
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Short chain chlorinated paraffins (SCCPs), also called polychlorinated n-alkanes, are mixtures of compounds of molecular formula $C_xH_{2x+2-y}Cl_y$ containing 10-13 carbon atoms and usually 30-70 % degree of chlorination. They have a range of industrial applications, and have been detected in numerous environmental compartments. There is concern regarding SCCPs due to their environmental persistence and their potential for bioaccumulation, adverse effects and long-range transport. SCCPs have been included in the UNECE LRTAP Convention, the priority substance list of the European Water Framework Directive, and are under consideration for the Stockholm Convention on Persistent Organic Pollutants. However, the behaviour and fate of SCCPs remain poorly understood, in part as the technical mixtures consist of thousands of isomers, enantiomers and diastereomers, which make analysis and modelling of these compounds very challenging. The purpose of this study was to explore a complementary modelling and monitoring approach to evaluate the overall understanding of the link between emissions of SCCPs, environmental levels and human exposure in the Nordic environment and to identify the more critical knowledge gaps. Data for emissions and physicochemical properties of SCCPs were gathered or estimated, and used to parameterize an integrated, non-steady state environmental fate and bioaccumulation model (CoZMoMan). Specific congeners of SCCPs were selected for the study to assess the extent of expected variation of environmental fate and behaviour within the multitude of compounds. Model results were next compared to reported environmental levels in the Nordic region. For compartments where environmental levels were scarce or lacking, targeted sampling and analysis was carried out to further evaluate the model predictions. Results from this study will be presented and discussed with emphasis on the more critical research needs with respect to the overall fate and exposure of SCCPs.

WE 041
Linking multimedia environmental and PBPK models to assess health risks of lead associated to drinking water - A case study
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Human exposure to chemicals through multiple pathways is classically estimated by the so-called 'multimedia models', calculating the distribution of contaminants among products of interest for humans (like drinking water, inhaled air, vegetables, meat, milk, etc). Combined to data describing human behavior (diet composition, time spent outside, etc), such multimedia models provide an estimation of the daily quantity inhaled or ingested by the population of interest. Once the exposure scenario is identified, the dose-response assessment is typically achieved by comparing exposure outputs (*e.g*, the daily intake) to reference doses, estimated from toxicological data. Coupling multi-media models for different exposure pathways with a generic physiologically based pharmacokinetic (PBPK) model for the human population enables to assess directly the impact of the exposure scenarios on the chemical's concentration in the target tissues.One aim of the European 2-FUN project (Full-chain and UNcertainty Approaches for Assessing Health Risks in FUture ENvironmental Scenarios) was to develop an integrated 'multimedia-PBPK' toolbox that also incorporates uncertainty and inter-individual variability analyses by Monte Carlo simulations, and different kinds of sensitivity analysis.In the present study, an integrated modeling approach was demonstrated for predicting internal tissue concentrations of chemicals by coupling a multimedia environmental model and a PBPK model. A case study was designed for a region situated on the Seine river watershed, downstream of the Paris megacity and for lead emitted from industrial zones in the region. The limited monitoring datasets of lead concentrations in bottom sediment and in raw river water, obtained at the downstream of Paris, were used to re-construct long-term daily concentrations of lead in river water. The re-construction of long-term series of lead level played a key role for the intermediate model calibration (conducted in multimedia model), and thus for improving model input to PBPK model. In order to take into account the parametric uncertainty in the model inputs, some input parameters relevant for the multimedia model were given by probability density functions (PDFs); some generic PDFs were updated with site-specific measurements by a Bayesian approach.This case study demonstrated the feasibility of a full-chain assessment combining multimedia environmental predictions and PBPK modeling, including uncertainty analysis.

WE 042
Scenario analysis for reducing priority pollutants in receiving water using integrated dynamic urban fate models
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The Water Framework Directive (WFD, 2000/60/EC) has the objective of a catchment oriented water quality protection for all European waters with the purpose of achieving a good ecological and chemical quality status by the year 2015. To that end, necessary measures should be identified and implemented, with the aim of progressively reducing pollution from priority substances. The objective of this poster is to demonstrate how a dynamic model of the integrated urban wastewater system (IUWS) can be used to test different emission reduction strategies for organic priority pollutants (PPs) in a semi-generic case study on DEHP. The IUWS is composed of coupled entities: sources, urban catchment surface (run-off/infiltration), sewer system, stormwater treatment unit, wastewater treatment plant (WWTP) including sludge handling, and receiving surface water (river). State-of-the-art dynamic fate models were selected from literature. Dynamic DEHP release profiles were estimated and inputted in the model to predict the fate and concentration of DEHP in each IUWS sub-system. The model was then used to test eight scenarios on environmental performance, namely (1) reduction of impervious urban area, (2) reduction of infiltration in the sewer system, (3) input reduction (excluding the main pollutant sources), (4) separating the combined sewer system, (5) treatment of stormwater by stormwater infiltration ponds (separate sewer systems), (6) placement of retention basins at main sewer junctions, (7) sand filtration of secondary effluent, and (8) pre-precipitation of phosphorous. The simulation results revealed that the most effective measure in terms of river water quality improvement for DEHP (annual average and spikiness reduction) and PP concentration in the disposed WWTP sludge heavenly

depends on the parameterisation of the scenarios. It can be concluded that dynamic modelling tools are very useful in the WFD context, as they allow to check compliance of the PP's river concentration with both types of environmental quality standards (the maximum allowable MAC-EQS and the annual average value AA-EQS). Furthermore, these models can be used to test the impact of different emission reduction strategies on the river water quality status. Testing different measures before their actual implementation in real life can help to make better choices in terms of resources, and as such costs can be saved.

WE 043

Multimedia assessment of pollutant pathways in the environment - European scale screening model (MAPPE-Europe)

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The work gives an updated overview of the structure, functions and algorithms used in development a modelling tool for pollutants pathways in air, soil and surface and sea water at the European continental scale. The algorithms are implemented as an extension for ESRI ArcGIS 9.3. The tool is called MAPPE after Multimedia Assessment of Pollutant Pathways in Environment of Europe.

The purpose of the model is to provide a user-friendly way to convey the wealth of geographic data available to model the fluxes and concentrations of pollutants emitted by industrial activities and other point emissions, or chemicals widespread use within households, urban environments or agriculture. The intended applications include organic contaminants such as pesticides, pharmaceuticals, VOCs, and other industrial POPs.

The maps of chemical concentrations and fluxes produced by the model can be used for screening level assessment, at continental scale, of risks to human health and ecosystems, evaluation of policies and scenarios. The model is a complement both to more detailed, site specific assessment procedures, and to generic tools such as EUSES, aimed at non-spatial risk assessment in contexts such as the management of priority substances of concern for soil, water and air, the control of effects of environmental pollution on human health and ecosystems, and the sustainable management of agro-chemicals.

The original version of MAPPE has been used in several case studies - e.g. for PCBs, dioxins, gamma-HCH, while model variants served in investigations of Pyrethroid pesticides, PFOS/PFOA or common surface water contaminants including pharmaceuticals and personal care products.

WE 044

Multimedia assessment of pollutant pathways in the environment: a Global spatially explicit fate and exposure model (MAPPE-Global)

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The work describes a spatially explicit fate and exposure model with global coverage for Multimedia Assessment of Pollutant Pathways in the Environment (MAPPE Global). The model computes removal rates of a substance with given physico-chemical properties in an evaluative environment, composed of atmospheric boundary layer, soil, inland and seawater, in the form of maps with a spatial resolution of 1°x1°, by capitalizing on a comprehensive global data set of landscape and climate parameters.

The maps of removal rates enable the computation of concentrations from emissions of a chemical in one or more environmental compartments using a simple box model, except for the stream network where a plug flow scheme is adopted. Four types of environmental processes are included, namely: chemical degradation; advective transport; diffusive transport; and sinking to deep ocean.

MAPPE Global does not compute explicitly chemical transport in space, but only the fate of a substance at each location by calculating mass fluxes of chemical that are available for transport outside of the cell, in addition to concentrations from local emissions.

Grounding on these features, the model can be only used when at least one of the following conditions is met: (1) emissions are uniform in space; (2) the effects of emissions away from the emission location are not of interest. However, the model could answer questions related to the spatial differentiation of fate in response to variability of emissions and chemical fate processes. Moreover, the estimates of mass fluxes available from each cell for global transport can be used to feed more complex global ocean and atmospheric transport models. Therefore, in principle the model output can be compared to any other spatially explicit fate model.

The model was run for a set of 34 representative organic chemicals under different emission scenarios, such as multiple source vs. point source emissions, in order to identify situations for which spatial differentiation in the life cycle impact assessment of toxic chemicals should be considered relevant, especially in the interest of practitioners.

WE 045

EUSES, ECETOC-TRA, IUCLID-CHESAR, EASYtra: which regulatory environmental risk assessment tool to use?

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In June 2007, the new European regulation REACH entered into force. One of its aims is to improve the protection of human health and the environment from possible effect of chemical compounds. In order to achieve this goal, the so called risk assessment has to be performed for each substance. In general, RA is based on the knowledge of the chemical hazard, i.e. the intrinsic properties of a chemical, and the so called exposure which depends on many parameters such as the amount used, the type of process, the percentage release. Briefly, once the amount of exposure and the hazard are known, their ratio constitutes the risk assessment: if the exposure is higher than the hazard there is a risk and further investigation are necessary.

Although the main principles of RA are easily understandable, there are a great number of parameters and scenarios to consider to obtain the risk assessment. For this reason, over the last years free, commercial and homemade software appeared to perform the risk assessment. Among them, which is the best software? Does it exist a software which is robust, easy to use and regularly updated?

To attempt to answer these questions a critical evaluation of three free softwares (Chesar, EcetoxTRA, EUSES) and a commercial one (easyTRA) using several environmental risk assessment scenarios has been carried out. Several parameters have been evaluated; among them user friendly, minimum number of data needed, external need for simulated data, output and cost. Our results indicate that an ideal software does not yet exist: the best choice still rely on the parallel utilisation of more than one software, thus increasing the weight of the "personal preference"factor in the final choice of the ERA tool.

WE 046

BETR Global - a geographically-explicit global-scale multimedia contaminant fate model

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Multimedia mass-balance models are well-established as scientific and decision-support tools for understanding the behavior of chemical pollutants in the environment, especially persistent organic pollutants. Global-scale multimedia mass balance models have been particularly important in establishing the link between chemical emissions in temperate, industrialized regions and their presence in the Arctic. Global models that were developed to study transport of persistent chemicals to the Arctic describe the environment as a set of latitudinal zones, and are spatially discretized only along a north-south axis. In 2005, the first version of the BETR Global multimedia contaminant fate model was introduced. It described the global environment as a set of linked multimedia regions on a 15o [GREEKX] 15o grid. Several modifications have been made to the BETR Global model since 2005, including changes to the model algorithms and updates of the model's database of environmental characteristics. Here, we introduce two new software implementations of the BETR Global model, BETR Global V.2.0, and BETR-Research. Both new versions incorporate all model updates made since 2005, and both are being made publically available for direct download as open-source software. BETR Global 2.0 is coded in Visual Basic for Applications (VBA) as an add-on for Microsoft Excel, and is based on the same code-base as the 2005 version of the model. BETR-Research is a new implementation of the model in Python that is more accessible for model modifications and thus more versatile, but that lacks the graphical user-interface of the VBA version. During development of these new versions of the model a thorough code-review was performed, which increased the quality and validity of both BETR implementations. We illustrate the new version of the model using a case study of the global fate and transport of decamethylcyclopentasiloxane (D5), which can be replicated using the VBA version of BETR Global 2.0 by following the tutorial on the BETR Global website, http://sites.google.com/site/betrglobal/home. The BETR-Research Python implementation of the model can be downloaded from http:// betrs.sourceforge.net.

WE 047

A dynamic vegetation model: development and integration with an existing dynamic air/soil model

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Plant biomass has been shown to exert a decisive influence on the fate of chemicals in the air/soil system. Uptake and clearing rates of a number of species have been experimentally determined in the past decade as well as the role of forest canopies, capable of filtering air and transfer chemicals from air to the litter/soil environment. Recently, plant/air partition coefficients for several species were determined in field conditions and the parameters describing the biomass development with time were measured for a number of forest types. Additionally, a multimedia fate model (AirFug) which incorporates a rather dynamic air system was developed and showed that air concentrations could considerably change during a diel cycle. AirFug allows to predict concentrations in soil and in two air compartments, which change in height and volume according to the variation of mixing height, predicted basing on meteorological observations. Such dynamic atmospheric compartment is capable to show rapid air concentration changes. To evaluate the influence of such rapid changes on vegetation behaviour, a dynamic bioaccumulation model of organic contaminants in vegetation biomass was developed in order to calculate leaf contaminants uptake from air. In this model, formulated in terms of fugacity, the vegetation compartment is composed by a multi-specific forest canopy. The bioaccumulation equations employed to simulate uptake by leaves calculate the plant/air partition coefficient (Kpa) using Koa (octanol-air partition coefficient) and some forest structural parameters such as specific leaf area (SLA) and leaf area index (LAI), which vary with time, environmental and ecological conditions. This vegetation biomass model was finally integrated with AirFug to predict the effect of plants in regulating the fate of organic contaminants in a system where a double-layered air compartment interacts dynamically with multi-layered litter and soil compartments. Simulations were performed for some Semivolatile Organic Compounds (SVOCs) in order to evaluate the role of air concentration variations and soil degassing in influencing the change of foliar biomass concentrations with time.

WE 048

Aquatic exposure models - Where do we stand in Switzerland?

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For the aquatic risk assessment in the context of pesticides registration, concentrations in surface waters are predicted using deterministic models. The idea is that a realistic worst-case exposure of aquatic organisms is calculated in order to assess a generalized risk and to formulate risk mitigation measures for the whole country.

In this poster we challenge such calculations by investigating the variability of the factors affecting run-off and drainage. The loading of pesticides to surface waters via run-off and drainage depends on numerous factors, such as the application rate, crop interception, degradation in soil, timing of rain events, distance to the water body, size of the water body and dilution due to rain water and flow velocity. Some of these factors vary with local conditions, such as intensity and frequency of rain events, topography, application technology and the size of the water body, but are treated as constants in the model calculations.

Switzerland is a country with very diverse climatic and topographic conditions. Therefore, a generalization of these diverse regions to a single scenario leads by default to over- or underestimation of the concentration in surface waters and potentially to inadequate risk mitigation measures. We show the range of some parameters and its effect on the predicted concentration in order to verify the assumption that the model leads to the calculation of a worst-case exposure.

WE 049

A model to predict the fate of chemicals in the soil/air/water system: development and evaluation of the effectiveness of in-situ treatment after a diesel spill

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The rupture of underground storage tanks and accidental oil and fuel spills are two of the most common causes of soil and groundwater contamination. Remediation often represents a time consuming and expensive task and requires different approaches depending on a number of parameters, such as the types of contaminants involved, their distribution in the impacted media and the present or future targets potentially exposed to hazards. Some in situ remediation technologies are directed to enhance the degradation of chemicals; however, a precise timing for such operations is often difficult to estimate and frequent measurements must be performed in order to verify the adequacy of the adopted measure. In this context, models could represent a helpful tool in planning management strategies. In order to develop and calibrate an integrated modelling approach capable of quantifying the effectiveness of different remediation technologies, a monitoring campaign was started in a site located in the Mantua Province (Po Valley, Northern Italy), where an accidental diesel spilled had occurred. Soil and groundwater samples were collected during three times, in order to assess (1) the role of natural attenuation after two months from the first sampling and (2) the effect of the multi-phase extraction (MPE) technology after two additional months. The site was also monitored for a number of parameters, such as groundwater level, dissolved oxygen and temperature, etc. Bench-scale biodegradation experiments in pulsed flow conditions were also carried out to assess the biodegradation of some of the hydrocarbons detected in soil under natural and oxygen-rich conditions. In the present work, a preliminary version of the model, which couples a multimedia fate box model describing the air/soil system (SoilPlus) and a water flow model (MODFLOW-2005) and incorporates equations capable of describing the diesel pure phase movement, is presented. The model can be used to evaluate the fate of single chemicals in soil unsaturated and saturated zones and to predict the enhanced degradation/volatilization consequent to an MPE treatment. Results of the model were also evaluated in the context of the risk analysis required by the Italian legislation.

WE 050
Predictive performance of mercury fate model for multimedia environment
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The predictive performance of Mercury Fate Model for Multimedia Environment (MFM-ME) was developed and evaluated for the mercury compounds. The MFM-ME Model is a multi-box dynamic multi-compartmental model to simulate the fate of the mercury compound. The modeling region is the Andongho watershed that is located in the eastern midpart of Korean peninsula. The model was evaluated by comparing the predicted relative concentrations with the measured relative concentrations. The relative concentration (Ci/Csoil) in this study refers to a ratio of the concentration in a medium i (Ci) to that in soil medium (Csoil) and was proposed to use to avoid the problems of uncertainties or lack of emission estimates. The concentrations of the mercury compounds in air, water, sediment, and soil media are predicted within an order of magnitude. The fluxes of the mercuty compounds between media are predicted within an order of magnitude. The predicted compositions of mercury compounds in air, water, sediment, and soil media is similar with those of the measured data of Andongho watershed. The assessment of MFM-ME model indicated that main source of mercury compounds in water is deposition process in air media and run-off process from soil media. The sediment of Andongho is final sink of the mercury compounds

WE 051
Screening and prioritizing organic chemicals based on far-field human exposure
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Globally, scientists and regulatory programs seek to identify chemical substances that may pose risks to humans and the environment. Measured chemical property and monitoring data are limited compared to the large number of chemicals requiring evaluation making it necessary to develop, evaluate and apply models and Quantitative Structure-Activity Relationships (QSARs) for screening and priority setting. The Risk Assessment, IDentification And Ranking (RAIDAR) model is an evaluative, regional scale, multimedia mass balance model that combines chemical emissions, fate, and aquatic and terrestrial food web bioaccumulation to estimate concentrations in ecological receptors and humans. This holistic modeling framework is being applied to screen and rank approximately 13,000 chemicals for far-field human exposure using concentrations in an adult human as a comparative endpoint. Guidance for improving mass balance model input requirements to reduce uncertainty in predicted human concentrations was obtained through sensitivity and uncertainty analyses conducted in concert with the initial screening and ranking. The analyses highlighted a need to reduce uncertainty in key parameters, most notably chemical emissions estimates and biotransformation half-lives in humans. New methods and models have been developed in an effort to reduce uncertainty in the model input parameters for emissions estimates and degradation half-lives. The updated ranking of the 13,000 chemicals is compared with the initial ranking results. Future plans to further reduce uncertainty in the combined mass balance and QSAR screening approach are discussed.

WE 052
Further development of open source routines for fitting kinetic models to chemical degradation data
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One of the cornerstones of environmental fate modelling is the derivation of kinetic rate constants from experimental studies. Two extension packages for kinetic evaluations written in the R programming language have been previously presented. They facilitate the evaluation of experimental data from environmental degradation and metabolism studies for the parent compound (kinfit) or for parent and metabolites (mkin) with a focus on the evaluation procedures used in the registration of pesticides in the European Union.
One feature not present in other kinetic modelling tools is the ready-to-use implementation of the single first-order reversible binding (SFORB) model for metabolites. This entails the possibility to use formation-decline kinetics for metabolites also in cases where the metabolite decline does not follow single first-order (SFO) kinetics.
Other kinetic models that describe a change in net degradation rate over time that are commonly used in the kinetic evaluation of pesticide degradation are not easily adaptable for the description of metabolite kinetics. The implications of the use of the SFORB kinetic model for environmental fate modelling of metabolites are discussed.

WE 053
A high-throughput method to screen organic chemicals in commerce for emissions
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"There are thousands of organic chemicals in commerce, yet the environmental fate and effects of most substances remain largely unknown". This is probably among the most well-known clichés within environmental chemistry and toxicology. Accordingly, a hot topic on both the research and regulatory agendas is trying to identify organic chemicals which may represent a hazard to the environment and humans, based on individual attributes such as persistence, bioaccumulation, toxicity and long-range transport potential - or combinations thereof. However, only chemicals which combine these harmful properties with emissions in significant quantities are expected to represent a real risk. Here, we present an attempt to develop a high throughput method to screen chemicals in commerce for emissions, building upon the approach outlined in the EU Technical Guidance Document. While doing so, efforts were directed towards proving estimates of the uncertainty in the resulting emission scenarios. This is considered vital, not only because important input data are fragmented or inaccessible, but also because screening or categorization methods which solely rely on threshold values are susceptible to generating a significant number of false positive and false negative categorizations. The emission screening tool is being integrated into a large effort to screen for exposure that also seeks to account for the uncertainty in other input parameters. The latter results are, in turn, expected to identify a sub-set of substances for which more accurate emission estimates are needed at consecutive tiers.

WE 054
Climate variability and POPs environmental behaviour: a focus on the organic carbon content in marine systems
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Understanding and predicting the environmental distribution of persistent organic pollutants (POPs) in the environment under different climate scenarios requires that both primary emissions and re-emissions to the atmosphere from reservoirs in the environment are taken into account. Another important factor to be taken into account in studying POPs fate and transport is given by the coupling of air and phytoplankton systems, where biologically driven cycles can act to remove POPs from the atmosphere into the deep sea.
A level III fugacity model was developed and applied on the Adriatic Sea to study the environmental fate and transport of polychlorinated biphenyls (PCBs) under a present climate scenario and under a future climate change scenario. The objective of this study was to evaluate the effect of a climate change scenario on the environmental PCBs distribution considering also PCBs' primary emissions and re-emissions. This study aimed also at quantifying the mass of PCBs associated with the carbon pool under the two climate scenarios in order to address the role of the marine compartment as a sink for atmospheric pollutants under a warming climate.
This model application highlighted a clear need to further improve our understanding of the fate of PCBs related to the organic carbon (OC) content trends in the marine environment.
There is indeed an ongoing discussion on the OC trend content under a climate change conditions, in fact biogeochemical models' results do not always agree on the estimation of the OC content under a warming climate. Model comparisons show that the reason for this is mainly dependent on the nature of observations taken into account by the different biogeochemical studies.
Results from the study on the fate and transport of selected PCBs in the Adriatic Sea under a climate warming scenario show that OC content plays an important role in driving chemicals' concentrations in the marine environment, hence it is of interest to identify the trend of OC content under warming climate conditions. Here we want to compare the different results found in literature starting a discussion on OC content trends under climate warming in the marine ecosystems.

WE 055
Long range transport versus source region exposure potential: effect of dynamic climactic scenarios
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Rainfall patterns exert a strong effect on the chemical fate and transport of a large range of water soluble and particle bounds chemicals. Heavy rainfall in source regions prevents long range transport of these chemical species to remote regions, and heavy rainfall in remote regions efficiently deposits them in surface media. However, long range transport is most effective if there are primarily dry conditions in the intermediary regions. When the correct rainfall conditions exist in the source, intermediary and remote regions then chemicals we would normally expect to be strictly source region problems can be quite effectively transported and deposited in remote regions. An attempt is made to quantify this effect and describe the sensitivity of chemical transport to variable climactic scenarios.
On a regional scale the interaction of climatic factors such as rainfall and temperature with the seasonal cycling of organic matter leads to peaks and valleys of chemical concentrations in air and other exposure relevant media. Extrapolated to a global scale this means that some climactic zones contribute more significantly to long range transport of chemicals during particular seasons but may be more susceptible to local emissions during other seasons. Chemical fate simulations have been performed on global and regional scales and the results are used to quantify the human exposure potential to remote and local emissions during different seasons and under different climatic conditions.

WE 056
Screening-level exposure scenarios for personal care products in China and India
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The development and application of models to predict in-river concentrations of down-the-drain chemicals (i.e. those used in household and personal care products and pharmaceuticals) are important components of the environmental risk assessment process. While such models (e.g. EUSES and GREAT-ER) exist in Europe, there are currently no available models to estimate exposure of personal care products many developing countries. To this end, work was performed to create a GIS-based system that develops scenarios used to predict the fate of "down the drain"chemicals into freshwater and marine ecosystems.
Predicted Environmental Concentrations in surface water (PECsw) were generated for China at the county level (sub-province) and for India at the district level (sub-province). These geographic units were considered the smallest appropriate spatial analysis unit for each country. PECsw were based on both user supplied product information, as well as other geographically-linked socio-economic and environmental information from official census and other data sources. Product information such as category (e.g., hair, skin, etc.), composition (e.g., ingredient fraction) and "take off "values (GDP threshold under which the product would not be purchased) were used to distribute total tonnes of individual ingredients used in each country. Product use information was available at a regional level (6 regions in China and 4 regions in India). These data were combined with county-level economic information, population density (including urban and rural separation), dilution factors, and disposal mechanism (e.g., STP, septic, direct discharge to river, etc.) to estimate ingredient-level PECs in surface water.
The method presented incorporates the inherent spatial variability of the model inputs so that patterns can be identified and used in the risk assessment. Results identify combinations of model inputs resulting in PECsw distributions, with the ability to identify realistic high-exposure scenarios in the context of the overall country-wide distribution. The results from this modelling are at a more spatially detailed level than previously possible in thes countries. The approach was standardized so it can be applied with very few changes to generate refined datasets for other developing countries with similar data availability challenges.

EC08 - Tracking community consumption of illicit drugs and other substances by measuring human metabolic residues in urban wastewater

WE 060

Year-long community level measurement of drug use using passive methods with in situ calibration

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The determination of the exposure of a population to a chemical by analysis of metabolites in wastewater (sewage epidemiology) is a relatively new field within environmental chemistry. Fluctuating, trace concentrations of these polar organic compounds in a complex environmental matrix presents a significant analytical challenge. Passive sampling is a monitoring tool that may overcome these difficulties by offering time integrated sampling over a period of several weeks and also achieving lower detection limits. Challenges in applying these techniques include the lack of both an exposure correction method and an adequate uptake model. The aims of this study were therefore to calibrate a passive sampling device in situ for narcotics and their metabolites and to furthermore use these methods to estimate community drug use over a period of one year. Polar organic chemical integrative samplers (POCIS) were exposed at the sewage treatment system for western Oslo (Norway) over a 5 week period. Subsequently POCIS were exposed in exactly the same manner at exactly the same place for two week periods over the course of a whole year in order to determine trends in drug usage. Following exposure, POCIS were extracted and analysed for target compounds by LC-MSMS. Most of the target compounds were accumulated in POCIS and showed linear uptake during the exposure period. Sampling rates were in the order of tens to hundreds of mL d-1 allowing detection limits below ng L-1 in most cases. Results from the year long survey are discussed in terms of the challenges faced by implementing these techniques in a quantitative way, i.e. from passive sampling accumulations to acceptably accurate estimations of drug use per unit population per unit time. Furthermore ongoing work characterising other similar compounds such as the tentative identification of controlled steroids in POCIS extracts is briefly presented. Passive sampling techniques may offer significant advantages over traditional grab sampling methods for measuring polar compounds in wastewater. The current sampling device was able to accumulate most of the target compounds, metabolites of commonly used narcotics and can likely be applied to many more similar compounds. However, more work is required characterising the uptake kinetics of these samplers under varying conditions in order that data can be used quantitatively.

WE 061

Selective determination of illicit drugs by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry

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In 2005, Zuccato et al. related sewage measured levels of benzoylecgonine, the main cocaine metabolite, to local drug abuse, beginning a trend that has been extended to other illicit drugs and followed by many other researchers. But indeed, the accuracy of this strategy depends on the quality of the analytical methodology employed for the measurement of drugs in wastewater. Most published analytical methods rely on solid-phase extraction (SPE) for analytes' enrichment and liquid chromatography-tandem mass spectrometry (LC-MS/MS) on triple quadrupole instruments (QQQ) for quantification. However, one of the main drawbacks of LC-MS/MS is the occurrence of matrix effects during determination. Also, at least a second confirmation transition is necessary in order to properly identify a positive on a QQQ system, which can also be problematic in complex samples, e.g. for amphetamines due to their low *m/z*. Thus, the main objective of this work was the increase in selectivity during the sample preparation process and LC-MS determination, in order to minimize the aforementioned problems, for the determination of 24 drugs of abuse and metabolites in wastewater. This was achieved by the use of a mixed-mode SPE sorbent (Oasis MCX) with a fractionated elution protocol, where the neutral and acidic compounds were eluted first with methanol and the basic analytes were eluted subsequently with basified methanol. This protocol resulted in less matrix effects (15% to 40% mean signal suppression) as compared to others employed in the literature (ca. 50% to ca. 60% mean signal suppression). Additionally, the performance of the quadrupole-time-of-flight (QTOF) instrument was evaluated, in order to improve the selectivity of LC-MS/MS determination. Due to its high resolution and mass accuracy, only a single MS/MS transition is required, but even the second one can be recorded with a higher mass accuracy than a QQQ. Moreover, the modern Agilent system tested provided an excellent linearity (R2>0.99) in the 1-1000 ng/mL range and LODs in the 0.2-4.3 ng/mL range. Overall, the method provided recoveries in the 80-120% range for most analytes and LODs, after SPE, between 1 and 20 ng/L. A further advantage of the QTOF system is the fact that it acquires simultaneously full scan MS and targeted MS/MS data. Thus, a database of accurate masses of more than 90 illicit drugs and metabolites was created and samples could be screened for the presence of new drugs reaching the black market.

WE 062

Monitoring of drugs of abuse in Dutch sewage water by LC-LTQ FT Orbitrap MS

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The presence of drugs of abuse in the water cycle has spurred researchers to monitor their occurrence in wastewater, surface waters and drinking water. Data obtained have been used to calculate environmental loads and removal efficiencies of sewage treatment plants (STPs), and also to estimate consumption and usage trends in the population. The objective in this work is to illustrate the potential of LC coupled to an LTQ FT Orbitrap MS for qualitative and quantitative analysis at ng/L level and to present, for the first time, results of an extensive monitoring study of 25 drugs of abuse and relevant metabolites in urban wastewater from the Netherlands.
From five different STPs of the Netherlands, 24-h composite samples were collected from 32 influents and 32 effluents, along one whole week. Sample pre-treatment consisted of SPE using Oasis HLB cartridges. The chromatographic separation was achieved within 30 min. Full-scan accurate mass spectra, from 100 to 600 Da, were obtained in positive-ion mode (ESI+) at a resolution of 30,000 FWHM. Along the complete chromatographic run, the mass spectrometer operated in a data-dependent-acquisition (DDA) mode, in which both MS and MSn spectra were acquired. In this way, highly confident information for identification, quantification and confirmation could be obtained in a single analysis.
In various influent wastewater samples, concentrations higher than 300ng/L were found for cocaine, benzoylecgonine, codeine, oxazepam and THC-COOH. Relatively high concentrations of amphetamine (~500 ng/L) and ketamine (~ 20 ng/L) were found in influents from a STP located in the south of the Netherlands. In general, concentrations of drugs of abuse in effluents were lower than those of influents, except for benzodiazepines and occasionally MDMA.
By performing additional PCA the analytical results could be evaluated semi quantitatively and allowed to have a better insight on drugs abuse and sewage water treatment efficiency in the Netherlands.

WE 063

Monitoring and uncertainty assessment of cocaine and benzoylecgonine wastewater loads in Switzerland

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To check the effectiveness of campaigns preventing drug abuse or indicating local effects of efforts against drug trafficking, it is beneficial to know consumed amounts of substances in a high spatial and temporal resolution. The analysis of drugs in sewage has the potential to provide this information. Here, we present two analytical procedures to determine cocaine (COC) and its main metabolite benzoylecgonine (BE) in sewage treatment plant (STP) influent and effluent water. One is based on solid phase extraction followed by gas chromatography-mass spectrometry (GC/MS), the other uses direct injection high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). In addition, we provide novel experimental data from several Swiss STPs and suggest a simulation-based method to assess the total uncertainty of the monitored substance loads from flow measurements, sampling, and analytics. The GC/MS method was then applied to acquire a 14-days profile of COC and BE in the STP wastewater (WW) of Berne. In addition, weekend and Wednesday samples were analyzed from the STPs of Zurich, Basel, Geneva, and Lucerne. With 1521 ng/L, the highest COC concentration was found in Geneva WW. With 2900 ng/L, the maximum BE concentration was measured in Zurich WW, collected after a mass rave event. For Berne, the estimated mean daily consumed amount was 107 ± 21 g of pure COC, corresponding to 321 g of street-grade COC. We were able to show that for WW loads of COC and BE in catchments with more than 100'000 inhabitants, the analytical uncertainty is the dominating influence factor and that random errors from sampling are expected to be in the same range as that of flow measurements.

WE 064

Potential of LC-QTOF MS for investigation of drugs of abuse in the aquatic environment

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In this work, liquid chromatography coupled to a quadrupole time-of-flight mass spectrometers (QTOF MS) has been used for the investigation of emerging contaminants (e.g. drugs of abuse (DOA)) in the aquatic environment. QTOF MS offers interesting features in this field: full-spectrum acquisition with satisfactory sensitivity, accurate mass measurements of the analyte molecule and its main fragments, strong potential for screening purposes, and the possibility to investigate fragmentation pathways in order to appoint specific fragments.
A large-scope screening was made in water samples. The use of a QTOF instrument enables the simultaneous application of two acquisition functions with different collision energies: a low energy (LE) function, where none or poor fragmentation takes place, and a high energy (HE) function, where fragmentation in the collision cell is promoted. Data obtained are processed after MS acquisition using specialized software and databases allowing the search of a large list of LC-MS amenable contaminants.
A compound database was created, including theoretical exact masses and empirical formulas of around 1000 potential emerging contaminants (including 80 DOA). Since the software first processes data of the LE function, the database mainly consists of exact masses of the parent compounds (typically the protonated molecule). Mass error and isotopic fit of accurate mass data compared with theoretical information indicate possible positives samples. Then, HE spectra with accurate mass fragmentation data are used for a reliable identification. Reference standards were available for 250 compounds (20 DOA), and experimental data (e.g. retention time and fragmentation data) could be obtained in these cases, facilitating an unequivocal confirmation. Besides strong identification purposes, accurate-mass data allows elucidation of the chemical formulae of product ions and is therefore of great value for correct interpretation in the study of

fragmentation pathways. Correct interpretation also permits more rational selection of structural specific fragments, which can be used for searching related compounds, such as metabolites and TPs. In this way, a possible TP of cocaine was discovered after performing chlorination under controlled laboratory conditions.
LC-QTOF MS is highly suited for screening of DOA in aquatic samples and to discover potentially hazardous related compounds.

WE 065
The stability of illicit drugs and metabolites in the aquatic environment - implications for sewage epidemiology
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The stability of nine illicit drugs and metabolites (cocaine (COC), benzoylecgonine (BE), ecgonine methyl ester (EME), amphetamine (AMP), methylenedioxymethamphetamine (MDMA), methamphetamine (METH), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and 6-monoacetylmorphine (6-MAM)) in surface and wastewater was evaluated in 36-hour experiments at representative pH-values and temperatures. Blank surface and wastewater was spiked with relevant concentrations of each compound, based on published concentrations in surface and wastewater. At fixed time intervals (e.g. each hour), 100 mL aliquots were collected, internal standards were added for quantification and the aliquots were stored at -20°C in the dark until analysis. Analysis was done with a validated method based on solid-phase extraction and liquid chromatography tandem mass spectrometry (LC-MS/MS) method. All collected aliquots were analyzed in duplicate the day after the collection. The stability for each compound at each time was calculated as a percentage of the initial concentration present in the water. Generally, BE, METH, MDMA, MTD and EDDP showed a high stability while for COC, EME, AMP and 6-MAM significant degradation was observed. As could be expected, a faster degradation occurred with higher temperatures of the water. Differences between surface and wastewater were observed; a higher degree of degradation in wastewater was observed possibly due to the higher content of micro-organisms. A thorough evaluation of the stability of illicit drugs and metabolites in the aquatic environment is of great importance in sewage epidemiology. If degradation during the transport from the place of excretion to the wastewater treatment plant occurs, measured concentrations in wastewater should be corrected for this. Ignoring stability issues in wastewater can lead to serious underestimations in sewage epidemiology.

WE 066
Estimation of the collective consumption of illicit and licit drugs in the city of Zagreb using sewage epidemiology approach
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The work presented in this paper reports on the application of sewage epidemiology approach for the estimation of the collective consumption of illicit and licit drugs in the city of Zagreb
The study was performed in 2009 at the central wastewater treatment plant of the city of Zagreb. Selected illicit and licit drugs were monitored in 24h-composite samples of untreated wastewater over a period of eight months. The analytical procedures used for the determination of selected drug target residues (DTRs) included solid phase extraction and subsequent analysis by liquid chromatography-tandem mass spectrometry (LC-MS-MS). The collective consumption of a number of selected drugs, including 5 illicit drugs (cannabis, heroin, cocaine, amphetamine, MDMA) and several licit drugs belonging to therapeutic opiates and antimicrobials was estimated for the city of Zagreb, based on the representative mass loads of the corresponding DTRs, and the average excretion rates, reported in the literature. The average daily mass loads of studied DTRs varied in the range 1.6 -122 g/day. The observed mass loads were relatively uniform during the investigated period for most of the studied drugs. However, stimulating illicit drugs, including cocaine and amphetamine-type drugs, exhibited a clear weekday-dependant pattern, characterised by an enhanced consumption during weekends. The consumption of the illicit and licit drugs determined in this study was compared with the available official figures and observed agreements and disagreements were discussed.

EP01 - Alternative flame retardants: Environmental exposure, fate and trends

WE 069
Biodegradability and toxicity of halogen free flame retardants
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Several halogenated flame retardants have been banned due to their persistency, bioaccumulation potential and toxicity (PBT) and probable environmental risk. Hence, alternative flame retardants are required and a range of new flame retardants have been proposed. The PBT properties of most alternative flame retardants, however, have not been tested. Therefore, the aim of the present study was to experimentally determine the ready biodegradability and aquatic toxicity of halogen free flame retardants (HFFRs). Thirteen HFFRs were selected, including 6 organic phosphates (Aluminium Diethyl Phosphinate, Bisphenol A bis(diphenyl phosphate), Dihydro Oxa Phosphaphenanthrene, Melamine Polyphosphate, Resorcinol bis(diphenyl phosphate), Triphenyl Phosphate) and 7 inorganic compounds (Aluminium Trihydroxide, Ammonium Polyphosphate, Antimony Trioxide, Magnesium Hydroxide, a silicate based nanoclay, Zinc Hydroxy Stannate and Zinc Stannate). To estimate the persistency of the organic HFFRs, we applied a modified standardized ready biodegradability test, using the RespicondTM apparatus to make the test suitable for a higher number of chemicals. To determine the acute toxicity of the 13 HFFRs, the compounds were subjected to 48 h immobility tests with Daphnia magna. To test the effects of the HFFRs on a primary producer, we performed a 4 h acute test with the alga Scenedesmus subspicatus, using Pulse Amplitude Modulation Fluorometry to quantify impairment of photosynthesis. These three tests combined provide a relatively fast and effective approach for screening a selection of HFFRs on ready biodegradability and acute aquatic toxicity. Testing the persistency and toxicity is the first step in assessing the environmental safety of these alternative flame retardants.

WE 070
Identification of the flame retardants Ethylene bis(tetrabromophtalimide), TBBPA diallyl ether and TBBPA bis(2,3-dipropyl ether) in environmental samples using LC-APPI-tandem-MS/MS
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An analytical method using liquid chromatography atmospheric pressure photoionization tandem mass spectrometry (LC-APPI-MS/MS) was developed for analysis of the current use brominated flame retardants (BFRs) Ethylene bis(tetrabromophtalimide) (ETBPI), Tetrabromobisphenol A diallyl ether (TBBPA AE) and Tetrabromobisphenol A bis(2,3-dibromodipropyl ether) (TBBPA DBPE). The developed method was applied on diverse environmental samples obtained from three regions in Norway, including waste water, sewage sludge, seepage water, and sediment. ETBPI was detected in seepage water taken close to a metal recycling facility; TBBPA AE was detected both in seepage water and sediment, and TBPPA DBPE in waste water and seepage water. The identities of the BFRs were verified by accurate mass measurements using LC-APPI coupled with high resolution mass spectrometry. The analytes were found in few samples in ng/L levels. To our best knowledge this is the first detection of ETBPI and TBBPA AE in environmental samples. The analyzed samples were taken during a survey of new BFRs in the Norwegian environment and in these samples we also quantified PBDEs and other more hydrophobic BFRs. Reported levels of ETBPI, TBBPA AE, and TBBPA DBPE are thus compared with the regularly monitored BFRs including BDE209.

WE 071
Hexabromocyclododecane (HBCDD) - a brominated flame retardant of high concern
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Brominated hexabromocyclododecane (HBCDD or HBCD) is used mainly as a flame retardant in polystyrene-based insulation products. Of particular concern is the capacity of this lipophilic and persistent organic pollutant to accumulate in the food chain, leading to progressively increasing levels in human tissues and in wildlife. The extent of accumulation correlates directly with its ever-more prevalent use. Despite this alarming trend, only limited toxicological information is available to assess its long-term implications for health or the environment. Nevertheless, HBCDD continues to be used despite the availability of alternatives. The European Chemicals Agency has recently in 2008 identified HBCDD as 1 of 14 substances of "Very High Concern", and in September 2010 HBCDD was added to REACH Authorisation List. In October 2010 a committee under the Stockholm Convention has assessed the risks from HBCDD and concluded that HBCDD fulfils the criteria of a persistent organic pollutant (POP), and the committee recommended a global ban of HBCDD use. This presentation provides a succinct up-to date overview of HBCDD's properties and discusses the risks associated with its prevalence in our homes and immediate environment.

WE 072
Levels and distribution of Dechlorane Plus in sediments from three coastal bays, North China
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Dechlorane Plus (DP), which was first synthesized in 1970s, has widely been used as a substitute for Dechlorane and brominated flame retardants. Both syn- and anti-isomer have widely been detected in air, water, sediment, biota and human serum, but little information is available for marine sediment which is one of the major sinks for persistent organic pollutants (POPs).
This study focused on DP levels and distribution in marine sediments from three Chinese bays ,i. e. Jiaozhou, Sishili and Taozi Bay in North China.
The top 5cm of the sediments were collected at 48 sampling sites of the three bays. The DP concentrations ranged from 0.6 to 189 ng/kg dw (mean 24.1 ng/kg dw) in Jiaozhou Bay, 0.3 to 138 ng/kg dw (mean 64.8 ng/kg dw) in Sishili Bay and 0.6 to 67 ng/kg dw (mean 34.1 ng/kg dw) in Taozi Bay, respectively. The contamination level was comparable with that of rural river sediments from China (~160 ng/kg dw) and of sediments from Lake Winnipeg (30±3.2 ng/kg dw), and it was dramatically lower than that in the sediments close to manufacturing plants, for example, Huai'an canal, China, (1860 to 8000 ng/kg dw) and Lake Ontario, Canada. (2.23 to 586 ng/g dw).
The fsyn value is defined as the syn-isomer/(syn-+ anti- isomer) to identify possible sources of DP in the environment. The fsyn values of sediments from Jiaozhou Bay (mean 0.29) were close to technical mixture (0.35 to 0.40), probably indicating a local usage of DP as a flame retardant. In contrast, sediments in Sishili and Taozi Bay showed much lower fsyn values (mean 0.17). Sishili and Taozi Bay were more open than the semi-closed Jiaozhou Bay, and receive sediment input from the Bohai Sea.
The sedimentation rates were 0.704 cm/a in Jiaozhou Bay and 1.24 cm/a in Taozi and Sishili Bay. The DP inventories from 2006 to 2010 were estimated to be 0.33 kg for Jiaozhou Bay, 0.49 kg for Sishili Bay and 0.37 kg for Taozi Bay, respectively.

WE 073
Seasonal trends in concentrations of organophosphorous flame retardants in coastal surface water
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In the late 1970s organophosphorous flame retardants (OPFR) were detected in the environment for the first time. Due to the fact that OPFR are used as substitutes for the prohibited polybrominated diphenylethers (PBDE) an increase in the OPFR production is expected.
Varying alkyl- and aryl-ester groups, some of them halogenated, lead to a large variation in the physico-chemical properties - ranging from very polar and volatile (e.g. trimethyl phosphate) to non-polar and non-volatile (e.g. tri(ethylhexyl) phosphate). Therefore they can be transported in different environmental media and have been detected in various environmental compartments. Especially the halogenated OPFR are supposed to be highly persistent in the environment. Several OPFR are known to be toxic (e.g. carcinogenicity) and additionally, the lipophilic OPFR

have the potential to bioaccumulate.
This study focused on the seasonality of OPFR concentrations in the estuary of the River Elbe and the German Bight (North Sea).
Surface water samples were taken during four cruises of RV Ludwig Prandtl in the estuary of the Elbe in March, May, August and October 2010 and three cruises of RV Heincke in the North Sea in March, July and September 2010. The samples were extracted using solid phase extraction (SERVA SERDOLIT PAD III, dichloromethane) and analysed by GC-(EI)-MS. Quantification of 19 different OPFR was done using 5 deuterated OPFR as internal standards (TMP-d9, TEP-d15, TPrP-d21, TBP-d27, TPhP-d15).
14 and 11 OPFR were detected in the Elbe and the German Bight respectively. The decreasing concentrations of OPFR towards the open sea is mostly due to mixing processes with sea water which is approved by the linear correlation of OPFR concentration to salinity. In August an additional depletion probably due to enzymatic reactions might occur. Concluded from the ratio of halogenated to non-halogenated OPFR halogenated OPFR seem to be more stable to this depletion. In the German Bight the sum concentration of OPFR is higher in spring than in summer (100-300 ng/L in March and 20-60 ng/L in July), but the number of individual OPFR compounds is lower. In the non tidal influenced part of the Elbe the sum concentration of OPFR ranged from 200 (May) to 700 ng/L (August).
In conclusion, this study shows a seasonal variation of OPFR in riverine and marine surface water. In summer, non-halogenated OPFR are affected by biodegradation leading to decreasing concentrations while halogenated OPFR are more stable.

WE 074
Occurrence of chlorinated organophosphate flame retardants in soil
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Restrictions on the use of some polybrominated diphenyl ethers (PBDEs) have resulted in an increased consumption of chlorinated organophosphate esters (OPEs) as alternate flame retardants. This resulted in a ubiquitous detection of chlorinated OPEs in the environment but, surprisingly, we have found no published data that address their occurrence in soil.
In this study, the spatial distribution of tris(2-chloroethyl) (TCEP) and tris(2-chloroisopropyl) phosphate (TCPP) in soil samples was investigated. Soil samples were collected between April and November 2010 from four different locations in Germany: (i) Frankfurt, city centre (ii) Osnabrueck, city centre, (iii) Osnabrueck, 6 km distant from the city centre, and (iv) 3 km distant from the village Jengum. The selected sampling locations were each surrounded by different building and traffic densities which decreased from sampling location (i) to sampling location (ii), (iii), and (iv).
Samples were freeze dried and sieved (2 mm), equilibrated for 24 h, and extracted for 12 h using 150 mL of toluene in a Twisselmann apparatus. Extracts were evaporated to dryness, resolved again in a mixture of 1 mL methanol and 13 mL of tap water, ultrasonicated for 60 min and filtered. 7 mL aliquots were spiked with TnBP-d27 and analysed by solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). A one way ANOVA ($p > 0.05$) was performed to analyse the influence of the sampling location on the concentrations of TCEP and TCPP in soil.
Mean concentrations (n=3) varied between 1.75 and 13.5 ng g-1 for TCEP between 1.23 and 8.33 ng g-1, for TCPP. Mean TCEP concentrations decreased from sampling location (ii) to (iii), and (iv) whereas mean TCPP concentrations (n=3) decreased from sampling location (i) to (ii), and (iii). Results of ANOVA demonstrated a significant influence of the sampling location on the concentration of TCEP (F2.6=127.6) and TCPP (F2.6=102.4). Our results demonstrated that atmospheric deposition leads to a contamination of soil with chlorinated OPEs with a significant influence of building and/or traffic density. However, these are the first data on OPEs in soil and more data are needed to clarify the atmospheric deposition processes. The results indicate that atmospheric deposition must be considered in existing risk assessments for TCEP and TCPP.

WE 075
Study of novel brominated flame retardants (nBFRs) in biota and sediment samples from the UK
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Brominated flame retardants (BFRs) have been widely used to avoid or control fires. The most used BFRs have been the polybrominated diphenyl ethers (PBDEs) and biphenyls (PBBs), tetrabromobisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD). In recent years, many studies have shown their widespread distribution, persistence and bioaccumulation capacity in different food webs [1]. For this reason, several directives and regulations have recently restricted or banned the use of some of these compounds. As their replacements, a range of 'novel' brominated flame retardants (nBFRs) are being used, including decabromodiphenyl ethane (DBDPE), bis(tribromophenoxy) ethane (BTBPE), ethylhexyl-tetrabromobenzoate (TBB or EHTBB), bis(ethylhexyl)-tetrabromo-phthalate (TBPH), tetrabromoethyl cyclohexane (TBBPA-DBPE), hexachloro-cyclopentadienyl dibromocyclooctane (HCDBCO), pentabromotoluene (PBT), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB). Although little is known about these nBFRs, recent papers have shown the presence of some of these compounds in the Artic [2] and also their bioaccumulation capacity in the aquatic environment [3].
The analysis of nBFRs is usually carried out using analytical methods optimised for other BFRs, making the results obtained less reliable. For this reason, the need for development of improved multi-residue analytical methods for nBFRs has been highlighted [4]. Our study has focused on the development of analytical methods for the analysis of 17 novel BFRs (including allyl-tribromophenyl ether (ATE), bromoallyl-tribromophenyl ether (BATE), tetrabromo-p-xylene (pTBX), pentabromobenzylacrylate (PBBA), pentabromobenzylbromide (PBBB), tetrabromo-o-chlorotoluene (TBoCT), pentabromochlorocyclohexane (PBCC), octabromotrimethylphenylindane (OBIND) and tris-(tribromophenoxy)-triazine) in environmental samples. Different extraction and clean-up methods have been evaluated, including PLE, SPE and GPC, and an optimised method has been produced. This validated method will be used for the analysis of biota and sediment samples from the UK.
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WE 076
Comparison of PBDE and brominated flame retardant toxicity in zebrafish
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New flame retardant chemicals are being developed and used in consumer products due to the banning and phasing out of polybrominated diphenyl ethers (PBDEs). Unlike PBDEs, however, the toxicity and potential for environmental release of these newer compounds is largely unknown. The purpose of this study was to investigate the toxicity of a wide range of PBDEs in comparison to several brominated flame retardants. A variety of PBDEs were chosen for evaluation with varying numbers of bromines and varying locations of bromines (BDEs 28, 47, 99, 100, 153, 183, and 209). In addition, several non-PBDE brominated flame retardant, such as tetrabromobisphenol A (TBBPA) and its ethanol-derivative (TBBPA-OHEE) as well as hexabromocyclododecane (HBCD) and tetrabromophthalic anhydride (PHT4) were also examined. Embryos were exposed to a concentration gradient from 6 hours post fertilization (hpf) until 168 hpf. There was an inverse correlation between LC50 and log Kow for PBDEs. BDEs 28, 47, 99, and 100 induced increases in spontaneous movement, curved body axis, and mortality. BDEs 153, 183 and 209 did not elicit an effect at any concentration tested (up to 20 ppm). Tetrabromobisphenol A (TBBPA) and TBBPA-OHEE decreased spontaneous movement in a concentration-dependent manner. Furthermore, these two compounds induced different malformations than typical malformations (i.e., fin malformation, pericardial and yolk sac edema) seen with PBDE exposures. TBBPA induced adverse effects at lower concentrations and earlier in development than TBBPA-OHEE. HBCD, however, elicited similar responses in zebrafish as did PBDEs, although it did not induce mortality at any concentration over the seven-day exposure study. PHT4 did not alter behavior or induce malformations at any concentration tested; however, at 20 ppm it induced 100% mortality. As PBDEs are continued to be phased out, the flame retardants that replace them may pose new risks with unknown modes of action and biological activity.

WE 077
Effects of latitude and diet on levels and patterns of PBDEs in the eggs of a marine top predator in the North Atlantic
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In contrast to organochlorines, little is known about the factors which affect concentrations and patterns of PBDEs in biota. PBDEs have been detected in temperate latitudes and the high Arctic, however few comparisons exist of PBDE concentration and pattern across a range of different latitudes in the same matrix. The Great Skua (Stercorarius skua) is a long lived seabird, and as a top predator has the potential to bioaccumulate high levels of persistent organic pollutants. Its breeding range across the North East Atlantic has allowed comparison of PBDEs in eggs from 3 colonies; Shetland, Iceland and Bjørnøya, ranging from 60 to 74°N, to investigate the effects of latitude. Confining comparisons to one species controls for potential differences in ability to metabolise PBDEs, however Great Skuas are generalist predators so diet composition may also affect PBDE concentrations and patterns. Here we analyse PBDE data from eggs collected in 2008, in conjunction with diet data from pellet collection and stable isotope (δ13C and δ15N) analysis to allow further exploration of PBDE variation between the three colonies. Concentrations of PBDEs in the eggs are generally higher than those found in similar species in the North Atlantic. Although diet varied within and between colonies no relationships were found between trophic level (assessed using δ15N) and PBDE concentration. The pattern of PBDE varied between colonies with proportionally more congeners with a higher degree of bromination in the most northerly colony, in contrast to predictions based on global fractionation theory. However great skuas are migratory and pollutants in eggs may reflect wintering areas in addition to breeding colony. Geolocator data has identified two main wintering areas for the Icelandic and Bjørnøya birds, the Grand Banks in North America and Southern Europe and North Africa. In contrast Shetland great skuas winter only in Southern Europe and North Africa. Greater variation in PBDE pattern within the Icelandic and Bjørnøya colonies may be an indication of the influence of members of these populations wintering in geographically diverse areas.

WE 078
In utero exposure to polybrominated diphenyl ethers (PBDE); hair analysis to examine link to cryptorchidism
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Cryptochidism, or undescended or maldescended testis, is the most common birth defect of male genitalia. Its prevalence has been increasing over the past few decades. This may be due to an increase in the prevalence of anti-androgenic chemicals such as polychlorinated biphenyls, organochloride pesticides, plasticizers and fungicides. A newer group of chemicals, brominated flame retardants (BFR), are being implicated as endocrine-disrupting chemicals these chemicals are used worldwide in polymers that are incorporated into a variety of consumer products (e.g., textile, computers and televisions, insulating foam, electrical equipment and kitchen appliances). In order to quantify BFRs we will use hair levels of polybrominated diphenyl esters (PBDE's) as a biomarker of systemic exposure. This approach will allow for the determination of BFR levels that the baby was exposed to in utero. Thus our hypothesis is that there may be a link between the incidence of cryptorchidism in new born males and chronic exposure of the pregnant mother to environmentally relevant BFRs. To evaluate this we have developed gas chromatography with mass spectrometry assay in which we analyzed the hair of children ranging in age from newborn to 15 years of age for the presence of BDE-28, 47, 100, 99, 153, 154, 183 and 209. Twenty to 50 mg of hair was used for the analysis. The greatest variability was found with BDE-47 (49.9 pg/mg) and BDE-99 (35.4 - 1180 pg/mg) and BDE-209 was found in 75% of the samples. Further BDE's were found in all of the newborn hair samples tested suggesting that transplacental transfer of these xenobiotics does occur.

WE 079
Estimation of the content of brominated flame retardants in end of life products in Mexico

and the potential emissions

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Brominated flame retardants (BFRs) are synthetic additives that have been used in a variety of manufactured products, including foam cushioning used in furniture and plastic housings for televisions and computers, automobiles and large and small household appliances. The polybrominated diphenyl ethers (PBDEs) are a group of chemicals used as flame retardants that have persistent organic pollutants properties and are suspected to cause adverse health and environmental effects. For this reason, in May, 2009 were included in the Stockholm Convention as one of the chemicals of global concern. Currently, a large number of goods manufactured or imported to Mexico contain an unknown percentage of PBDEs since many of them are manufactured in other countries. In addition, the environmental fate of these substances is unknown and an inappropriate management might represent a potential risk to human health and the environment. In this study, the patterns of consumption, use and disposal of PBDE containing materials and products in Mexico from 1998 to 2008 in order to estimate potential risk from emissions along the life cycle of these materials. The methodology included: 1) review of official databases (INEGI, Customs, Economy Bureau, PRTR, US and Canada databases), 2) Official reports (OECD, Basel & Rotterdam Conventions, 3) Industry reports, 4) Polymer applications, 5) substance flow analysis, and 6) International regulatory framework. Preliminary results estimated that PBDEs released at the end of life of products might range from 5.6 tons in 1997 to 457.5 tons in 2008 with a mean yearly value of 237.40 tons for the period 1996-2009. As conclusion, restrictions on the import of PBDE containing materials might be implemented and disposal in landfills might be restricted.

WE 080

Polybrominated diphenyl ethers analysis in plastics using ultrasound assisted extraction

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Polybrominated diphenyl ethers (PBDEs) are a group of compounds used as additives in a wide range of materials to reduce the risk of fire. PBDEs comprise a total of 209 isomers. The IUPAC nomenclature consist in assigning a number to each compound (BDE-1 to BDE-209) conventionally called congeners and they are organized into 10 groups (mono to deca brominated). Three commercial mixtures of PBDEs are applied as flame retardants, Penta-BDE (45% Tetra-BDE, 45% penta-BDE, 10% other isomers), Octa-BDE (45% hepta-BDE, 33% octa-BDE, 12% hexa-BDE, 10% nona-BDE) and Deca-BDE (98% deca-BDE and 2% other isomers). PBDEs have been used in a variety of manufactured products at different proportions according to the manufacturer and according to the product they are used in. The aim of this study was to determine the content of polybrominated diphenyl ethers in samples of the plastic casing of a laptop computer using Soxhlet & ultrasound-assisted extraction (UAE). Analysis was developed by gas chromatography coupled to mass spectrometry (GC/MS) using toluene, ethyl acetate and methanol-isopropanol (1:1 v/v) as extraction solvents. The PBDEs: BDE-47, BDE-99, BDE-100, BDE-153, BDE-209 were characterized and quantified with good linearity (0.987-0.999) and repeatability (RSD, 0.7-2.8. The toluene was shown to be the best solvent for extraction of PBDEs in the analyzed samples. Different content of PBDEs were found in the samples ranging from 4.8 µg g-1 to 23.98 µg g-1. UAE showed equivalent results to Soxhlet with the benefit of requiring of a reduced time for the extraction stage.

WE 081

A comprehensive analysis of brominated, chlorinated and organophosphorus flame retardants by Gas Chromatography/Mass Spectrometry: optimization of ionization and detection techniques

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A multiresidue method based in gas chromatography coupled to quadrupole mass spectrometetry was developed to determine organophosphorus flame retardants (TCEP, TCPP, TDCPP, TPhP), PDBEs (BDEs 28, 47, 99, 100, 153, 154, 183 and 209), non-PBDEs flame retardants (PBEB, DPTE, HBB, HCDBCO, EHTBB, BTBPE, BEHTBP, DBDPE), bromophenols, bromoanilines, bromotoluenes and bromoanisoles. Two ionization techniques (electron ionization, EI and electron capture negative ionization, ECNI) and two acquisition modes (selected ion monitoring, SIM, and multiple reaction monitoring, MRM) were compared as regards to identification, sensitivity and quantification capabilities. Highest sensitivity, at expenses of identification capacity, were obtained by ECNI MS/SIM for most of the compounds analyzed, mainly for PBDEs and DBDPE, while 3 and 4 bromophenol, and TCEP and TCPP were not detected. The GC-MS in MRM was the most selective technique and permitted the identification of target compounds at the pg/uL level, and identification capabilities increased when real samples were analyzed. This method was further used to evaluate the presence and degradability of flame retardants in water within a waterwork facility. TCEP, TCPP, TDCPP and TPhP were detected in influent waters, and TCEP and TCPP were not degraded throughout the different treatment stages and were identified in finished water.

EP02 - Antimicrobial resistance in the environment

WE 084

Occurrence of antibiotic resistance and characterization of resistant genes and integrons in gram-negative bacilli (GNB) isolated from integrated fish farms in Zhongshan, southern China

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Gram-negative bacilli (GNB) isolated from four integrated fish farms in Zhongshan, South China were tested for antibiotic resistance, tetracycline resistance genes, sulfonamide resistance genes and class 1 integrons. The Kirby-Bauer disk diffusion method and PCR assays were carried out to test antibiotic susceptibility and resistance genes. High antibiotic resistances were found, especially for ampicillin (86%), tetracycline (73%), oxytetracycline (73%) and trimethoprim (58%). Out of 477 GNB isolates, 98.5% were resistant to one or more antibiotics tested. Multiple antibiotic resistance was found highest in animal manures. Tetracycline resistant genes and sulfonamide resistant genes were detected; tetA, tetC and sul2 were detected with a percentage of more than 50%. The intl1 gene was found in 416 isolates (87.2%). Four gene cassettes, aadA5, aadA22, dfr2 and dfrA17 were detected. To the best of our knowledge, this is the first report for molecular characterization of antibiotic resistance genes in gram-negative bacilli isolated from integrated fish farms in China and gene cassette array dfrA17-aadA5 was first detected in integrated fish farms in China. Results of this study indicated that fish farms may be a reservoir of highly diverse and abundant ARGs and gene cassettes. Integrons may play a key role in multiple antibiotic resistances with the antibiotic selective pressures, posing potential health risks to the general public and aquaculture.

Keywords: fish farm, antibiotic resistance, resistance genes, integrons, gene cassettes.

WE 085

Pollution Induced Community Tolerance (PICT) as a tool for environmental risk assessment of antibiotics

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Pollution Induced Community Tolerance (PICT) is a concept where toxicant-induced succession in a microbial community is quantified by an increase in tolerance of the total community to the compound in question. This increase is thought to reflect three possible effects; (i) the disappearance of sensitive species through direct intoxication and the proliferation of more tolerant species, (ii) the physiological changes that render the organisms less sensitive or, (iii) genetic changes such as acquiring mobile genetic material encoding more resistance. Traditionally, PICT has been used to study microbial communities based on physiological profiling or growth parameters and is often presented as dose-response curves. However, the causative effects explaining the PICT-responses are not uncovered using the traditional methods. In this presentation, we evaluate PICT as a tool for assessing the risk of introducing antibiotics into the soil environment using the synthetic antibiotic sulfadiazine as a model compound. The potential hazard of exposure of SDZ on a time scale was calculated using experimental and predicted properties of motility, volatility, persistency, bioconcentration and solubility of the compound. Finally, a preliminarily ecotoxicological and human risk assessment was conducted using both traditional methods and the PICT assays.

WE 086

Antibiotic resistance profiles of bacterial strains isolated from a treatment plant receiving waste-water from the manufacture of pharmaceuticals

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Environmental antibiotic contamination presents the potential to select for resistance factors that can be transferred between bacteria, contributing to the establishment of multidrug-resistant variants. In the present study we have investigated the antibiotic sensitivity pattern of bacterial strains isolated from a treatment plant that receives waste-water from 90 different Indian bulk drug producers. In the treated effluent from this plant, we have previously reported very high levels of antibiotics, predominantly fluoroquinolones, at concentrations up to 1,000,000 times greater than those normally observed in treated sewage effluents. The taxonomic identities of all isolates were determined by 16S rRNA gene sequencing, followed by screening their sensitivity to 39 antibiotics belonging to 11 different classes. All isolates were resistant to, at least, 4 antibiotics, thus conferring a 100% multi-drug resistant population, and the majority were resistant to more than 20 antibiotics. Our study suggests that the waste-water treatment plant studied is potentially serving as an enrichment site for multidrug-resistant bacteria, which could pose a major public health issue in the future.

WE 087

Influence of sulfadiazine on the dynamics of bacterial resistance genes in manure and manured soil

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In the last years, the relation between the use of antibiotics in animal production and the environmental occurrence of antibiotic resistance genes (ARG) has received growing interest. The implication of using manure from pigs treated with the sulfonamide antibiotic sulfadiazine (SDZ) as fertilizer for arable fields and pastures was investigated in a recent study (Heuer *et al*, SBB 2008). SDZ was administered as ^{14}C-labeled compound to pigs to investigate the fate of the drug and its metabolites and the antibiotic effect on resistance genes in manure and manure-amended soil. In soil, the extractable amounts of the compounds decreased to less than 1 mg kg^{-1} within 11 days after manure amendment. The abundances of SDZ resistance genes *sul1* and *sul2* were determined by qPCR relative to 16S rRNA genes in total DNA. With manure, high amounts of *sul1* and *sul2* were introduced into the soil, which were reduced by more than 10 times within 24 days. Thereafter, *sul1* was stable in soil, while *sul2* further decreased between day 60 and day 165. A mathematical model was developed that could well explain the time course of *sul* gene abundance by considering the cost of sul genes, horizontal gene transfer, and selection of resistant populations in the presence of SDZ. Modelling revealed a selective effect of SDZ on *sul2* even at low concentrations down to 0.15 mg kg^{-1} soil.

This study showed that an increase of ARG levels in the environment can be a direct consequence of current practice in animal production. The simultaneous application of ARG and antibiotics leads to a prolonged persistence of ARG in soil. The risk for ecosystems posed by ARG may be rather low, because the accumulation of resistance genes in the environmental reflects an adaptation of microbial communities to selective pressure. Adaptation of microbial communities lead to stabilized soil functions. Examples for an adaptation of e.g. ammonium oxidation in soil as key step in nitrogen turnover are known (Schauss *et al*, Env.Microb. 2009). For human health, however, increasing frequencies of ARG in the environment lead to a higher exposure towards ARG via raw foods or farmers with known consequences for health and economics. Application volumes of antibiotics in animal production should be reduced by downsizing stocking rates or immediate separation of single sick animals instead of preventive antibiotic treatments of complete livestocks. This would possibly reduce the selection of ARG in the environment.

WE 088

The genome of an extensively drug-resistant bacterium isolated from a wastewater treatment plant receiving effluent from antibiotic manufacturing

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Antibiotics save millions of lives every year and are crucial for fighting disease worldwide. How-

ever, the heavy usage also leads to antibiotic resistance - one of the most important challenges for the health care sector. As bacteria can move between environments, and resistance genes often are transferred horizontally between species, it is important to protect also the environmental bacteria from excess antibiotic exposure. We have sequenced the genome of a multiresistant strain of *Ochrobactrum intermedium*, a common environmental bacterium and opportunistic pathogen. This particular strain was isolated inside a treatment plant in India receiving waste water from 90 bulk drug manufactures and was found to be resistant to 36 of 39 tested antibiotics. Massively parallel pyrosequencing (454 sequencing) of its genome resulted in an average sixteen-fold coverage. Comparative genomics were used to analyze the genome of the resistant strain in relation to the already sequenced reference strain (*O. intermedium* LMG 3301). The analysis revealed structural differences between the strains including both larger deletions and insertions. Several point mutations were also detected in coding sequences as well as in ribosomal RNA. We identified twelve resistance genes in the isolate, compared to two in the reference strain. The quinolone target enzymes DNA gyrase and topoisomerase IV showed nine amino acid changes in the protein sequences for the isolate, one of which is known to cause quinolone resistance in *Escherichia coli*. There is also a considerable number of reads that do not map onto the reference genome, suggesting that the isolate has acquired large regions of novel genetic material. The results presented here demonstrate the power of second generation sequencing technology as well as the need for sustainable management of antibiotic waste.

WE 089

Survival and leaching of Tetracycline resistant bacteria and fecal indicators from manure in field scale experiments.

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The spreading of manure on agricultural land is an economic and practical solution for improving soil quality; however, animal manure frequently contains zoonotic pathogenic bacteria, such as certain Eschericia coli, Salmonella spp. and Campylobacter spp. The present experiment was conducted as a large multidisciplinary project. Pig manure with a natural content of Tetracycline resistant bacteria and fecal indicator organisms was followed in soil columns and a field scale experiment.

In the field experiment pig manure was injected into agricultural soil. The distribution and survival of natural occurring indicator bacteria around a manure slurry slit in the soil was followed. During a period of two months, sections of soils with different distance to the manure string were assayed to obtain information on survival and spread of bacteriophage, faecal indicators (Enterococci, Bacterioides, E. coli) and Tetracycline resistant bacteria. The die-off of the different organisms was quantified showing an extended survival close to the manure string. Genomic DNA from 400 Tetracycline resistant bacteria was isolated and their phylogenetic relationship was established using BOX PCR showing that the main Tetracycline resistant bacterial species is E. coli.

Drainage water from the field sites were collected weekly from one year prior to manure application, where no Tetracycline resistant bacteria were detected. For a period of 11 months following the first manure application, drainage water was sampled proportional to the flow and collected weekly. Selected storm events were intensively monitored by the collection of subsamples for every 2 mm of drainage runoff, using a refrigerated ISCO sampler. Drainage samples were tested for Tetracyckline resistant bacteria and fecal indicators. The highest concentration was found in the first drainage sample following manure application; however a fast decrease in cell numbers in the following drainage samples was seen. For the Tetracyckline resistant bacteria concentrations up to approximately 100 CFU ml^{-1} was detected.

In conclusion, the survival and environmental spread of indicator organisms show that the upper soil and drainage water are impacted by the microorganisms natural originating from pig manure under natural conditions.

WE 090

Relationship between geochemistry and antibiotic resistance in Scottish soils

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Links exist between heavy-metal and antibiotic resistance. Basically, these genes can either be found on same transferrable genetic elements (co-resistance) or a single gene confers resistance to both chemicals (cross-resistance). Therefore, the selection of one trait tends to confer resistance for the other. Most evidence exists in clinical samples (e.g., dental amalgams), but little evidence exists in the environment. Is it possible for background metal levels in the natural environment to promote the presence of resistance genes? Here, we examined the relationship between antibiotic resistance and certain geochemical conditions in Scottish soils, and we found that antibiotic-resistance gene abundance directly correlated with Cu, Zn and clay levels. The study suggests that geochemical conditions can impact the potential for resistance among soil micro-organisms. The extensive survey of soils done here, over wide range of geologic conditions, will help inform risk assessors understand background environmental conditions, which is essential to help drive policy and decision-making to protect agricultural and human health.

EP03 - Emerging per- and poly-fluorinated compounds: Source identification, environmental fate, and remediation strategies

WE 093

Physicochemical properties of poly- and perfluorinated alkyl substances (PFAS) calculated using COSMOtherm®

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Many man-made poly- and perfluorinated alkyl substances (PFAS) have been detected in the environment and human food, but there is still incomplete understanding of how they cycle and transform in the environment. To address this knowledge gap, environmental fate models have been applied to analyze the sources and distribution of some PFAS. However, lack of physicochemical data that are required as input data for the models has limited the focus of model studies to C8-C13 PFCAs and PFOS, and a limited number of their precursor compounds. Experimental determination of properties of PFAS is challenging and there are many compounds of potential interest. Therefore, computational methods are an attractive alternative for estimating properties of PFAS. A quantum chemistry-based software, COSMOtherm®, was applied to calculate properties for some PFAS in a previous study by Arp et al. [1]. In this work, we evaluate the performance of COSMOtherm® and extend its application to a much larger set of PFAS, including the most widely used perfluoroalkyl acids, their precursors and some important intermediates that have been identified in degradation studies. We evaluated the performance of the method using fluorotelomer alcohols as model chemicals, since measurement data exist. For these compounds, property data calculated by COSMOtherm® agree within one order of magnitude with the experimental data. We additionally applied the method to other PFAS for which no or very few data exist. Specifically, we calculated octanol-air, air-water and octanol-water partition coefficients (*Koa*, *Kaw* and *Kow*) for homologous series of sulfonic and sulfinic acids, phosphonic and phosphinic acids, olefins, iodides, phosphate esters, sulfonamides/-ethanols, and saturated and unsaturated aldehydes with poly- and perfluorinated chain lengths from 4-14 carbons. Consistency checks between different properties were made to assess the quality of empirical and modeled property data. The result of the calculations described here are a set of physicochemical property data that provide insights into structure-property relationships for PFAS. Within the same group, both *Kaw* and *Kow* increase with increasing fluorinated chain length, whereas for the same fluorinated chain length, the polarity of the functional group contributes significantly to the partitioning; e.g. *Kaw* decreases with increasing polarity of the functional group.

[1] Arp, H.P. et al. (2006). Environ Sci Technol. 40, 7298-7304

WE 094

Analysis of 18 perfluorinated compounds in blood by on-line turbo flow-LC-MS/MS

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Perfluorinated compounds (PFCs) have been manufactured since 40s due to their thermal, chemical and biological stability. The PFCs are persistent and bioaccumulative due their affinity to proteins. The aim of the present work has been: I)to develop an on-line methodology turbo-flow extraction to analyze 18 PFCs in biological samples; II)to assess the presence of PFCs in 30 serum samples.

150µl serum sample was taken and introduced into PP eppendorf with 150µl Acetonitrile, shaked 1 min and centrifugated at 4000rpm, 10min, in order to precipitate the proteins from samples.100µl of supernatant was introduced into PP insert vial. The extraction and separtation was carried out in a Thermo Scientific Aria TLX-2 system utilizing TurboFlow. Different extraction columns were tested during optimization. The final methodology include two extraction columns in tandem: Cyclone and C18 XL. The injection volume was 20µl at turbulent flow of 1.5ml/min water pH 3.4. The loop elution (250µl) was performed with water pH 3.4:methanol (20:80). Separation was carried out in a LC-column Hypersil GOLD PFP (50x3) and an extra-column was used after LC pumps in order to remove the contamination from the pumps. Triple mass spectrometer for analysis, equipped with a Turbo Ion Spray source (negative mode) and single reaction monitoring.

Lamp blood was used as a blank material during optimization process. Method limits of detection were established between 18-1114ng/L. The CCα error and CCβ error were calculated according ISO 11843 (CCα = 39 to 2030ng/L and CCβ = 53 to 2690ng/L). Recoveries were established between 50 - 169%.

The developed method was applied to 30 serum samples. The levels of PFCs were between 0.09 and 26.99 µg/L. The highest ones corresponded to perfluoropentanoic acid and perfluorohexanesulfonate (PFHxS). One of the most ubiquitous patten was observed for ion perfluorooctanesulfonate.

WE 095

Fully automated analytical method for the determination of perfluorinated compounds accumulation in human hair

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Perfluorinated compounds (PFCs) are ubiquitous contaminants in humans and animals worldwide [1]. Furthermore, concern has increased about the toxicity of these compounds. In addition, different studies have shown that PFCs affects the lipid metabolism, disturbs the immunity system, can cause liver cancer and can be a cause of human infertility [2]. Therefore, monitoring human exposure to PFCs is important.

In the present work a high-throughput method for measuring trace levels of 21 PFCs in human hair have been developed. The method consists of an ultrasonic extraction with acetonitrile, followed by an on-line clean-up step utilizing the TurboFlow[TRADEMARK] technology coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method is sensitive, with LOD between 0.025 and 1 ng/g of hair, involves minimal sample preparation, and it is suitable for large epidemiologic studies to assess human exposure to PFCs. This approach involves the use of two extraction columns in tandem: Cyclone and C18 XL. The injection volume was 20 µl. The influent solvent consisted in water pH 3.4 at turbulent flow of 1.5 ml/min, 20 s. An extra clean-up step with water at 0.5 ml/min, 10 s, was included. The loop elution (250 µl) was performed with (water pH 3.4 : methanol (20:80)) and followed by (water at pH 3.4 : methanol (70:30)) at flow of 0.2 ml/min, 2 min. Separation was carried out in a LC-column Hypersil GOLD PFP (50 x 3) (Thermo Scientific). Chromatographic mobile phases were (A) aqueous ammonium acetate 20 mM, and (B) MeOH, and the total run time was16 min. Thermo Scientific TSQVantage mass spectrometer, coupled to TLX-2, was used for analytical purposes, equipped with a Turbo Ion Spray source operated in the negative mode and working in SRM. The analytical method was validated using fortiufied blank hair samples. The method showed high recoveries rates in the range from 60 to130%, and good reproducibility and repeatability were also shown. The applicability of the method was tested with 20 real samples of hair.

[1] Giesy JP, et al. (2001) Environmental Science and Technology 35:1339-1342.
[2] Fei C, et al., (2009) Human Reproduction 24:1200-1205.

WE 096

Liquid chromatography-tandem mass spectrometry analysis of perfluorooctane sulfonate and perfluorooctanoic acid in fish fillet samples

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two environmental contaminants, characterized by a fully fluorinated hydrophobic chain and an hydrophilic head.

Their specific properties, especially high chemical and thermal stability and low surface free energy, make these chemicals unique for their ability to repel both water and oils, and useful in several applications, such as surface treatments for coatings, clothes, carpets, packaging products, cookwares, and food contact papers. As a result of their widespread use, they are globally distribuited and detected in environmental and biological samples. Moreover, they are extremely persistent, bioaccumulative and of toxicological concern because they have been shown to be carcinogenic in experimental animals.
In this study we present a new and fast analytical method to quantify these compounds in the edible part of fish samples. The method, based on isotope dilution with 13C labeled internal standards, uses a simple liquid extraction by sonication, followed by a direct determination using liquid chromatography - tandem mass spectrometry and avoids a solid phase extraction step (SPE), resulting in a lower sample contamination by extraction solvents and in a shortening of the analysis time. The linearity of the instrumental response was good, since the average regression coefficients of the calibration curves were always close to 1. The repeatability, expressed as average coefficient of variation, was 14% and 20% (inter-day) and 8% and 19% (intra-day) for PFOS and PFOA, respectively. The method was applied to samples of homogenized fillets of wild fish from Mediterranean Sea. Most of the samples showed low or contamination below limit of detection values (LOD = 0.04 ng/g), which was the same for both the analytes. The highest concentrations of PFOS (5.96 ng/g f.w.) and PFOA (1.89 ng/g f.w.) were found in an anchovy (Engraulis Encrasicolus) and in a norway lobster (Nephrops Norvegicus), respectively.
The developed analytical methodology can be used as a tool to monitor and to assess human exposure to perfluorinated compounds through sea food consumption, which seems to be one of the main routes of exposure to these pollutants for human population.

WE 097
Different quantification methods to calculate PFC concentrations in biota samples
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In this study we investigated the applicability of different quantification methods to calculate the concentrations of twelve perfluorinated compounds (PFCs) in biota samples. Four perfluorinated sulfonates (PFSAs), seven perfluorinated carboxylates (PFCAs), and perfluorooctane sulfonamide (PFOSA) were analysed using HPLC-ESI-MS/MS. Four liver samples of red-throated divers were prepared and analysed by an optimised and validated extraction and clean-up method. Biota samples were quantified by standard addition using two spiking levels. These calculated PFC concentrations were assumed to be 'correct'. Accuracy and precision of the standard addition method were verified before. Additionally, a calibration in solvent and a calibration in reference matrix (turkey liver) were used for quantification of PFC concentrations in the liver samples of the red-throated divers. All PFC concentrations were calculated by the external calibration approach as well as the internal calibration approach using mass-labelled standards. Results obtained by the different quantification methods were compared and evaluated for their applicability to calculate PFCs in biota extracts on the basis of influence on ionisation or relative recovery rates of native substances, i.e. the ratio of the native substance's recovery to the recovery of the mass-labelled counterpart. Using the t-test accuracies of all calculated PFC concentrations were verified and compared. Detailed results will be presented on the poster.

WE 098
Occurrence of perfluorinated surfactants in the Korean water system and human exposure
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Perfluorinated surfactants (PFSs) are an emerging pollutants globally detected in human. Their human exposure and uptake mechanisms are unclear. To elucidate the relationship of ambient water PFSs with human exposure, we investigated tap water and the river/stream water in eight main Korean city basins. The tap water examined reflected the contamination pattern and levels in their corresponding source waters. Tap water from the two cities along the Nakdong River contained more than 10-fold higher perfluorooctanoate (PFOA) (13-33 ng L-1) than those of other cities. For Korean adults, the mean daily intake of PFOA and perfluorooctanesulfonate (PFOS) via tap water was estimated to be 5.03 ng kg-1 d-1 and 10.8 ng kg-1 d-1, respectively. However, these intake rates of PFOA and PFOS accounted for only 2.4% and 0.2% respectively of the total daily intake estimated from reported serum concentrations, indicating the existence of other more important exposure pathways.

WE 099
Distribution and sources of polyfluoralkyl substances (PFASs) in the river Po (north of Italy)
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The river Po (north of Italy), the major Italian river which flows in the Adriatic Sea, was recognized as the dominant source of PFOA in the Europe. Preliminary studies on the PFASs in the river Po and in its major tributaries underlined the presence, in the Po rivers watershed, of point and diffuse sources of PFASs. The origin of PFOA was related to the presence of a point industrial emission in the Tanaro/Bormida watershed but available data were scarce and sporadic.
In this study sampling campaigns of the surface waters of river Po, collected downstream its main tributaries, were carried out in order to identify emission sources of PFASs in the Po watershed and to calculate PFASs annual discharges into the Adriatic Sea.
Analysis of perfluorinated carboxylates (from C5 to C10) and perfluorinated sulphonates (C4 and C8) were achieved by HPLC with ion trap mass spectrometry detection. Recovery for PFASs was, for all analytes, above 70% at µg/L levels. Limit of quantification (LOQ) ranged from 40 to 140 pg injected.
Concentration levels at the mouth of the river Po are comparable to the levels of densely populated and PFAS heavily impacted areas of northern Europe. PFOA and PFBS are the main compounds (47±39 e 65±51 ng/L respectively) contributing with about 30% (1.4 t/y) and about 40 %, (1.9 t/y) respectively, to the total PFAS load (4.5 t/y) discharged into the Adriatic Sea.
In general PFOS concentrations in the riverine water samples are low (5±2 ng/L) compared to riverine concentrations reported in previous studies and might be a result of the decreasing usage of PFOS and concurrent reduction of its emissions into the aqueous environment. Though the industrial source in the Tanaro/Bormida watershed was confirmed as an important source of PFOA (0.6 t/a; 50% of the total discharge), other diffuse or industrial sources of this compound are present in the Po watershed.
The river Adda, a Po tributary which drained one of most densely and industrialized region of the north of Italy, was identified as an important emission source of PFPeA e PFHxA for the Po watershed. The PFPeA and of PFHxA Adda fluxes were estimated to be about 82% (13 mg/s) and 65% (7.8 mg/s) of PFPeA and of PFHxA fluxes at basin closure of the river Po. Unlike PFBS e PFOS, it was not possible to identify emission point sources but their origin is probably related to urban sources since their fluxes correlates rather well with river basin population.

WE 100
Perfluorinated compounds: from urban wastewater to the river
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Our everyday life is a source of environmental pollution through domestic wastewater. The wastewater treatment plants (WWTPs) have been conceived to biologically eliminate pollutants, such as organic matter or nitrogen. However, the used treatments are not effective for the elimination of many compounds that have been increasingly appearing in our everyday use for several decades: e.g. pharmaceuticals, biocides present in body care products or non-adhesive surfacing of frying pans.
This study aimed at assessing the concentration of two perfluorinated compounds (PFCs), perfluorooctanoic acid (PFOA) and perfluorooctan sulfonate (PFOS) in the wastewater of an urban area. We have followed the wastewater issued from a residential area on the one hand and from a mixed area (administrations, hospital, houses, etc.) on the other hand, both at the inlet of the WWTP (300 000 eq. inh.), which collects the wastewater from different municipalities, and at the WWTP's outlet.
The samples' aqueous phase was extracted by solid phase extraction (SPE) and the extract was analyzed by UPLC/MS/MS. The PFCs concentrations measured in the residential and mixed area were similar: PFOA was around 6-8 ng/L and PFOS around 4-8 ng/L. The concentrations measured at the inlet and at the outlet of the WWTP were identical: 10 ng/L for PFOA and 25 ng/L for PFOS. This showed that the biological treatment did not eliminate these compounds. The daily mass flow of PFCs to the river was of around 600 mg/d of PFOA and 1600 mg/d of PFOS.

WE 101
Contribution of wastewaters to PFCs contamination in ambient river waters of South Korea
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We selected and analyzed water from the outlet of wastewater and/or along an upstream-downstream transect of the outlet, to elucidate the contribution of industrial/municipal wastewaters to PFCs contamination in ambient river water. Waters were collected from 31 sites of eight water bodies receiving varying wastewater from LCD, semi-conduct, fabrication factories, two local airports, and municipal wastewater treatment plants. Total PFC concentration (sum of C6-C13 PFCAs and C4-C8 PFASs) ranged from 6.3 to 1,160 ng/L with a median of 30.4 ng/L. Dominant compounds were PFHxA (median = 5.8 ng/L), PFOA (median = 5.7 ng/L), and PFOS (median = 6.3 ng/L) in most waters. In comparison of waters along the longitudinal transect, the waters did not show an increase in PFC concentration after than before receiving wastewaters. This indicates that the contribution of wastewaters could not be significant enough to exceed water dilution effect in Korean waters if excluding some exceptions.

WE 102
Ombotrophic peatbogs as natural archives to investigate the historical deposition of polyfluorinated compounds (PFCs)
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As they get their pollution from the atmosphere only, ombotrophic peatbogs have been identified as suitable natural archives for the investigation of historical depositions of persistent organic pollutants such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls or polybrominated diphenylethers. In order to find out if ombotrophic peatlands are also suitable natural archives for the reconstruction of atmospheric PFC pollution, two peat cores were taken in fall 2009 at Mer Bleue Bog, an undistrurbed ombotrophic bog close to Ottawa, Canada. For analysis, cores were cut into 5 cm segments. Dating of the peat segments was performed at a separate core using the 210Pb method. The peat cores roughly covered the past 100 years. Peat samples were extracted using a modified QuEChERS method and analysed for 25 PFCs comprising perfluoroalkyl carboxylates, perfluoroalkyl sulfonates as well as several neutral PFCs by HPLC-MS/MS. Analyses were performed in duplicates. By this approach, intra-core and inter-core variations could be determined. Of 25 PFCs, 15 were detected in the ombotrophic peatland. Perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorobutanoate (PFBA) dominated the PFC profile with total depositions (entire core) of 10.4 +/- 2.5 µg/m², 7.4 +/- 0.7 µg/m² and 38.3 +/- 9 µg/m², respectively. The extremely high value for PFBA remained unclear. Whereas intra-core variations of PFC deposition rates were low, inter-core variations were not. Compared to the temporal trend of PFC production, chronological sequence of PFC deposition was shifted towards deeper, i.e. older peat segments. These temporal trends will be presented on the poster and indicate that PFCs migrate downwards to a certain extend or that there were problems with the peat dating. In conclusion, analyses of peat cores as natural archive for PFC pollution may help reconstructing the overall atmospheric input. Their suitability to provide authentic and reliable temporal trend data has to be evaluated carefully.

WE 103
Perfluorinated compounds in ice core samples from the Alps
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Since 1950, per- and polyfluorinated compounds (PFCs) have been used in several industrial and consumer products due to their unique properties. Neutral PFCs are semi-volatile and are globally transported through the atmosphere. Remote areas, such as high alpine mountains, can be contaminated with PFCs by wet and/or dry atmospheric deposition. High altitude sites are usually not influenced by direct sources of PFCs and receive these contaminants by atmospheric deposition, only.
The aim of the present study was to investigate the occurrence and temporal variations of perfluorinated compounds in an high mountain area of the Alps. Furthermore, annual deposition rates and fluxes of PFCs to the high alpine environment were estimated. For this purpose, a 10 m shallow ice core was drilled at Colle Gnifetti (4452 m above sea level, Italy, Swiss) and was dated using the delta18O method. Overall, the ice core covered a period from 1996 to 2008. The ice core was cut according to annual and/or seasonal layers prior to the PFC analyses. Each ice segment was extracted using solid phase extraction and were separated and detected by HPLC-ESI-MS/MS. The target analytes included 17 PFCs (perfluorinated carboxylates (PFCAs) and perfluorinated sulfonates (PFSAs)).
We will present the results of PFC contamination from Colle Gniffetti. Concentrations, vertical distribution and deposition rates of PFCs were determined in the different ice core layers. The results will be compared to previous findings of PFCs in a shallow firn core from Mount Ortler (2004 - 2009, 3852 m a.s.l., Italy). There, 12 of 17 PFCs were detected with ∑PFC concentrations between 1 ng L-1 to 5.8 ng L-1. Most frequently observed individual PFCs were PFBA (0.3 - 1.7 ng L^{-1}), PFNA (0.1 - 2.1 ng L^{-1}) and PFOA (0.1 - 0.9 ng L^{-1}). Increasing concentrations from 2004 to 2009 were only demonstrated for PFBA (0.3 to 1.4 ng L^{-1}).

WE 104
Mass balance analysis of fluorinated compounds in wet precipitation
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Thousands of tons of organic and inorganic fluoride are emitted to the atmosphere by various sources including releases from industrial processes, domestic applications and power plants. Per- and polyfluorinated compounds (PFCs) are organic fluoride chemicals that have received worldwide attention due to their environmental persistence. The ubiquitous distribution of PFCs has been documented globally in the atmosphere and in wet precipitation. However, there is currently a lack of robust analytical methods that can be applied to determine levels of all of the PFCs. In this study, mass balance analysis involving the determination of total fluorine (TF), inorganic fluorine (IF) and extractable organic fluorine (EOF) using combustion ion chromatography (CIC-F) was carried out to gain a comprehensive understanding of the fate of both known and unknown PFCs in wet precipitation.
Concentrations of TF and IF were measured in rain (n=50), snow (n=14) and ice core samples (n=6). EOF was extracted from several rain (n=10) and snow samples (n=6) using solid phase extraction (SPE) procedures. Concentration of 19 known individual PFCs were also determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The contribution of EOF and IF to TF for different kinds of wet precipitation samples will be discussed. Interestingly, a high percentage of non-extractable organic fluorine was found in precipitation samples, in contrast to what was observed in seawater. This may due to the forms of fluoride present in the atmosphere and water-fluoride tends to bind to particulates in the atmosphere and exist as organic fluoride, while it dissolves in water and ionizes. Known PFCs were found to contribute around 0.0005% to EOF, suggesting the need to identify the unknown fluorinated chemicals present in the precipitation samples. Finally, geographical distribution of TF and IF among several locations can be compared, and source determination can be carried out by using the ratio of known PFCs to TF to provide more information on the fate and transport of PFCs in the atmospheric environment.

WE 105
Long-term environmental fate of perfluorinated compounds after accidental release at Toronto Airport
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Concerns about perfluorinated compounds (PFCs) have been raised during the past 15 years due to their persistent, bioaccumulative and toxic characteristics. Although perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride were listed in Annex B of the Stockholm Convention in 2009 to restrict their worldwide uses, very few studies have simultaneously measured PFCs in various environmental media as well as assessed their long-term fate in aquatic systems. Since application of perfluorooctane sulfonate (PFOS) based aqueous firefighting foam (AFFF) can be a major source of PFOS to nearby aquatic systems, we monitored PFCs in water, sediment, fish tissue and liver in an upstream-downstream transect of Spring and Etobicoke Creeks near Toronto airport over the 10 years following an accidental release of 22000 L of PFC-containing AFFF in 2000. In 2005, another incident involving fire at the Toronto airport resulted in a release of 48000 L AFFF, which was likely different in composition compared to the earlier release.
The results indicate that PFOS is the predominant PFC in all four media. PFOS concentrations were approximately 10 times higher near the airport than the other sites in the water sampled in 2003 and 2009; however, PFOS was lower in 2006 than the other years at this. This could be a result of dilution due to precipitation prior to the sampling date. PFOS level in water measured by another study ~5 months after the spill in 2000 at a sampling site ~5 km downstream were over 5 times higher than our measurements in the 2003 at a site close to the sampling site of the other study. Unlike traditional neutral POPs with a high affinity for sediment, most PFCs could not be detected in the sediments. However, PFOS concentrations and detection rate in sediments were highest near the airport than other sites. No significant spatial trend could be observed for PFCs in fish tissue and liver since the exposures are not necessarily confined in one segment of the creek. Comparing the profile of PFCs in fish and water, higher Bioconcentration Factors for the long-chain PFCs were observed. Assuming no site specific loading of PFCs to the creek occurred since the import of PFC-containing AFFF to Canada ceased in 2002 and the regulation on existing stocks proposed in 2006, the elevated level of PFOS at the site with the spill 10 years ago raises concern about its persistence in aquatic system and/or contribution of secondary sources.

WE 106
Determination of perfluorinated compounds in various consumer products
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Perfluorinated compounds (PFCs) are a subject of increasing environmental concern. PFCs are used in many industrial and consumer products, such as polymers and surfactants, because of their unique and useful properties (stability, chemical resistance and generally low reactivity). The presence of residual unbound fluorotelomer alcohols and perfluoroalkyl sulfonamido ethanols was confirmed in technical fluorinated materials. Moreover, other perfluorinated compounds (PFOA and other PFCAs) may be present in various consumer products because of their usage in production of fluoropolymers. Monitoring of above mentioned perfluorinated compounds is crucial for identification of indoor environment contamination. An assessment of PFCs contamination of various consumer products was a target of the study presented here.

WE 107
Sources of Perfluoroalkyl substances present in groundwater and drinking water
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Several PFASs (perfluoroalkyl substances) appear to have persistent, bioaccumulative and toxic (PBT) properties. They have been found to be present in the low ng/L concentrations in drinking water. Water is the beverage consumed in the largest quantity on a daily base and therefore can be a significant dietary exposure pathway of PFCs to human beings. Because in many countries the majority of drinking water is abstracted from groundwater, PFASs in groundwater could be a possible source of contamination to drinking water. This especially because the treatment of groundwater dedicated for drinking water is often marginal compared to surface water treatment. This study explores the presence of PFAS in groundwater contaminated by landfill leachate. Two groundwater abstraction sites in the Netherlands with a landfill in the area of the water abstraction were sampled and analyzed for 18 different PFASs. Total concentrations in groundwater in one location ranged from a maximum of 5459 ng/L in the groundwater travelling towards the abstraction wells to a minimum concentration of 2.4 ng/L. In finished drinking water made from this groundwater a Σ PFASs concentration of 7 ng/L was observed. Patterns of PFASs indicated that the major source of the PFASs was the landfill leachate. However, other locations sampled within the groundwater abstraction area showed a different finger print indicating that different sources might be involved. Mechanisms determining the observed patterns were explored. Dominating compounds were perfluorooctanoic acid (PFOA) and perfluorobutanoic acid (PFBA). This study is part of the EU-sponsored PERFOOD project which aims at assessing the sources of and exposure to PFCs in the European diet.

WE 108
Impact of treatment processes on the occurrence of perfluoroalkyl acids in the drinking water production chain
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Perfluoroalkyl substances (PFASs) have been detected in drinking water at concentrations typically in the low ng/L range, with occasionally higher concentrations (lower µg/L level) in some contaminated areas. These findings suggest that PFASs are not or poorly removed during drinking water treatment. The behavior of polyfluoralkyl substances (PFASs) from raw source water to finished drinking water was assessed by taking samples from influent and effluent of the several treatment steps used in a drinking water production chain. This consisted of intake, coagulation, rapid sand filtration, dune passage, aeration, rapid sand filtration, ozonation, pellet softening, granular activated carbon (GAC) filtration, slow sand filtration and finished water. In the source water taken in at the Lek canal (a confluent of the Rhine), the most abundant PFAS were PFBA (Pefluorobutanoic acid) 52 ng/L, PFBS (Perfluorobutane Sulfonate) 42 ng/L, PFOS (perfluorooctane sulfonate) 10 ng/L and PFOA (perfluorooctanoic acid) 5.1 ng/L. During treatment longer chained PFAS such as PFNA and PFOS were readily removed by the GAC treatment step and their GAC effluent concentrations were <LOQ. However, more polar shorter chain PFAS (especially PFBA and PFBS) were not removed by GAC and their concentrations remained constant through treatment. A decreasing removal capacity was observed with increasing carbon life time. The finished water contained 26 and 19 ng/L of PFBA and PFBS. Other PFAS were present in concentrations below 4.2 ng/L. The concentrations observed are no reason for concern for human health whatsoever as margins to existing guidelines are sufficiently large

WE 109
Uptake of perfluorinated alkyl substances by hydroponically grown lettuce
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Perfluorinated alkyl substances (PFAS) are bioaccumulative persistent, organic pollutants (POPs). They can be detected ubiquitously in the environment and they may pose a risk to human health due to accumulation in the food chain. The occurrence of PFAS in animals, such as fish, birds and mammals including humans is fairly well documented, but little can be found in the literature about crops or plants in general. However, humans are possibly exposed to PFAS through consumption of vegetables and other plant-related food items.
Plants appear to accumulate PFAS differently, as has become apparent from published data on PFAS concentrations in crops, e.g., potatoes or cereals. The objective of this study was to increase the understanding of the accumulation process of PFAS in crops.
In a first experiment lettuce was grown hydroponically in a greenhouse with a contaminated nutrient solution to make sure the offered PFAS were completely bioavailable. The lettuces were exposed to a set of 13 perfluorinated carboxylic acids and 4 perfluorinated sulphonates in four different concentrations to assess the difference in behavior between PFAS and concentration dependencies. Furthermore, the uptake of PFAS during the growth period of lettuce was investigated in a 4-5 days interval in a second experiment.
Because of the high water solubility of the compounds, we assumed that the PFAS will be taken up by the root system of the plants and will be distributed through the water system. Hence it can be assumed that evaporation plays an important role in the uptake, therefore bioaccumulation hypothetically takes place especially in the leaves of the plants. To confirm this hypothesis correla-

tions of the PFAS uptake with the water uptake were examined.
The contribution presents the results of the greenhouse experiments.

WE 110

Uptake and depuration of PFOS in liver and muscle tissue of bluegill sunfish under environmentally relevant conditions

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Previous studies have indicated that diet is one of the important routes of human exposure to perfluorinated compounds (PFCs), particularly perfluorooctane sulfonate (PFOS). Among food items, fish are frequently found to contain relatively high concentrations of PFOS and other long-chain PFCs. However, little is known about the uptake and depuration rates of PFOS in fish tissues under environmentally relevant condition, making it difficult to determine how fish are likely to be contaminated. In the current study, bluegill sunfish were placed in aquaria containing an environmentally relevant concentration of PFOS (50 ng/L) or in PFC-free well water for a period of 20 days followed by a 19 day depuration period. Control and exposed fish were sacrificed throughout the exposure and depuration periods. Fish fillets and livers were extracted by alkaline digestion and analyzed by LC-MS/MS. PFOS concentrations were found to slowly increase with time during the exposure and depuration periods for both control and exposed fish, suggesting that contaminated water is not the primary exposure route for bluegills. Instead, the analysis of the brine shrimp fed to control and exposed fish indicated that this dietary exposure was more likely to have lead to the small cumulative increase of PFOS concentrations observed.

WE 111

Perfluorooctanoic acid toxicity in zebrafish (Danio rerio)

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Perfluorooctanoic acid (PFOA) is a common surfactant with wide use, and has been detected as a contaminant in soil, air, water and biota. Previous studies have shown that PFOA has detrimental effects on different life stages in zebrafish (*Danio rerio*).
We have evaluated the effects of PFOA toxicity covering different life stages of zebrafish through three tests that were based on OECD guidelines. In the Fish Embryo toxicity (FET) test, fertilized embryos were used to calculate effective concentrations (LOEC and NOEC) based on early life stage lethal and sub-lethal endpoints. Based on the FET test, a Fish Sexual Development Test (FSDT) was conducted and effects on vitellogenin (VTG), body and liver somatic indices and gonad histopathology including sex ratios were evaluated. A reproduction test (Fish Screening Assay: FSA) was also performed where effects on reproductive parameters like spawning, fecundity and fertilization rate were evaluated, as well as effects on histopathology, liver enzyme activity VTG-levels and gene expression levels in the adult fish.

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WE 114

Macro algae, Ecklonia cava - Ecotoxicogenomic approach for environmental stress responses

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Environmental stress dramatically induces stress responsiveness in an organism and it affects the related genes expressions. The marine forest consisted of the kelp (macro algae) is identical to the ground forest and is worthy of spawning place as well as shelter for various marine organisms. Also they are very important for marine resources and environment-economic values. This study aimed to screen the differentially expressed genes induced by marine environmental changes at genomic level and to prove those gene expressions represent the individual organism's health as well as real environment. The targeted seaweed is Ecklonia cava known as dominant, plentiful and easily collected in Korean coastal regions. First of all, the RNA extraction method was established E. cava before screening of differentially expressed genes by environmental differences and the DNA sequences of several stress responsive genes were found. Massive screening of the differentially expressed genes by environmental changes was attempted by the construction of E. cava cDNA library. As a result, approximately 2700 genes were collected from the standard library and 11 oxidative stress responsive genes were cloned. Now we are comparing the gene expression profiling of E.cava collected in non-contaminated and contaminated sites in polluted areas and investigating that profiling differences could respond to the health status in the real environment.

WE 115

Transcriptomic analysis of Japanese medaka exposed to mixtures of sex steroids

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In this study, the effects of mixtures were evaluated for an estrogen and androgen. Larval Japanese medaka, Olyzias laptipes, within 12h post-hatch, were exposed to mixtures of 17β-estradiol (E2) and 17β-trenbolone (TB). Chronic experiments showed that estrogenic and androgenic effects appeared to cancel each other at low concentration of mixture (30ng/L of E2 and 20ng/L of TB), resulting in no reproductive impairment. At high concentration of mixture (100ng/L of E2 and TB), reproductive activities were severely damaged for both sexes, although sex reversal did not occur. Gene profiling of medaka was performed using oligonucleotide-based DNA microarray containing 36,398 probes (Agilent Technologies). The gene expression profiles obtained for the mixtures were in agreement with biological experiments and histological analyses. Our study demonstrates that the exposure experiments of binary mixtures would be helpful to provide more insight into the mechanism of endocrine disruption compared with single exposure experiments.

WE 116

Transcriptional responses of Enchytraeus albidus (Oligochaeta) to metal exposure: effects of zinc and cadmium

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Enchytraeus albidus is an important and typical inhabitant of a wide variety of soil types and fulfils vital functions such as improvement of the soil pore structure and indirectly the degradation of organic matter. Traditionally, in soil Environmental Risk Assessment (ERA), several biological tests are commonly performed with E. albidus (ISO No 16387, 2003; OECD 202, 2004), providing information about effects on survival and reproduction. Such traditional test methods are important to predict realistic threshold values for policy makers and to screen the overall toxicity of polluted soils. Nevertheless, such bioassays are time-consuming and consider limited endpoints. It is commonly agreed that this information should be complemented with responses at lower levels to better understand the underlying response mechanisms but so far, very little is known about the effects induced by different toxicants on these organisms at the molecular level. Recently, substantial efforts have been made to further develop and enrich the existing microarray for this soil invertebrate species. In the present study, E. albidus were exposed during 2, 4 and 8 days to concentrations of zinc and cadmium known to cause 50 and 90% effects on their reproduction. The main goal of this study was to elucidate the early molecular responses and the toxic mechanisms of these metals in E. albidus with the use of transcriptomic analysis. The gene expression analysis was performed through hybridization of fluorescently labelled probes from the exposed and control organisms on the newly developed Custom Gene Expression Microarrays 8[GREEKX]15 k format (Agilent Technologies). Results show differently expressed genes involved in functions like transcription and translation, immune and stress response, protein metabolism, energy metabolism, development, cell structure and carbohydrate and fat metabolism. This study demonstrates that the determination of effects at different levels of biological organization allow a more in depth evaluation of the mechanisms of toxicity that may contribute to reproduction impairment by metals.

WE 117

DNA-expression in roots of Oryza sativa exposed to sediments spiked with Ni - linking expression profiles to phenotype

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Sediments play an important role in the assessment of the environmental quality of a river because of several reasons. The matrix sediment is considered an ecosystem (benthos) with organisms reacting to particle bound pollution. The benthic habitat constitutes an integral and dynamic part of aquatic ecosystems, whose processes are important for the whole water body. Sediments can act as both, potential sinks but under other conditions as potential sources for many hazardous substances. Particle-bound contaminants are problematic due to their particular persistence and liability to bio-accumulation.
Taking this into account several methods for sediment contact tests with various species were developed in the recent years. Sediment contact assays have the advantage to cover various routes of exposure, namely direct exposure by particle contact as well as exposure via the sediment pore water. In the present study sediment contact tests with rice (*Oryza sativa*) were performed with Ni-spiked artificial sediments. *Oryza sativa* can be grown under emerse conditions and has - as a prerequisite for a comprehensive expression analysis - a sequenced genome.
The most sensitive end point for exposure to Ni was the root length; in addition to growth inhibition a different morphology of the root was observable. Roots of exposed plants were less branched and thicker compared to the unexposed control. In order to establish an endpoint with ecotoxicological relevance that is based on gene expression it is crucial to correlate expression data with an adverse phenotype - e.g. inhibition of root development. With this rationale sprouted seeds of *Oryza sativa* were exposed to different Ni-doses, i.e. EC_0 at 200 mg/kg and EC_{50} at 600 mg/kg, with a subsequent analysis of the genome wide expression profile by DNA-arrays. The objective of the study is to determine if it is possible to discriminate between exposed / non-exposed samples but as well between affected (exposure at EC_{50}) and unaffected (control and exposure at EC_0) samples based on the gene expression data. If such a differentiation is possible, robust molecular markers should be identified the expression levels of which can be used as predictors for exposure and/or adverse effect. The successful correlation of molecular markers and adverse phenotypes in studies like the one presented here, is an essential prerequisite for the use of techniques like expression profiling in the field of regulation in the future.

WE 118

Transcriptional responses of exposure to oil, mercury and CO2 in the marine zooplankton Calanus finmarchicus

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Calanus finmarchicus is one of the most common marine zooplankton in parts of the Norwegian-, the North- and the Barents Seas. C. finmarchicus is a pelagic species continuously exposed to the surrounding water, and would inevitably be exposed to pollutants originating from anthropogenic activities and changes in environmental factors. The biological effects of these stressors may be manifested at various levels (e.g. biochemical, behavioral and functional effects), although it is often assumed that alterations at the molecular (transcriptome) level occur prior to more adverse endpoints and therefore may serve as a early-warning biological marker (biomarker).
The main aim of this work was to develop a custom oligonucleotide microarray (oligoarrray) for C. finmarchicus and utilize this tool for assessing transcriptional effects of multiple stressors originating from different pollution sources. The Calanus oligoarray were constructed of contigs and singletons obtained from the pre-processing, clustering and assembly of 11k NCBI Expressed Sequence Tags (ESTs). Resulting contigs and singletons were subjected to blasting and annotation, sequence and probe redundancy reduction, and 6 k presumably non-redundant sequences were subjected to 60-mer probe production for inclusion on a 15k Agilent custom oligoarray. The performance of the Calanus oligoarray was evaluated with RNA from C. finmarchicus exposed to sublethal concentrations of CO2, inorganic Hg and the water soluble fraction (WSF) of a North Sea crude oil for 48h (10_C). The data obtained from analysis were normalized and filtered, followed by statistical analysis and identification of differentially expressed genes that were further subjected to analysis of functional enrichment analysis.
The results from the studies show that exposure to the different stressors gave rise to substantially different transcriptional patterns which were characterised by different Gene ontology functions. The Calanus oligoarray is a promising new tool in our genomic toolbox linking exposures to low concentrations of anthropogenic pollutants with an early biomarker response in the important marine organism Calanus finmarchicus.

WE 119

Gene expression profiling in the Model Yeast Saccharomyces cerevisiae to characterize and

compare the toxicity of six pesticides and identify biomarkers of toxicity

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Short-term bioassays using non-animal eukaryotic models are required for the preliminary screening of the toxicity of chemicals and to early-warn potential toxic effects of xenobiotics in whole environmental samples. The yeast Saccharomyces cerevisiae is a useful experimental model of the eukaryotic cell; it is widely distributed, easy to cultivate, and has a vast amount of genomic knowledge and resources available. This work aims to identify molecular biomarkers in the yeast model that may be useful for assessing and diagnosing pesticide toxicity, and to get insights over mechanistic aspects of the yeast responses to pesticide-stress. Affymetrix GeneChip Yeast Genome 2.0 arrays were used to examine modifications occurring in the transcriptome of an exponential population of cells due to exposure to the 20%-inhibitory concentration (IC20) of each of four herbicides (alachlor, s-metolachlor, diuron and MCPA-methyl ester), the insecticide carbofuran and the fungicide pyrimethanil. The total number of genes with expression significantly altered by the herbicides varied between 350 and 750 (<12% of whole genome), while 1072 (~20% of genome) were responsive to the insecticide carbofuran; interestingly, most of the latter (~85%) seem to be carbofuran-specific. To try to understand correlations between gene expression profiles and chemical structure/mode of action, differences in the profiles of the six different pesticides are being further analysed based on clustering approaches. For each pesticide under study, the differentially expressed genes are also being categorized into overrepresented functional classes, predicted metabolic pathways and interaction networks within the encoded proteins. Search for proteins from diverse higher eukaryotes (e.g. relevant as representatives of ecosystems inhabitants or models in ecotoxicological studies) that are homologous to yeast proteins encoded by the differentially expressed genes is also in course. The modifications in the expression of a number of selected genes that may be relevant as biomarkers of pesticides toxicity is being further examined by quantitative RT-PCR. Significantly, the magnitude of the expression change in pesticide-stressed cells appears to increase in a concentration-dependent manner and to show a good correlation with pesticides growth inhibitory effects.

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WE 120

Transcriptomic approach to assess the effects of two Active Pharmaceutical Ingredients (APIs) in Mytillus galloprovincialis

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DNA region or gene specific oligonucleotides immobilization in ordered matrix allows attaining DNA microarrays. Recently, these DNA microarrays have been applied in relevant aquatic organisms exposed to diverse contaminant levels. However, there is no knowledge of any work reporting changes on mussels, M. galloprovincialis to nonsteroidal anti-inflammatory drugs at molecular/gene level.

Ibuprofen is commonly used as analgesic/antipyretic and diclofenac to treat rheumatic diseases and inflammations of the musculoskeletal. Their physic-chemical characteristics namely polarity, non-volatile low biodegradability favor their persistence in the aquatic ecosystems.

The main aim of this study was to assess the gene expression profiles using a 1.7 K cDNA microarray (Mytarray V1.1) in the digestive gland of mussels exposed to environmental realistic concentrations of ibuprofen and diclofenac (250 ng/L) during short-term exposure.

Total RNA (10 μg) from individual mussels (either drug exposed or not exposed ones) was reverse-transcribed using M-MuLV RNase Hðð reverse transcriptase primed by an anchored oligo-dT and in the presence of a modified dNTP mixture including amino-allyl dUTP. Fluorescence labeled nucleic acids probes were prepared by further coupling with the fluorescent Cy3 or Cy5 dyes. Tester and control cDNA labelled probes (20 pmoles each) were used for dual color competitive hybridization in a "dye-swap" experimental design. Microarrays were scanned at high resolution with an Agilent scanner and raw fluorescence intensities extracted with the Genepix 6.0 software. Further processing and statistical analysis of microarray were performed using the R-package Linear Mode for Microarray Analysis (LIMMA) implemented through a web user interface (TD_Hand).

This preliminary study demonstrated that both APIs induced significant alterations in gene transcription patterns of the exposed mussels. Ibuprofen exposure induced a significant expression of 33 (Benjamini and Hochberg adj_p < 0.05, N=4) genes which were mostly down-regulated. Furthermore, 29 differentially expressed genes were reported for the effects of diclofenac. About 40% of differentially expressed genes were shared between treatments, possibly indicating a common mode of action.

WE 121

Dithiocarbamates induce craniofacial abnormalities and downregulate sox9a during zebrafish development

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Dithiocarbamates (DTCs) have a wide variety of applications in diverse fields ranging from agriculture to medicine. DTCs are teratogenic to vertebrates but the mechanisms by which they exert these effects are poorly understood. Here we show that low nanomolar exposure to three DTCs, tetraethylthiuram (thiram), tetramethylthiuram (disulfiram) and sodium metam (metam), leads to craniofacial abnormalities in developing zebrafish embryos that are reminiscent of DTC induced bone and cartilage abnormalities found in higher vertebrates. In order to better understand the molecular events underlying DTC teratogenesis, we exposed embryonic zebrafish (PAC2) cells to thiram and disulfiram and measured changes in gene expression with microarrays. We found differential expression of 166 genes that were specific for exposure to DTCs and identified a network of genes related to connective tissue development and function. Additionally, we found eight downregulated genes related to transforming growth factor β-1 (TGF-β1) signaling, including an essential transcription factor for zebrafish craniofacial development; SRY-box containing gene 9a (sox9a). Finally, we show that sox9a expression is perturbed in the ceratobranchial arches in DTC exposed developing zebrafish suggesting that this is an important event in the development of DTC induced craniofacial abnormalities. Together, we provide evidence for a novel teratogenic endpoint and a molecular basis for a better understanding of DTC induced teratogenesis in vertebrates.

WE 122

A proteomic study in zebra mussels (D. polymorpha) exposed to benzo(a)pyrene: role played by gender and exposure concentrations

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Despite the fact that zebra mussel (*Dreissena polymorpha*) is considered a reference model for freshwater ecosystems, the investigation of proteomic approach in this organism has not been deeply investigated. This work represents the first attempt to verify the suitability of the proteomic approach also in this biological model. This aim was realized by evaluating the changes in protein expression profiles in gills of *D. polymorpha* specimens after an in vivo exposure (7 days) to benzo(α)pyrene (B[α]P). In order to provide a more in-depth evaluation of this approach, we investigated the role played by gender in proteome response: to this end, we evaluated the changes in protein expression analysing separately male and female mussels. Moreover, we choose to test the effects played by two different concentrations of B[α]P (0.1 and 2 μg L-1) in order to evaluate possible dose-dependent changes in the proteome. We identified proteins changed in response to the B[α]P exposure by MALDI-TOF/TOF mass spectrometry. Finally, B[α]P bioaccumulation in mussels soft tissues was also assessed to ensure a real effect of the selected chemical. On the whole, we found a total of 28 proteins differentially expressed. The most significant identified altered proteins are related to oxidative stress, signal transduction, cellular structure and generic metabolism. Although gill tissue was expected to be less prone to respond in a sex specific manner, males and females responded differently to exposure, because B[α]P affects different proteins in the two genders, with the exception of only two proteins. Surprisingly, the comparative analysis of protein data sets showed no proteins varying commonly between both experimental conditions. The present report shows not only the utility of proteomic techniques to study differences in protein expression in the freshwater mussel *D. polymorpha*, but above all that the interpretation of data set from this approach should be carefully checked. For instance, the role played by gender on protein modulation and the responses generated by different toxicant concentrations surely need to be deeply investigated. Finally, our preliminary results confirm the need of increasing the number of proteomic studies in invertebrates to allow a rise of their representation in databases and the successfully identification of the most relevant proteins.

WE 123

Ecotoxicoproteomics tests on Daphnia pulex : preliminary steps

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Drugs administered in humans are eliminated through the excreta in either intact or metabolized form leading pharmaceuticals and their derivatives to reach sewage treatment plant. Several drugs are able to induce genetic and cell cycle changes and the current burgeoning of new therapeutic agents with original mechanisms of action call for a comprehensive assessment of their potential effects on the aquatic fauna and flora. It is therefore crucial to find out robust biomarkers to identify early noxious effects of these compounds. We assume that proteomics approach will allow us to identify at the protein level such biomarkers, which then may be used to assess the risk of drugs and their metabolites for aquatic life.

Toxicoproteomics is a field of science that seeks to understand changes, at the protein level, in cells or organisms exposed to toxins such as pollutants or drugs. Nowadays, toxicoproteomics can be used in environmental toxicology to find early ecotoxicological markers at the protein level; this field is called ecotoxicoproteomics. In this study, proteomics analyses were performed on waterflea Daphnia pulex. The sample preparation (i.e. daphnid homogenisation, cell lysis, protein extraction and fractionation) was optimized in order to dramatically reduce the amount of daphnia tissue needed. Three different procedures for homogenization and cell lysis were thus tested: (a) ceramic mortar; (b) ultra-sonic extraction; (c) FastPrep system. Preliminary results are presented here as a proof-of-principle of the developed method. The goal is to find out the best procedure for a relevant and reproducible protein extraction.

WE 124

Convergent and specific responses of the flounder Platichthys flesus to different stress: a proteomic approach

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The flounder Platichthys flesus spends the earlier years of its life in estuarine environments, along the European East Atlantic coasts. Estuarine environments are strongly subjected to anthropogenic stress factors such as hypoxia linked to eutrophication, chemical pollution especially in industrial area, or thermal stress in the context of global warming.

This study aims at understanding the molecular mechanisms allowing flounder to adapt to different stress factors and multi-stress conditions, and to determine whether a local adaptation to a specific stress could modify the ability of the individuals to cope with other stress.

Flounders were fished in different estuaries, one located in the southern limit of this species (the Mondego - Portugal), the others being chemically polluted (the Seine - France), or displaying hypoxia events (the Vilaine - France) or considered as a control area (the Canche-France). Some farm flounders were also raised in the laboratory under contaminated conditions (PAHs and PCBs cocktail at a concentration representing 10 times the concentration found in the Seine). They were then subjected to a second stress (thermal or hypoxic) to study the way they can cope with the multiple stress; thus, a proteomic approach by 2-dimensional electrophoresis has been performed on these animals. This allowed us to identify differentially expressed proteins by MALDI TOF-TOF mass spectrometry. Our main objective will be here to explore the molecular responses of a marine fish to single versus multiple stress, looking for specific versus convergent pathways.

WE 125

Effects of heavy metal contamination on the expression of immune- and stress- response genes in the Sydney rock oyster, Saccostrea glomerata

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Environmental contamination by chemical pollutants is a serious threat to the biological sustainability of coastal ecosystems worldwide. Our current understanding of the biological effects of chemical pollution in these ecosystems is poor. Extensive chemical maps of pollution exist, but there is little corresponding biological effects data. New, more sensitive biomonitoring methods are needed to provide an early warning of biological harm that can assist in the management of sensitive marine environments and prevent permanent damage.

The current study tested the expression of immune- and stress- response genes after exposure to heavy metal contamination in Sydney Rock oysters (Saccostrea glomerata). Target genes (HSP70, HSP90, Metallothionein, Superoxide dismutase, Defensin and Ferritin) were selected from

previous studies that investigated the effects of contaminants on immune- and stress- responses in marine molluscs. Quantitative (real time) PCR analyses of gene expression showed that laboratory exposures to different metals (cadmium, copper, lead and zinc; 100µg/l) elicited different gene expression profiles. The expression of defensin, an antimicrobial peptide, was up-regulated in response to lead exposure, but was down-regulated in the presence of zinc. The bacteriostatic, metal-binding protein, ferritin, was up-regulated by exposure to cadmium, copper and lead, but not by zinc. Heat-shock protein 90 (a generic stress-response protein), ficolin (a lectin involved in host defense against pathogens), and the metal-binding protein, metallothionein, were up-regulated in response to all four metals. Conversely, superoxide dismutase (an antioxidant enzyme involved in phagolysosmal defence) was down-regulated by exposure to all four metals.
These results suggest that metal exposure may have complex, differential effects on the immune- and stress- responses of oysters and can potentially provide a mechanism for identifying the specific stressors responsible for changes in gene expression.
The environmental significance of this research will be assessed using oysters transplanted to a polluted estuarine environment so that their expression profiles can be analysed in the same way.

WE 126

Molecular Biomarkers of exposure in marine diatom Thalassiosira pseudonana upon exposure to polycyclic aromatic hydrocarbons (PAHs): from in vitro to field studies

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Marine diatoms have a key role in the global carbon fixation and therefore in the ecosystem. Diatoms are unicellular, eukaryotic algae that inhabit marine and fresh waters worldwide and are responsible for about 40% of the total carbon fixation in oceans [1]. We used Thalassiosira pseudonana as a model organism to assess the effects of exposure to environmental pollutants at the gene expression level. Diatoms were exposed to polycyclic aromatic hydrocarbons mixture (PAH) from surface sediments collected at a highly PAH contaminated area of the Mediterranean Sea (Genoa, Italy), due to intense industrial and harbor activities. The gene expression data for exposure to the sediment-derived PAH mixture was compared with gene expression data for in vitro exposure to specific PAH compounds.
Genes involved in stress response, silica uptake, and metabolism were regulated both upon exposure to the sediment-derived PAH mixture and to the single component. Complementary monitoring of silica in the diatom cultures provide further evidence of a reduced cellular uptake of silica as an end-point for benzo[a]pyrene (BaP) exposure that could be linked with the reduced gene and protein expression of the silicon transporter protein. However some genes showed differences in regulation indicating that mixtures of structurally related chemical compounds can elicit a slightly different gene expression response compared to that of a single component [2]. Furthermore, based on the specific pathways affected by PAH exposure, some selected genes (silicon transporter and silaffin 3) involved in silica uptake and metabolism could be suitable molecular biomarkers of exposure to PAHs. This study aims to confirm the suitability of these genes as molecular biomarkers of phytoplankton species exposed to PAHs in contaminated aquatic environments. It also seeks to uncover molecular differences between the modes of action of complex PAH mixtures and the highly carcinogenic BaP as an isolated compound [2].
References:
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[2] Carvalho,R.N.,et al., (2010). Gene biomarkers indiatom Thalassiosira pseudonana exposed to polycyclic aromatic hydrocarbons from contaminated marine surface sediments. Aquat. Toxicol, doi:10.1016/j.aquatox.2010.10.004

WE 127

The use of molecular responses of crayfish Procambarus clarkii as biomarkers of cadmium and uranium contamination

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Uranium's (U) and cadmium's (Cd) distribution in ecosystems is currently increasing due to anthropogenic activities. Therefore, knowledge in describing the potential impact of these metals on ecosystem components is essential so that proper decisions can be made to protect the environment. Our study aims first to better understand the impact of U and Cd on the molecular level of crayfish Procambarus clarkii by studying modifications in gene expressions after an acute and chronic exposure, then link these changes to the bioaccumulation. Second, this work aims to identify relevant biomarkers of Cd and U contamination depending upon the type of exposure (chronic/acute). Groups of crayfish were exposed to 0 (controls), 0.05, 0.5, 5, and 10 mg/L of Cd and others with 0.3, 0.6, 4 and 8 mg/L of U for 10 days, whereas different groups were exposed to 0(controls), 40 µg/L of U or 10 µg/L of Cd for 60 days. The expression of genes involved in response to oxidative stress (sod(Mn), mt), in heavy metal detoxification (mt) and mitochondrial metabolism (cox1, atp6) were studied in the gills and the digestive glands of crayfish using real-time RT-PCR. 12S gene expression was used as an indicator of the amount of mitochondria in the cell. mt and atp6 genes had to be cloned and sequenced. Gene expressions were altered after short exposure duration and seemed to be sensitive tools to evaluate an early metals toxicity. Data suggest that the expression of mitochondrial genes and genes involved in oxidative stress responses are modified in presence of U and Cd in the gills and the digestive glands of crayfish in both experiments. However, the correlation between the expression of most studied genes and metals bioaccumulation were different in both experiments showing that the type of exposure influences molecular responses. Results proved that mt gene expression is a useful biomarker of Cd contamination no matter the type of exposure. This work also confirms the need of a mutliple biomarker approach.

WE 128

Combined Cu-temperature effects in the Mediterranean mussel Mytilus galloprovincialis: gene transcription profiling associated to physiological responses

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Little is known about the combined effects of heat with pollutants in tissues of marine organisms such as mussels. To get more clues on potential interactions, we carried out short term exposure (4 d) of *Mytilus galloprovincialis* specimens in natural sea water added with increasing amount of copper (10-40 microg/L) across a temperature gradient (16°C-24°C). Core lysosomal biomarkers responses, i.e. lysosomal membrane stability, neutral lipid and lipofuscin content, were examined in the digestive tissue along with transcriptomic profiling in the same organ and in gills.
At molecular level, a clear response to heat and unfolded proteins took place in the digestive gland of mussels exposed to the highest temperature (75 differentially expressed genes found, 5 hsp genes belonging to 3 different classes identified;moderated t-test,p<0.005,N=4).Gene expression profiles in tissues of animals exposed to 40 µg Cu across the temperature gradient, however, showed different patters respect to those obtained for the effects of temperature alone. A of bell shaped response with maximum effects -in terms of differentially expressed gene number- was observed at 20°C, and, indeed, in these tissues, the expression signature associated to heat response appeared to be suppressed.
The genuine response to Cu in digestive gland (23 genes, moderated t-test, p value < 0.005, N=4) encompassed some mitochondrial and extracellular matrix genes, the latter possibly indicating a response to inflammation. However no specific stress response genes, such as MT or oxidative stress related sequences, could be identified.
At physiological levels, biomarkers showed significant lysosomal alterations only at the highest copper concentration; moreover few differences could be ascribed to the temperature gradient per se.
In conclusion, our data demonstrated that the interference of temperature with copper did not produce effects at organismic level, such as mortality. Differences on biomarkers responses to copper were negligible across the temperature gradient, confirming the the ability of mussels to adapt to a changing environment and their role as robust biological indicators. Molecular analysis depicted a clear temperature dependent response and also the ability of Cu to affect such process. To get more clues on such molecular interactions, the effects on other tissues such as gills, where copper is preferentially accumulated during short term exposures, will be investigated.

WE 129

Using metabolomics to identify class-specific changes in Chironomus tepperi larvae exposed to metal and non-metal contaminants

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Metabolomics can be defined as the analysis of the complement of small molecules associated with metabolism. Metabolic profiles can be used to investigate the underlying biochemical response of an organism following exposure to chemical or non-chemical stressors. The aim of the first experiment was to establish whether metabolomics techniques (Nuclear Magnetic Resonance spectroscopy (NMR)) could measure the metabolic profile of third instar *Chironomus tepperi* larvae. Results showed that a suite of metabolites in *C. tepperi* larvae could be measured, such as pyruvate, valine and histidine, from a number of biochemical pathways. A second study was conducted to investigate whether metal-specific changes in the metabolic profile of *C. tepperi* larvae could be identified following exposure to metal and non-metal contaminants. Third instar *C. tepperi* larvae were exposed to two sub-lethal aqueous concentrations of zinc chloride, copper sulphate and ammonium chloride for 24 hours. Larvae were sampled after 2 and 24 hours exposure. These exposures were repeated three times to ensure there was enough tissue for the analyses (based on results from the pilot study). There were three replicates per treatment, per time period. Controls (artificial water) were sampled at 2 and 24 hours exposure and at the start of the experiment. Changes in the metabolite profile following exposure to metals and non-metals are linked to the different mechanisms of toxicity of these chemicals. The value of this technique for identifying pollution impacts on aquatic ecosystems and understanding the mechanistic basis of pollution responses will be discussed.

WE 130

Using the genotoxicity biomarker micronuclei piscine in Bathygobius soporator (Valenciennes, 1873) (Teleostei, gobiidae) in the coast of Salvador (Ba), Brazil

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The aim of this study was to use the genotoxicity biomarker through micronucleus test in Bathygobius soporator in an urban coast environment. Herein we evaluated the metals Cr, Cd, Mn, Pb, Hg, Zn and 16 polycyclic aromatic hydrocarbons in the sediments. The dissolved oxygen, the pH and tide-pools temperature were measured as well. Micronucleus analyses were carried out on peripheral blood erythrocytes of Bathygobius soporator. There were significant differences (p<0,05) in the frequencies of micronclei betwen the negative and positive controls and the treatments (ANOVA one-way). Significant correlation was observed (r = -0,743, p = -0,023) between average frequency of micronucleus and the first axis of principal components analyses (PCA), which explained most of data variation (70,56%). We concluded that were a correlation between the micronucleus frequency and metals. The technique was effective in demonstrating cellular changes in B. soporator, that together with the correlation indicate stress conditions for the fish.

ET12 - Metals and metalloids in the environment: adaptation, bioavailability and speciation

WE 134

Cadmium adsorption in soils employed for pasture and sugarcane plantation with and without vinasse application from Northwest of São Paulo State, Brazil

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The northwest area of São Paulo's state (Brazil) has been presenting an expansion of the sugarcane culture in the last years (responsible by 18% of Brazilian ethanol production), where the vinasse application has been a practice common. Vinasse is a subproduct (wastewater) of sugarcane industry. Cadmium has been a metal present in some investigation in environment compartments in this region. Adsorption plays a key role in the remediation areas studies, since each soil has his own adsorption capacity. Thus, the main objective of this study was determine adsorption capacity for cadmium (II) in three different soil uses (natural soil, argissol and soil from sugarcane plantation with and without vinasse application), as well as the effect of pH and ionic strength on metals adsorption capacity. For this, soils samples were collected and homogenized employed official methods. The parameters pH (H2O, CaCl2), organic matter content, potential acidity, content of Ca^{+2}, Mg^{+2}, Al^{+3}, Na^{+}, K^{+}, cation exchange capacity-CEC, particle size and Cd total contents were quantified in each soil samples. Soil cadmium (II) adsorptions capacity were obtained by batch experiments with metal concentration ranging from 0 to 200 mg L^{-1} for Cd.

Langmuir and Freundlich isotherms models were employed to obtained cadmium adsorption. It was observed that the Langmuir model fit better than Freundlich to the metal cadmium (II). The increasing of pH showed a positive correlation to the adsorption, while the ionic strength showed a negative correlation to the adsorption. Sorption maximum capacities of cadmium (II) per gram of soil found were 2.5 mg/g; 0.7 mg/g and 2.5 mg/g respectively for natural, with and without vinasse soil. It can be concluded that vinasse application decrease soil adsorption capacity turning other environmental compartment more susceptible to pollution. Acknowledgments: FAPESP and CAPES.

WE 135

Characterization of soil trace metal availability : DGT and kinetic extraction vs. conventional sequential scheme

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Numerous approaches are proposed in the literature about the mobility and bioavailability of soil trace metals (TMs). The conventional approach, principally based on the measurement of the metal fraction extracted from the soil solid phase using single or sequential extraction schemes, is carried out at equilibrium where kinetics aspects are generally not considered, while the soil media is a dynamic environmental system. For this reason, some authors have attempted to study the kinetic aspects of TMs transfer. The most frequently used kinetic methods correspond to kinetic fractionation by single extractive schemes and Diffusive Gradient in Thin layer (DGT) technique.

However, both equilibrium and kinetic approaches have been subject to some criticisms like as the operational character and the reliability of each methods to predict the bioavailable fractions. Furthermore, the extractive schemes and DGT techniques are scarcely compared in the literature. In this paper, a comparative study is proposed for estimating the potential availability of Pb and Cd in three polluted urban and industrial sites of old Hanoi in Vietnam. A modified BCR sequential extraction scheme, a kinetic single extraction scheme (EDTA) and DGT technique were used. The kinetic behaviour of of Cd and Pb desorption in soil/EDTA systems were compared with metal concentrations extracted within different steps of BCR scheme and those transferred from the soil solid phase to the resin sink in DGT probes. Kinetic parameters like as kinetic constants rate of desorption were employed to assess the potential availability of each metal. Different kinetic trends have been observed for Pb and Cd.

WE 136

Selective binding of target heavy metals using imprinting technique

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The principle of "adjustment"of humic-based polymeric complexes by the substrate (template) on the stage of their synthesis or formation of three-dimensional structure has been used for selective binding of the heavy metals and radio-nuclides from migration cycles. The point is in the recognition and binding of ions of those metals, which were used as template ions in the synthesis of macro-complex. The principle of the Molecularly Imprinted Polymer (MIP) production is the formation of HA complexes with the corresponding cations of metals and the subsequent cross-linking of their chains, that allows to fix the conformations of the molecules favourable for the binding of these ions. It will result (after the removal of template ions) in the growth of sorption capacity and the speed and selectivity of sorption on the MIPs. Previously such approach was used for the selective binding of strontium ions in the regions contaminated as a consequence of Chernobyl disaster, adjusted polymeric sorbents on the basis of co-polymers of diacrylate strontium with sterol, methylmetacrylate, acrylic acid and cross-linking agent - dimethacryl ether ethylene glycol, etc. (1).

A specific MIPs for targeted Cu (II), Ni and Co metals have been designed and prepared for each metal of interest. MIPs used as a selective sensing material integrated in a sensoric system. Batch equilibrium studies using imprinted and non-imprinted polymer solutions were conducted to determine metal-binding capacities. As a result, such co-polymers possess significant speed of sorption (the balance is set up in several minutes) and sorption capacity (0.5-3.0 mg-eq. M/g). Imprinted sorbents showed increased binding to metals compared to non-imprinted. Factor of the selectivity (ratio of number of sorpted "own"ion to the number of "foreign"ion) is to 1:20- 1:25. Acknowledgements. This work supported by grant of the International Science and Technology Centre (#ISTC KR-1316) and FTP (2010-1.1-222-144).

WE 137

Measuring mercury speciation in multi-component solutions using Donnan membrane technique

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The DMT method has the advantages of simultaneous measurement of different elements in a single sample and it has less disturbance and interference of the sample equilibrium than most other methods.Even though the Donnan membrane technique has been applied successfully to measure several free metal ions in the environmental system, it's has never been used for mercury species measurement yet. Therefore, we tested the application of DMT to mercury measurement in multi-component solutions which contain different mercury species as the dominating components. The results showed that mercury species in solutions containing different ligands can be measured by a dynamic DMT approach at various pH values and ion strengths with a high agreement between calculated and measured mercury species. However, the most important factor which restricts the applicability of DMT to mercury species measurement is mercury adsorption to the cation exchange membrane in the form of Hg2+, HgOH+ and Hg(OH)2. This restriction can be partly solved by shortening sampling time and adding ligands to the acceptor side which complex strongly with mercury.

It was found that when 0.01 M CaCl2 was used as background solution, HgCl2, HgCl3- were main contributors to the flux of Hg transport through the cation exchange membrane. The rate-limiting step of Hg transport from donor side to acceptor side is Hg diffusion in the membrane. There is strong chemical adsorption of Hg in the membrane besides electrostatic adsorption of Hg, which plays as an important role restricting the applicability of DMT on Hg species measurement. The chemical adsorption of Hg can be reduced by shorten the sampling time and Hg species in the donor side can be calculated using the dynamic DMT method. 0.002 mol L-1 Ca(NO3)2 was also tried as background solution in which Hg2+ or Hg(OH)20 were dominative Hg species depending on solution pH. A much stronger Hg sorption phenomenon was observed compared with HgCl2-CaCl2 solution. The Hg loss in the donor side was more than 50% because of strong chemical Hg adsorption on the cation exchange membrane. A retardation factor was introduced to account for this retardation of Hg in membrane, but it could only help to solve this problem in some degree.

WE 138

Effect of copper exposure on the activity of Na+/K+-ATPase in Eisenia foetida coelomocytes

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Na+/K+-ATPase is an ubiquitous plasma membrane pump playing a fundamental role in the intracellular ionic homeostasis. Its sensitivity to pollutant exposure was previously demonstrated in several aquatic organisms. A previous work on the earthworm Eisenia fetida (Calisi et al., Ecotoxicol. Environ. Saf., 72: 1369-1377, 2009) demonstrated copper sulphate exposure to induce a dramatic cells swelling in coelomocytes. The aim of the present work was to test the hypothesis of the possible impairment of E. fetida coelomocyte Na+-K+-ATPase by copper exposure in either in vitro or in vivo conditions.

First, the presence of a specific Na+-K+-ATPase activity was demonstrated in the coelomatic fluid of E. foetida, using the spectrofotometric method of Bartlett (J. Biol. Chem., 234: 466-468, 1959). Then, the coelomatic fluid was in vitro exposed to copper sulphate (1 _M), or ouabain (0.2 mM) (specific inhibitor of Na+-K+-ATPase), or ouabain plus copper sulphate (0.2 mM + 1 _M) respectively, for a time interval of 5 hours. A significant increase of coelomocyte size was observed in the three experimental conditions. After 5 hours of exposure the effect of copper sulphate on cell welling was quite similar to the effect induced by ouabain or by the mixture of ouabain and Cu2+, suggesting that the in vitro inhibition of Na+-K+-ATPase is able to induce cell swelling in earthworm coelomocytes.

Thereafter, specimens of E. foetida were exposed to CuSO4 1 µM utilizing the standard acute toxicity test "Filter paper test"for 48 hours. A significant ($P<0.05$) increase of coelomocytes dimension was observed in exposed organism with respect to the control group paralleled by a significant ($P<0.05$) inhibition of Na+/K+-ATPase activity.

In conclusion, the results demonstrated that the Na+-K+-ATPase of E. fetida celomocites is inhibited by the exposure to Cu2+. This, in turn, is able to induce impairment of intracellular ionic homeostasis and cell swelling. Due to the important immunological role of ceolomocytes which mediate many of the innate immune responses in earthworms, the observed copper inhibition of colomocyte Na+-K+-ATPase activity and the following cellular swelling may increase the susceptibility of animals to diseases and reduce their survival capability. Moreover, results suggest that the pollutant-induced Na+-K+-ATPase inhibition in earthworm coelomic fluid can represent a non destructive biomarker of effect which may be directly linked to organism health.

WE 139

Complexation of nickel by natural ligands and influence on Ni biovailability in aquatic ecosystems - kinetic considerations

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Among the priority pollutants that need to be monitored according to the European Union Water Framework Directive, relatively few studies about nickel fate and effect in freshwater ecosystems are available. The main reason is that the complexation kinetics of Ni is known to be slow, in contrast to those of other metals like Cu and Cd. Consequently we expect that speciation and bioavailability models for Ni require specific studies that take into account exchange kinetics and not only equilibrium status in the environment.

We propose here to present a case of study of the bioavailability of Ni, studied by the mean of Fontinalis Antypiretica. These aquatic mosses were chosen because of their resistance, but also their fast accumulation of metals, allowing working at low level of contamination. Moreover, previous successful studies on Cu have been conducted with them, showing that more than inorganic copper was available for the mosses (Ferreira et al, ET&C, 2008).

The Diffusive Gradient in Thin film technique (DGT) proposed by Davison and Zhang (1994) was chosen as speciation tool. DGT collects inorganic species and a variable part of metal complexes formed with organic ligands. Previous studies highlighted that DGT is suitable for monitoring bioavailability of Cu and Cd when we compare the fraction sampled by DGT and by aquatic organisms (Fontinalis Antypiretica and Daphnia magna).

The bioaccumulation experiments were conducted at environmentally realistic concentrations of Ni (5 to 15 µg.L-1) in natural water. We varied the speciation by adding different type of ligands (EDTA, humic acids, natural organic matter). Bioaccumulation kinetics was monitored from Ni concentration decrease in water. A preliminary study let us verify that Ni decrease corresponded to Ni bioaccumulation in mosses. After a 5d-exposure, bioaccumulated Ni in mosses was measured too. . .

Two behaviours were highlighted in our results depending on the composition of water: without organic ligands, a simple first-order decrease of Ni in the mineral medium, whereas in experiments with organic matter, Ni concentrations in water exhibited a plateau after a first decrease and then a second step decrease.The plateau might be due to the slow dissociation of organic complexes as bioaccumulation in mosses modifies Ni speciation in water.

WE 140

Relationship between metals bioavailability and ecotoxicity in Catalan (Spain) river waters

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It is well known that the toxicity associated to heavy metals is not directly caused by the total amount of metal, but only by its bioavailable form, being this closely linked with the fraction of free metal. The main objective of this study was to assess the toxicity of metals in Catalan (Spain) river waters and to correlate it to several parameters which may have a certain influence on metals bioavailability. For this purpose, the concentrations of 8 potentially toxic elements (As, Cd, Cr, Mn, Ni, Pb, V, and Zn) were analyzed in freshwater and filtered water samples from 11 Catalan river sections. Diffusive Gradient in Thin-Films (DGTs) passive samplers were also used to estimate the bioavailable fraction. Acid extracts of DGTs were used to assess the acute toxicity by using the photo-luminescent bacteria Vibrio fischeri (Microtox*). The knowledge of the tendency of metals to remain in the filterable fraction, adsorbed into suspended material or into DGT devices provides was used to acquire useful information for the prediction of metal speciation in waters. Furthermore, the Mineql+ software, a chemical equilibrium modeling system, was applied to check the consistency of experimental data. The evaluation of metal concentrations with competitive factors, such as water hardness or dissolved organic matter, mostly explained the amount of free ion metals responsible for toxicity. In general terms, toxicity levels in Catalan riv-

ers were found to be relatively low, excepting for 2 sites presenting an important industrialization. On the other hand, the oxidation state of the elements in natural conditions was confirmed to be a critical aspect when assessing toxicity in fluvial ecosystems. The integration of modeling (chemical software) and monitoring (passive methods) seems to be crucial to understand the hazard of aquatic compartments for the river ecological status.

WE 141

In situ validation of chronic biotic ligand models of Cu, Ni and Zn with caged Daphnia magna

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Twelve brooks covering a range of metal contaminations (PEC/NEC from <1 to 80) were selected for validation of chronic biotic ligand models of Cu, Ni and Zn in a complex outdoor multi-contaminant environment. The sites were located in the Dommel catchment in The Netherlands, which is historically contaminated with metals due to the activities of zinc smelters. Data of other organic and anorganic contaminants and general water quality parameters were also taken into account. The effects of pesticides and other organic contaminants were of minor importance, since sites were located in semi-natural areas, dominated by forest and heathland alternated with pastures and maize culture.

Four cages on each site with 20 juveniles of Daphia magna were exposed to natural outdoor conditions for three weeks. Survival and reproduction of Daphnids were determined after three weeks. Contents of Cu, Ni, Zn as well as Co, Cd, Se, Ca, Mg, Na, Fe and Mn were determined with HR-ICP-MS. The level of expected metal risks is reduced by application of the biotic ligand models. The level of observed effects on Daphnia reproduction corresponded well with the effects predicted by the added risk of metals (corrected for bioavailability). The explained variance further improved by taking into account the pH, DOC, PO_4, and O_2. Results of this experiment including accumulation data will be presented on the poster.

WE 142

Models characterizing the nickel bioaccumulation in Gammarus pulex and influence of water cationic composition

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Nickel (Ni) is a common contaminant in freshwaters because of its industrial and domestic applications, with potential ecological consequences. Although chemical analyses allow to determine the total contamination levels in freshwaters, they do not give information on the bioavailability of the metal and consequently, on its toxicity and impact of environmental health. The biodynamic and saturation models characterizing the metal bioaccumulation offer promising perspectives to predict the metal bioavailability in freshwaters. However, the accumulation strategies have to be defined for each metal/organism couple in controlled conditions to construct these models.

This study aims at assessing the waterborne bioaccumulation of Ni and the influence of geochemical properties of water on this process in a crustaceous widely distributed in Europe, *Gammarus pulex*. In the laboratory, the organism was exposed to several concentrations of Ni (from 0.001 to 100 mg L^{-1}) in aquatic microcosms. Then, bioaccumulation of Ni was recorded in waters exhibiting various concentrations of three major ions (Na, Mg and Ca).

Our results showed that *G. pulex* was greatly tolerant to Ni ($LC50_{48h}$ = 477 mg L^{-1}). Time course experiments allowed to construct the biodynamic model by determining the uptake (*ku*) and elimination rate constants (*ke*). For the exposures above 1 mg L^{-1}, the metal uptake saturated. Thus, the maximal capacity of organism to accumulate the metal (*Bmax*) and the half-saturation constant (*K*) were determined to establish the saturation model. It was verified that the two models are comparable for the lowest exposures (< 0.1 mg L^{-1}), with $ku/ke = Bmax/K$. Furthermore, only an increase in the concentration of Ca decreased the bioaccumulation whereas Na and Mg had no effect until 80 mg L^{-1}.

This study reports for the first time the bioaccumulation of Ni in *G. pulex* and suggests that this crustaceous could be used as an integrative tool to quantify the Ni bioavailability in freshwaters. Taking into account the geochemical factors influencing the metal bioavailability allows to improve the prediction of bioaccumulation models.

WE 143

Variability in Cd uptake by Pseudokirchneriella subcapitata among freshwaters containing dissolved organic matter with contrasting characteristics

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The Free Ion Activity Model (FIAM) predicts that the free ion is the preferred species for uptake from solutions with the same inorganic composition. It has been shown previously that FIAM not holds for the cadmium (Cd) uptake by the green algae Pseudokirchneriella subcapitata. The Cd uptake increased in the presence of inorganic (e.g. Cl-¬) at the same free Cd2+ activity. Here, we investigated the influence of natural dissolved organic matter (DOM) and synthetic organic ligands (e.g. NTA, EDTA) on the Cd uptake by green algae. Eighteen freshwater samples of natural, agricultural and urban areas were isolated by reverse osmosis. The waters were analysed for ionic composition, DOC concentration, SUVA and metal binding capacity. The Cd binding DOM properties per unit C varied tenfold among the different freshwater samples. The DOC concentration varied also a factor tenfold among waters. This suggested that both DOC quantity and DOC quality are equally important to predict Cd availability. We exposed algae for 4 days to different DOM samples and synthetic ligands at a constant free Cd2+ activity. The DOC concentration in the test medium was 25 mg C/l. In this design, we observed a higher Cd uptake in the presence of DOM/ synthetic ligands than in absence of complexes, in contrast with FIAM that predicts no change. As a result, Cd uptake by green algea increased with increasing metal binding capacity of the freshwater DOM samples.

WE 144

Relevance of water chemistry to assess lethal and sublethal effects of metals using Daphnia magna

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The aquatic toxicity of metals is a current and widespread environmental problem. The toxicity of metals is known to be dependent on the water chemistry parameters (pillar of the Biotic Ligand Model). The majority of the existing works focus either on the acute or chronic toxicity of metals. However, few models, integrating water chemistry, address the toxicity of metals on feeding activity. The feeding activity constitutes a sensitive indicator of toxicity and is an ecologically relevant endpoint. Thus, in this work we studied the relative importance of hardness and alkalinity in the toxicity of copper and zinc to the feeding activity of *Daphnia magna*. Concomitantly, we studied the effects of both these water chemistry parameters on the acute lethal responses.

The establishment of functional relationships between water chemistry-metals and the feeding rates in *D. magna* is essential to provide a better and more realistic understanding of potential long-term effects of the metals on cladocerans under ecologically relevant conditions. Both hardness and alkalinity were found to influence the lethal and sublethal effects of metals, although the effect of the former was more relevant. Additionally, regression analysis showed that the toxicity of copper and zinc was well described by these water chemistry parameters.

WE 145

Mitigating effects of humic substances in the polluted environments caused by chemical binding to ecotoxicants

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Humic substances (HS) are natural organic compounds comprising 50 to 90 % of the organic matter of peat, lignites, sapropels, as well as of the non-living organic matter of soil and water ecosystems. Being the products of stochastic synthesis, HS have elemental compositions that are non-stoichiometric, and structures, which are irregular and heterogeneous. The structural heterogeneity of HS may explain their resistance to biodegradation: the longevity of humics in soils is typically on the order of thousands of years. This is of particular practical relevance if objective is to develop soil/aquifer remediation technology predicated on a recalcitrant matrix derived from HS.

The complex structure provides a very diverse reactivity of HS. They are able of binding both heavy metals and organic xenobiotics. The most common metal binding occurs via carboxylic and phenolic oxygen, but nitrogen and sulfur also have a positive effect on metal binding. The various binding mechanisms between organic contaminants and HS include hydrogen bonding, van der Waals forces, ligand exchange, and charge transfer complexes representing weak binding energies and formation of covalent linkages representing chemically stable bonds.

The binding to HS causes a change in speciation of heavy metals and organic xenobiotics followed by a change in their toxicity and bioaccumulation.

The goal of the research was to develop detoxicants of integrated effect on the basis of humic substances and to conduct the feasibility studies on their production. 8 samples of parent humic materials have been isolated and about 30 humic-based de-toxicants have been synthesized. The experimental approaches undertaken to produce humic-based de-toxicants included enrichment with different groups (O-, OH-, CO-, hydrophobic groups). It has been demonstrated from both chemical characteristics and detoxifying ability point of view that all the produced de-toxicants possessed equal or higher detoxifying potential in relation to heavy metals or herbicides. Derivatives of humics have been studied at both express bioassay, laboratory vegetation experiments and field trials scales as well. All the experiments confirmed not only high detoxifying potential of those de-toxicants in relation to heavy metals but also their prolonged activity in the environment. Producing of the above de-toxicants at pilot plant scale demonstrated an opportunity to synthesize them at industrial scale.

WE 146

Assessing fate and toxicity of copper from re-suspended sediments in rainbow trout (Oncorhynchus mykiss)

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Sediments can act as a sink as well as a source for environmental pollutants. One interesting field of recent scientific studies is to investigate possible ecotoxicological effects due to the uptake of resuspended particles and contaminants during flood events. In this work, juvenile rainbow trout (Oncorrhynchus mykiss) were exposed to resuspended natural sediments from Ehrenbreitstein (River Rhine) spiked with copper at a nominal concentration of 500 mg kg-1 dry weight. Furthermore a control test without spiking was used in this experiment. Samples of water, suspended particles, fish tissues and livers were taken at days 0, 1, 2, 3, 6, 8, and 12. The aims of this study are twofold. The first approach is to look into the distribution of copper between the compartments water, suspended particles an fish tissues using different methods of digestion (UV, microwave) and ICP-MS. Secondly biomarkers, i.e. measurement of lipid peroxidation, as an indicator for oxidative stress, and quantification of metallothionein using Western immunoblotting, as an indicator for metal exposure, were used. The presentation will include the results of the experiments, which are currently being conducted. The RWTH Aachen University Undergraduate Funds Programme provided funding for performing ICP-MS analyses at the University of Heidelberg by a personal travel grant to the first author.

WE 147

Effect of DOC concentration and type on the bioavailability of copper in Lambro River (Italy)

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The interaction with physicochemical parameters, including pH, hardness, cationic competition and dissolved organic carbon (DOC), greatly affects the toxicity of metals to aquatic organisms in ambient water. Standard procedures for deriving environmental quality criteria are recognized inadequate, since toxicity data generated by tests set-up using standard media may be poorly representative for assessing metal toxicity in surface waters. The water-effect ratio (WER), based on the direct ecotoxicological evaluation of metal toxicity in site-water, is the only approach which measures the water-chemistry effect on metal toxicity at specific sites. Bioavailability adjustment using WER can thus help improving the development of mathematical models to predict the toxicity of dissolved metals in freshwater systems with different chemical composition.

Since important changes of the riverwater chemistry are expected up to downstream over the course of rivers draining highly anthropized areas, the aim of this work was to evaluate changes of metal toxicity over the River Lambro, which drains one of the most densely populated area of

Northern Italy. The experimental design was mainly focusing on the complexing properties of DOC, as differently affecting the toxic bioavailability of copper over the river-course.
The ecotoxicological WER procedure, involving the simultaneous evaluation of Cu toxicity in riversite-water and in standard-water, was set-up using the alga growth-inhibition test with Pseudokirchneriella subcapitata. Changes of Cu-toxicity were evaluated both in different river-flow, and over the river-course, up to downstream from the most urbanized area comprising the city of Milan. Following DOC enrichment, mainly originated from urban sewage, the protective effect of the organic-ligands was amplified in the lower part of the river. The experimental removal of organic ligands by SPE extraction of the downstream samples also proved the protective role of DOC in the control of Cu-bioavailability. Finally, changes of DOC type, at similar DOC concentration, have been suggested by the important enhancement of WERs measured in conditions of high river-flow, possibly involving the river-sediment release as an additional DOC-type source.
The obtained results highlight the importance of the WER-derived information to improve predictive models for metal bioavailability, suggesting to take account of the effect of DOC type, which is hardly predictable at present.

WE 148
Spatial and temporal variation in bioavailability and species sensitivity of Cu, Ni and Zn in surface waters of The Netherlands
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Geographical and temporal variations in metal speciation were calculated and water-type specific sensitivities were derived for a range of aquatic species, using surveillance water chemistry data that cover almost all WFD-surface water types in The Netherlands. Biotic ligand models (BLMs) for Cu, Zn and Ni were used to extrapolate chronic NOECs determined in test media towards site-specific NOECs for 372 sites sampled repeatedly over 2007-2010. Site-specific species sensitivity distributions were constructed accounting for chemical speciation. The sensitivity of species as well as the predicted risks shifted among species over space and time, due to changes chemical speciation and biotic ligand binding. The sensitivity of individual species (NOECs) and of the ecosystem (HC5) for Cu, Ni and Zn showed a spatial variation up to two orders of magnitude. Seasonality of risks was shown, with an average ratio between lowest and highest risk of 1.3, 2.0 and 3.6 for Cu, Ni and Zn respectively. Maximum risks were predicted to occur in the winter months (peaking in February) and minimum risks in September. A risk assessment using space-time specific HC5-values of Cu and Zn resulted in a reduction of sites at risk, whereas for Ni the number of sites at risks increased.

WE 149
Delivering a practical methodology to account for metal (bio)availability in the Water Framework Directive - tools and techniques
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Significant scientific progress has been made for assessing metals in surface waters. Recently, the current state of the science has been incorporated into the new EU Technical Guidance for deriving Environmental Quality Standards (EQS) under the European Water Framework Directive (WFD). The guidance supports the use of a tiered compliance assessment regime for metals that incorporates, notably, (bio)availability and recommends using Biotic Ligand Models (BLMs). This approach was recently supported by the EU Scientific Committee on Health and Environmental Risks (SCHER). However, the guidance does not provide practical tools to implement a bioavailability-based compliance regime (e.g. individual member state compliance assessment). To this end, simple screening tools based on full BLM calculations have been developed for several metals (e.g. Cu, Zn and Ni). The tools require the input of the following abiotic water parameters: Dissolved Organic Carbon, pH and Hardness/Calcium concentration. As such, they capture most of the variability in metal (bio)availability caused by the chemistry of a given water body. The tool can be fully automated in laboratory information management systems. Extension to other metals is anticipated. Screening tools, when used as part of a tiered approach, provide water managers with an opportunity to efficiently account for metal (bio)availability in a transparent way and deliver a robust metric of potential environmental risk. A website (www.bio-met.net) acting as a portal for all aspects of metal (bio)availability and its implementation, including the multi-metal tool for assessing bioavailability, will be demonstrated at this meeting and will be made publically available in the course 2011.

WE 150
Spatial risk assessment of nickel in surface waters of Great Britain
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The potential risks due to nickel in surface waters can be assessed by comparing the local dissolved Ni concentration against a local water quality standard calculated using the Ni Biotic Ligand Model (NiBLM). Data for dissolved Ni exposure and the required input data for the NiBLM are often unavailable for the same location, so kriging, a spatial interpolation approach, was used to estimate data for unsampled locations. The approach reproduces general trends in the data, and interpolated local water quality standards identify areas of high and low Ni bioavailability, which are driven predominantly by local dissolved organic carbon concentrations. Interpolated exposure data identify areas of current and historic metalworking industries, former mining areas, and areas of high soil background concentrations of Ni, all of which contribute to elevated Ni exposures. The predicted risks due to nickel in surface waters also follow general trends, although predictions for specific sites are poor. This approach is useful for identifying areas where increased monitoring effort may be required, but is not sufficiently reliable for more detailed assessment purposes.

WE 151
Water quality standards for silver in Dutch surface waters - a proposal in accordance with the Water Framework Directive
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Over the last years, the use of silver in consumer products has rapidly increased. Most of these applications are in the form of nano-silver, which is used as anti-microbial agent in many consumer products. This increased use is expected to lead to increased emissions of silver into the environment. The current water quality standard (QS) for silver in the Netherlands originates from 1999. In view of the expected increase in environmental concentrations, updated QSs for silver and nano-silver in surface water are needed. The number of aquatic ecotoxicity studies with nano-silver is still limited, but there are indications that silver ions determine the effect. Also for "non-nano"silver, dissolved ions are considered most relevant from an ecotoxicological point of view. Therefore, QSs for dissolved silver were derived in accordance with the methodology of the European Water Framework Directive (WFD). Public literature on aquatic ecotoxicity of silver was reviewed with respect to scientific reliability and relevance for this purpose. Only toxicity data which relate to dissolved silver were selected for QS-derivation. Most available studies are performed with silver nitrate, but also studies with other species like silver chloride and silver thiosulfate were reviewed. Following the requirements of the WFD, secondary poisoning of predatory birds and mammals was considered as well, since this could be a significant route of exposure. Human exposure through fish consumption is not considered relevant for the derivation of QSs, because human toxicological risk limits for silver are relatively high and this route is most likely not critical. For naturally occurring compounds like silver, QSs are expressed as maximum concentrations that may be added to the background level. Monitoring data are not available for Dutch surface waters, but literature data indicate that environmental concentrations can vary from 2 ng/L to 2 µg/L. This means that the background levels will have a significant influence when compliance to the new QSs needs to be evaluated.

WE 152
Revised method of hazard and ecological risk assessments for calculating Comparative Toxicity Potentials of metals for which BLMs are not available
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Current practice in chemical hazard ranking and toxic impact assessment estimates fate and toxicity assuming the chemical exists in dissolved and particulate phases and, for metals, that all dissolved species are equally bioavailable. This treatment of metals, similar to organic chemicals, introduces a significant error in their estimates of hazard ranking since metal bioavailability and ecotoxicity effects are related to truly dissolved phase and free metal ion within it, not the total dissolved phase. We previously addressed this concern by introducing a Bioavailability Factor (BF) to the calculation of Comparative Toxicity Potentials (CTPs) for hazard ranking of chemicals (Gandhi et al. 2010 ES&T). In this method, we showed that the aquatic ecotoxicity Effect Factor (EF) for metals are calculated using the Biotic Ligand Models (BLMs) to correct for water-type specific bioavailability to aquatic organisms. However, the method application is currently limited to cationic metals for which BLMs are available (e.g., Ag, Cu, Ni, and Zn). To address this issue, we extended the method of calculating CTPs by proposing the use of the modified Free Ion activity Model (FIAM) to replace BLMs for other metals. We first compare EFs and resulting CTPs for several distinct water-types around the world to show that the variations between the estimates from the two models are within the reasonable range for the purpose of hazard ranking and risk assessment. Our results stress that it is more important to select a generic freshwater archetype on which the analysis should be based than the type of ecotoxicity model itself. We then applied the extended method to calculate CTPs of metals like B, Cd, Co and Pb for which the BLMs are not publicly available yet. We present the variability in CTPs of these metals using the aquatic chemistry of several global freshwater systems.

WE 153
Development of a methodology for the ecotoxicological analysis of air samples. Correlation with chemical pollutant levels.
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The human being is exposed to multiple environmental chemicals, rather than to specific agents. Pollutant mixtures may pose important adverse health effects, not only because of the effects of individual substances, but also as a result of the potential synergisms/antagonisms among them. Despite that, and because of the difficulties to assess the hazard potential of these mixtures, regulation measures to control the levels of pollutants are traditionally focused on determining the levels of contaminants individually. Air inhalation is known to be an important exposure pathway to some chemicals, such as heavy metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). In recent years, a number of surveillance programs have been performed in order to monitor the air quality in industrial (close to incineration and cement plants) and urban areas in Catalonia (Spain). Data on the concentrations of metals (As, Cd, Cr, Hg, Mn, Ni, Pb, Tl, U, and V), PCDD/Fs as well as particulate matter (PM10), obtained by means of high-volume active sampling devices, are available. However, an assessment of the exposure to air pollutants has not been carried out from an integral point of view; this is health risks have not been fully characterized from an integrative way. Microtox® bioassay utilizing Vibrio fischeri bioluminescence bacteria is a suitable method to evaluate the ecotoxicity of environmental samples. Although it has been largely used in aquatic and solid extract studies, its applicability in other environmental compartments has been merely occasional. The objective of the present study was to develop and validate a Microtox®-based ecotoxicological method to evaluate the air quality. To obtain a first approximation of air samples ecotoxicity, the light emission was measured when the organisms were exposed to the PM10 retained in Quartz Fibber Filters (QFF) from different areas of Catalonia. The suitability of combination different solvents with different polarity such as, ethanol, methanol, acetone, hexane, DMSO was firstly checked and compared with corresponding QFF acid extracts neutralized with NaOH. Furthermore, toxicity values were correlated with the levels of metals, PCDD/Fs and PM10.

WE 154
Challenges for the ecological assessment of organometallic substances under the Canadian Environmental Protection Act, 1999
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As part of Canada's commitment to address the ecological and human health risks associated

with chemicals in commerce by 2020, Environment Canada needs to assess the ecological risks of a variety of metal-containing substances. One important group of metal-containing substances are the organometallics. These are defined as substances that contain at least one metal atom that is covalently bound to a carbon atom or that has multiple bonds with oxygen, nitrogen, sulphur, or phosphorus atoms. Organometallic substances are often not well studied in the realm of ecotoxicology. They are chemically complex and often fall outside of the domain of applicability of current models. In addition, their extent of degradation and contribution to the environmental loadings, and influence on the speciation of the metal moieties are not well characterized and may be variable. Organometallics may therefore require nuanced and specific approaches for individual cases or subgroups. This poster presentation will outline important data gaps for scientific questions and possible approaches for grouping organometallic substances and assessing their ecological risks. New information and understanding of these substances would greatly benefit current and future ecological assessments in Canada and elsewhere. Environment Canada will continue to engage the international community, researchers, stakeholders and partners to refine the evaluation methodology for organometallic substances.

WE 155

Empirical model of As bioavailability to earthworm, Eisenia fetida; approaches using geochemistry of soils

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Arsenic contamination in soils has been issued because of the toxic properties of a number of its compounds. Earthworm has been kown to bioaccumulate As from soils and found to be highly elevated in As. It is generally known that the inorganic forms of As are more toxic than the organic forms and readily accumulate in living tissues due to their affinity for proteins, lipids, and other cellular components. Geochemistry of soil has been reported to affect metal accumulation in earthworms. Understanding the As accumulation in earthworms with regard to both kinetics and metabolisms can make it possible to determine the bioaccessibility (or environmental availability) of As in soils. The study was aimed to determine the effects of soil characteristics on As toxicity, accumulation and metabolisms in earthworms and derive the geochemical factors on As accumulation. Field soils which were contaminated with As were collected and pre-treated in the lab. The soils were characterized for several geochemistry of soils. Earthworms, Eisenia fetida, cultured in the lab were exposed to field soils for 70 days and worms were collected at appropriate time interval to measure As body burdens. One compartment model was applied to estimate uptake rates. Calculated uptake rates were correlated with various soil properties and empirical models were created to predict uptake rates. Sorbed As on soils were significantly correlated with uptake rates implying bioavailability could be estimated by As fraction in soils. Fe-oxide contents were the second predictor of uptake rates. Multivariate regression was also applied to calculated uptake rate constant with various soil properties and sulfate, Fe-oxide, and DOC in soils were the predictors of uptake rate constants. All predictors in the empirical models were highly important in As fate and transport in soils implying As transport in soils played a significant role in As bioavailability in soils.

WE 156

Ecotoxicological assessment of catalytic heavy metals leaching from waste fuel cells

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Transportation relies on liquid fuels derived from fossil fuels with many associated environmental impact. Therefore, a lot of hopes are being placed on the future of fuel cells to replace today's batteries. However fuel cells are composed of separator (made from metals such as Fe), electrolytes (made of polymeric materials), electrodes (carbon), catalysts (metals such as Co, Ni). Leaching of Co, Ni, and other catalystic heavy metals from waste fuel cells poses possible environmental risk during dumping. This study will be very useful in us for making decisions on the type of fuel cells which should be used in the engineering of environmentally friendly batteries. The individual Co and Ni toxicity to the Daphnia magna (D. magna) and green alga Pseudokirchneriella subcapitata (P. subcapitata) were investigated. Acute 48h immobilization assays with juvenile D. magna (<24h old) were performed according to the OECD ecotoxicity test guideline 202. Acute 72 h growth inhibition assays with P. subcapitata were performed according to the OECD ecotoxicity test guideline 201. NiCoFe/C fuel cell catalyst was assessed for their leaching properties based on the Japanese Leaching test 13 (JLT13). The ecotoxic properties of the leachate obtained by leaching method was determined by the use of the freshwater crustacean D. magna. Toxicity of Co and Ni leached from the real fuel cells were also observed with D. magna and P. subcapitata. Our result indicates that the tested fuel cell catalyst was of the toxicity evel equal to that of D. magna used in our assay.

WE 157

Assessment of some environmental pollutants in Beni-Sueif Governorate, Egypt

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Concentrations of cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were measured in water, Ceratophillyum demersum (C. demersum) aquatic plant, and the muscle, gill, liver, blood and kidney of Claries lazera fish (C. lazera) collected from nine sampling stations (districts), Beni Suef, Elfashn, Beba, Somosta, Ehnasia, Elwasta and Naser, along El Ebrahimia canal and two districts located at the east bank of the Nile (Bayed El-Arab and Sanor) in the province of Beni Suef, Egypt during 2009-2010 using Solar Atomic Absorption spectrometer M6.The results reveal that the studied metals were detected in all the examined samples. In water, Pb had the highest concentration among the metals detected in Elfashn, Beba, Naser, Elwasta, Somosta, Bayed El-Arab and Sanor; Mn presented the highest concentration in Ehnasia, while Fe had the highest concentration in Beni Suef. The concentrations of Pb, Fe, and Mn were above the maximum permitted limits in all the districts. Cd concentration was above the permitted limit, except in Somosta and Naser, while Zn and Cu concentrations were below the permitted limits in the nine districts. The metal levels in water were compared with national and international water quality guidelines, and with the literature values reported for rivers and streams. Comparisons were made of the metal concentrations in water and aquatic plants with those in the catfish tissues obtained from water. The metal concentrations found in the C. demersum aquatic plant samples taken in the nine studied districts were distributed in this order; Mn > Zn > Cu >Pb >Fe > Cd. and were higher than the water. In fish, metals accumulated in the various examined tissues at several levels, but the metal concentrations in muscles (edible part) were below the metal levels in the other organs (nonedible) in the fish samples. The concentrations of Cd, Pb and Fe in fish tissues were above the international standard, while the concentrations of Mn, Zn and Cu were below this standard. The high concentrations of these metals in water, aquatic plants and fish in El Ebrahimia canal may be the result of both anthropogenic activities producing industrial, agricultural and domestic waste and accidental pollution incidents.

WE 158

Bioavailability of trace metals in contaminated soils (Northern Portugal)

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A chemical sequential extraction of heavy metals (Fe, Mn, Cu, Zn, Pb, Cd, Sn, W, Bi, Ni, Cr, Mo, Co) and As in tailings and soils samples around a five W/Sn mines (Northern Portugal) was realized using a 7-steps fractionation procedure: (1) water-soluble; (2) exchangeable; (3) bound to Fe oxyhydroxides (easily reducible); (4) bound to Fe oxides (moderately reducible); (5) bound to organic matter and secondary Cu-sulfides; (6) bound to primary sulfides; (7) residual. The extracted element contents were measured by ICP-MS. Samples were also analysed for pH, electrical conductivity and organic matter.
The results allowed us to notice that: 1) The pH was the main factor for controlling the geochemical distribution of the studied elements. The tailing and soil samples were very acid, with an average pH of approximately 4.37. Some metal cations (Mn, Cd, Cu, Zn, Pb, Co, Cr, Ni) behave in a similar way, revealed important enrichments in the most bioavailable fractions (water-soluble and exchangeable fractions). In contrast, oxyanions as Mo and As show low mobility through adsorption to Fe(III) oxyhydroxides dissolved in the two reducible fractions. These results reflect the pH dependent adsorption on the clay minerals, Fe and Mn oxyhydroxides and the co-precipitation with these secondary minerals; 2) Residual fraction was the most important fraction for Sn, Mn, Cr and Zn. These results suggest that in these soils these elements are in a detrital, non available form; 3) Scavenging of mobilized elements (mainly Fe, Mn, Cu, Zn, Cd, Pb, W, Bi, Mo, Cr, Ni, Co and As) in secondary mineral phases and sulfides could be considered a temporary mechanism of metal retention. These metal fractions are susceptible of being set free depending on some changes of environmental conditions.

WE 159

Monitoring data for trace elements in soil and their use in risk assessment: the GEMAS project

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A sound risk assessment for trace elements in soil must preferentially take into account the variation in natural background concentration and the differences in bioavailability across soil types. Data availability for both aspects differs largely across different countries or regions, hampering a consistent approach on a large (e.g. continental) scale. The European REACH Regulation specifies that industry must prove that it can produce and use its substances safely and risks, due to the exposure to a substance during production and use at the local, regional and European scale, all need to be reliably assessed. The GEMAS (geochemical mapping of agricultural and grazing land soil) project provides good quality and comparable exposure data of metals in agricultural and grazing land soil; in addition soil properties known to influence the bioavailability and toxicity of metals (and other elements) were determined in soil at the European scale. The GEMAS project was carried out by the EuroGeoSurveys (EGS) Geochemistry Expert Group in cooperation with Eurometaux and the aim of this project was to produce harmonised data with respect to the spatial scale (sampling density), analytical methodology and land-use (comparable level of diffuse emissions). These data provide a strong basis for taking into account the spatial variability of both exposure (metal concentrations) and effect concentrations (considering bioavailability through variation in soil properties) in a risk assessment for metals in soils. The results of the GEMAS project further allow a harmonized country"ðspecific regional risk characterization and correction for the correlation between exposure and effect concentrations in the development of a generic risk assessment. The use of GEMAS data in a local and regional risk assessment of metals in soil will be discussed based on a case study for Cu.

WE 160

Metals dynamic from ferti-irrigation in soils in the northwest region of the São Paulo state, Brazil

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Brazil is the world leader in the sugarcane production, where the São Paulo state is responsible by 60% of the Brazilian production and the northwest area of the state contributes with 18% of this amount. For alcohol production from sugarcane two types of wastewater are generated, one of the alcohol distillation (vinasse) and another of the industry machines cleaning, from sugarcane washing, floor washing, among others. All these residues are employed for ferti-irrigation, being this practice accomplished following technical norm P4.231 of the São Paulo State Environmental Control Agency, which establishes guidelines for the wastewater soil application. Northwest area of São Paulo state possesses two aquifers (Bauru and Guarani) employed main by water supply. In this region there are 15 sugarcane agro industries, with an area of approximately 400.000 hectare with sugarcane plantation. This study conducts a study of the metal dynamics for the several environmental compartments of the northwest area from São Paulo state. During one hydrological year water samples (underground, surface and sugarcane agro industry wastewater) and soils (with and without vinasse application) were collected, preserved and conditioned following to standard methods recommendations. Total metal (Cd, Cr, Zn and Cu) in all these samples were quantified employing a Graphite Furnace Atomic Absorption Spectrometer (GFAAS, Varian Z-280) or a Flame Atomic Absorption Spectrometer (FAAS, Varian FS240). Values found of Cd, Cu, Cr, and Zn in the surface water samples were <0.05, 3.8, 2.6 and 4.4 ug/L, respectively. For underground water the values obtained were <0.05, 3.6, 5.4 and 2.7 ug/L, respectively for Cd, Cu, Cr and Zn. Wastewater from sugarcane agro industry presents high values of metal concentration (Cd, Cu, Cr, and Zn of the 0.1, 35, 27 and 115 mg/L, respectively). According to the technical norm 86.486 L are applied for each soil hectare. Then 0.001, 0.3, 0.3 and 1.1 mg/m^2 of the Cd; Cu; Cr and Zn, respectively, are disposals during the sugarcane harvest season. Adsorption capacity of the soil of the region is 2.5 mg/g and 1.2 mg/g for Cd and Cr, respectively. It can be concluded that ferti-irrigation contributes as a metal sources for several environmental compartments. Finally, we waited to create a vulnerability index for the soils, as well as to infer on which environmental compartments will be more susceptible to the contamination. Acknowledgments: FAPESP and CAPES.

WE 161

Assessing the relationship between elimination and detoxification in fish and shellfish exposed to cadmium, copper, and zinc

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The purpose of this study was to estimate physiological traits related to Cd, Cu, and Zn bioaccumulation, compartmentalization, and susceptibility in fish and shellfish based on recent publish data. The subcellular partitioning was incorporated into metal influx threshold hypothesis to estimate the detoxification rate constant that is based on exposure concentration and elimination rate constant. Then elimination-detoxification relationships were constructed to predict the detoxification capacity by known elimination rate constants. Our results show negative relationship between elimination rate constant and % metal in metabolically detoxification pool for all aquatic species exposure to metals. Interestingly, a species-specific difference in relationship of detoxification rate constant and % metal in metabolically detoxification pool was found indicating a positive correlation for fish, and a negative correlation for shellfish. We found fish had higher bioaccumulation in metabolically active pool (MAP) when exposed to essential metals of Cu and Zn (~ 60 - 90 %), whereas ranged between 10 - 50 % accumulation for non-essential metal of Cd. A species-specific difference was also found in relationship between elimination rate constant and detoxification rate constant, indicating fish had negative relationship and shellfish had positive relationship. The combination of the biokinetics of metal accumulation with an understanding of the relationship between elimination and detoxified capacities can be applied for environmental risk assessment in the future study.

WE 162

Applications of non-biting midge larvae (Chironomus riparius) for sediment toxicity testing - a critical assessment

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The non-biting midge Chironomus riparius larvae are routinely used in subchronic (10-day) and chronic (lifecycle) sediment toxicity assessement tests. The chironomid response to stress caused by environmental contaminants may be affected by other factors like organic matter content, age of larvae or feeding regime. In our studies, we investigated the chironomid responses in 10-day and lifecycle experiments in both artificial sediments and natural sediments collected from several sampling campaigns in the Czech Republic. Further, variability in responses of test organisms originating from three distinct culture lines were evaluated. The chironomids showed high tolerance to contamination in the natural sediments and it was complicated to find a clear correlation patterns between the toxicity results and analytical data of sediment contaminants. Our results of 10-day tests further demonstrate the decrease in chironomid larvae sensitivity to toxicity with their posthatch age. The 2nd instar larvae were at least 100fold more sensitive than their 3rd-4th instar counterparts, and these differences must be carefully considered during experimental design. We also recorded a significant difference in the absolute values of larvae body length (sublethal growth endpoint) among individuals originating from different culture lines. In another experiment with cadmium contaminated artificial sediment, application of chironomids for bioaccumulation and biomarker assessment was demonstrated. Our results contribute to a critical evaluation of the sediment toxicity tests using chironomids. Various sources of variability have been described, and these should be carefully considered in the experimental design.

WE 163

Derivation of sediment effect concentration and site specific evaluation of potential ecotoxicity of metals in Korean freshwater sediment

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This study was implemented with objective of assessing potential ecotoxicity of sediment-associated metals from Korean freshwater using sediment effect concentration (SEC10 and SEC50) showing the chemical concentrations that correspond to a 10% and 50% probability of observing sediment toxicity to Hyalella azteca. A total of 540 sediment samples from rivers in 13 cities and counties were collected in July and November 2007, and analyzed for three metals (As, Cr, Ni) and one conservative element (Li) for normalizing the bulk sediment metal data. The average concentration (range) of As, Cr, Ni and Li are 68.5 (<0.002-2268), 60.1 (2.8-450), 30.7 (<1.9-360), and 20.8 (4.3-56) mg/kg, respectively. There were significant linear relationships between three metals and Li in most of areas, except for some areas located near heavily industrialized areas. The percentage of sediment samples exceeding SEC10 and SEC50 were 22.8% and 6.7% for As, 32.3% and 6.9% for Cr, and 12.5% and 3.8% for Ni, respectively. However, site specific background levels for Cr and Ni in some areas were higher than SEC10. This result indicates that it is necessary for a new SEC to be derived from the site specific background metal concentration in sediment.

WE 164

The effect of increased oxygen conditions on metal bioavailability, uptake and growth of Myriophyllum aquaticum.

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Despite a gradual improvement of the surface water quality of many European waters, historically polluted sediments may still pose an environmental risk. Metals, precipitated with sulphides under anaerobic conditions, can become available when exposed to higher oxygen concentrations. In a 60 day lab experiment, metal availability to the aquatic plant *Myriophylum aquaticum* was compared between a low (40%) and a high (95%) oxygen level treatment. Sediment metal and AVS-SEM concentrations, surface water metal concentrations, plant root and above ground metal concentrations and metal availability with DGT membranes were measured at different depths during five sampling campaigns. A significant decrease in superficial AVS concentrations following surface water aeration was measured. An increase in Pb availability and release to the surface water can be expected. However, no significant higher Pb concentrations in surface water or above ground plant tissues were found in the 95% O_2 treatment. An AVS/SEM value higher then 1, even at the end of the experiment and adsorption to Fe- and Mn-hydroxides might be an explanation. *Myriophyllum sp*. are often used in sediment contact tests with growth as end point. Altering bioavailability by oxidation of anaerobic sediments by plant growth or aeration might be a potential risk fot the tests. The slow uptake compared to biosorption and small biomass of the plants makes this species not very suitable for phytoremidiation of contaminated organic rich sediments.

WE 165

Comparing the performance of DGTs and SEM-AVS models of Ni bioavailability in lotic sediments

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For heavy metal contaminated sediments, diffusive gradients in thin films (DGTs) have been promoted as potential monitoring tools to measure metal bioavailability and thus predict adverse effects in biota. We conducted two manipulative field experiments using Ni-amended sediment to compared DGTs to the currently used acid volatile sulfide simultaneously extracted metal (AVS-SEM) models of bioavailability in their ability to predict changes in the colonizing benthic macroinvertebrate community. In total, we used 7 sediment types differing in AVS, organic carbon (OC), and Mn and Fe oxide concentration. Each sediment type was amended with Ni (concentrations ranged from control sediments with little Ni to potentially highly toxic Ni concentrations) and placed in baskets within a streamside mesocosm or flush with the stream bottom. Metal flux measured by DGTs correlated well to total Ni concentrations, but was not affected by AVS, OC, or Mn and Fe oxide concentration. The benthic macroinvertebrates responded to the sediment Ni with reduced abundance and diversity at higher Ni concentrations. The SEM-AVS bioavailability models outperformed the DGT-based models in predicting changes in the macroinvertebrate community. Neither the SEM-AVS nor DGT-based models of bioavailability accounted for the protective effects of Fe and Mn oxides, which greatly reduced the bioavailable Ni fraction at later sampling points. These results suggest that for lotic sediments with significant binding capacity (i.e., AVS, OC and Fe and Mn oxides), DGTs may not be the best tool for measuring Ni bioavailability.

WE 166

Uptake and accumulation of sediment-bound heavy metals and effects on gene expression in zebrafish (Danio rerio) embryos

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Predicting the bioavailability and effects of heavy metals in sediments is of major concern within sediment risk assessment. The present study investigated the bioavailability and bioaccumulation of heavy metals from spiked sediments in zebrafish (*Danio rerio*) embryos. In addition, effects on the transcript abundance of metallothioneins (MT1 and MT2) and the transcription factor MTF as molecular biomarkers of metal pollution were investigated. Furthermore, transcript abundances of the genes sod1, hsp70 and hsp90α1 as indicators of cellular stress as well as GST and CYP1A as markers of dioxin like compound exposure were examined.

Firstly, toxicity tests with heavy metal solutions and direct sediment contact tests on spiked sediments were carried out with *Danio rerio* embryos. For further experiments, zebrafish embryos were exposed to a natural and an artificial sediment, both spiked with cadmium, copper, nickel and zinc as single substances and as a mixture at concentrations between 150 and 3000 mg/kg. Following exposure, embryos were UV-acid-digested and metal concentrations were determined by means of ICP-MS. Changes in transcript abundance were investigated by Q-RT-PCR.

In the toxicity tests, the impact of heavy metals from sediments on zebrafish embryos, measured as LC_{50} values, was up to 10^2 times less compared to aqueous solutions. In the uptake-experiments, Danio rerio eggs accumulated heavy metals from spiked sediments in concentrations up to a few hundred times higher compared to the spiked concentrations. With a BAF of 275 ± 42, copper spiked to artificial sediment was accumulated at significantly greater rates than the other metals. The presence of a mixture of heavy metals reduced the uptake of individual metals. All heavy metals accumulated to a greater extent in embryos exposed to the artificial sediment compared to natural sediment exposure. This is likely due to greater contents of organic matter and bacterial activity which reduce bioavailability of metals spiked to the tested natural sediment. Conforming to this, artificial sediment spiked with zinc and the mixture of metals resulted in significantly greater transcript abundances of both MT1 and MT2 as well as hsp70 in zebrafish embryos compared to the control. MT1 showed up to 30 fold changes. Transcript abundance of hsp90α1 was significantly greater after exposure to the natural sediment spiked with cadmium and copper. Transcript abundances of other genes were not altered significantly.

WE 167

Toxicity of nickel in freshwater sediments: sediment spiking methodology, sensitivity of invertebrate taxa, and influence of sediment characteristics on bioavailability

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The toxicity of nickel (Ni) in European freshwater sediments is being evaluated under EU Existing Substances and REACH regulations. We conducted toxicity studies with Ni-spiked sediments to evaluate the variation in the sensitivity of invertebrate taxa to Ni toxicity and variation in Ni bioavailability among sediment types. The studies consisted of three tasks: (1) develop methods for preparing Ni-spiked sediments; (2) characterize toxicity of Ni-spiked sediments to 8 species of benthic invertebrates; and (3) evaluate variation in Ni bioavailability among 8 Ni-spiked sediments.

A three-stage spiking and equilibration process resulted in stable partitioning of Ni between sediment and pore water and avoided high Ni concentrations in overlying water during toxicity tests. Chronic toxicity values for sediment Ni (e.g., 20% effect concentrations or EC20s) varied widely among invertebrate taxa. The most sensitive taxa were two amphipods (*Hyalella* and *Gammarus*) and a mayfly (*Hexagenia*), with lowest EC20s ranging from 221 mg/kg (*Hyalella*) to 572 mg/kg (*Gammarus*). Nickel EC20s for sensitive taxa varied up to 8-fold among the sediments tested. These differences in bioavailability corresponded to Ni distribution coefficients (Kd=sediment Ni/pore-water Ni) and to concentrations of sediment constituents (acid-volatile sulfide or AVS, organic carbon, iron, and manganese). Normalization of Ni concentrations to AVS substantially reduced variation in EC20s among sediments.

These results will be used in an Integrated Sediment Effects Assessment for Ni (see related presentation by Vangheluwe et al.), which will estimate probable no-effect concentrations for a 'reasonable worst case scenario' (i.e., low Ni-binding sediment) and develop bioavailability models based on sediment characteristics.

WE 168

Acid-volatile sulfide and simultaneously extracted metals in sediments: results from an inter-laboratory comparison and recommendations for obtaining reproducible results

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Acid-volatile sulfide (AVS) can be an important factor in controlling the bioavailability of certain metals in sediments. However, there are concerns that measurements of AVS and the accompanying simultaneously extracted metals (SEM) are irreproducible among different laboratories. In support of a multi-component research program to determine nickel partitioning behavior and toxicity in sediments, we conducted an inter-laboratory comparison of acid-volatile sulfide and simultaneously extracted nickel (SEM_Ni) in spiked sediments. Five independent laboratories participated, but each was provided structured sample preparation and analytical guidelines to perform the measurements. The AVS and SEM_Ni measurements from this inter-laboratory study were in close agreement, indicating that reproducible results are obtainable among different laboratories. Results of the inter-laboratory comparison will be presented and the potential sources of variability will be discussed. In addition, quality control results that span nearly 20 years of SEM-AVS analyses conducted at the USGS Columbia Environmental Research Center will be presented. Finally, we offer specific recommendations for processing samples and for performing the SEM-AVS measurements.

WE 169

Assessment of bioaccumulation of platinum group metals in a river system in close proximity to mining activities in South Africa

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The emission of heavy and precious metals from mining, smelters and other industrial activities in the South African environment has been receiving considerable attention, due to the potential threat to human health and the environment. These metals have the potential to accumulate in sediment and biota, posing a serious threat to aquatic ecosystem health. It is further known that some of these elements are toxic to living organisms even at quite low concentrations, whereas others are biologically essential and natural constituents of the aquatic ecosystems and only become toxic at very high concentrations. Studies conducted on the toxicity of platinum group metals (PGMs), its environmental bioavailability and increased concentrations in biologically relevant media have indicated that environmental exposures to PGMs may indeed pose a health risk, especially at sublevel concentrations. The wide application and increasing use of palladium (Pd) in catalytic converters in motor vehicles and in some industrial processes, has seen increasing levels of Pd in the environment. Since Pd is toxic, the monitoring of this metal in surface waters, soil surfaces, plant and particular matter samples has been getting increasingly important. Research has further shown that aquatic macro-invertebrates form an integral part of the diet of freshwater fish and can be considered an important step in the aquatic food chain, playing an important role in the trophic transfer of pollutants, e.g. trace metals. The present study considered the bioaccumulation of trace metals such as cadmium (Cd), lead (Pb) and zinc (Zn), including precious metals such as platinum (Pt), palladium (Pd) and rhodium (Rh) in freshwater invertebrates. Preliminary results have shown that the metal concentrations were considerably higher in the sediment than in the water, whereas the benthic invertebrates showed differences in PGMs concentrations, taxon richness, number density and biodiversity for the different sampling sites evaluated in the Crocodile (West) Marico Water Management Area, North-West Province, South Africa.

WE 170

An integrated effects assessment of nickel in freshwater sediments

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A multi-component study was conducted to examine the effects of nickel bioavailability and toxicity of sediments in the laboratory and under field conditions. The goals of the study were to: 1) determine Predicted No Effects Concentrations (PNECs) for sediment-associated nickel (PNECsed) and 2) identify relationships between important sediment parameters and the toxicity of nickel to sediment-dwelling organisms. Using laboratory and field data from this testing initiative, an Integrated Sediment Effects Assessment was conducted to evaluate the use of laboratory generated toxicity thresholds (e.g., 5th Percentile Hazard Concentrations (HC5) and PNECs) in the regulatory context (e.g., REACH, Water Framework Directive) and to explore the possibility of incorporating sediment bioavailability models within the EU risk assessment framework. The Integrated Sediment Effects Assessment for nickel explored the following issues: 1) the derivation of a Reasonable Worst Case (RWC) HC5 sediment value of nickel for the freshwater environment; 2) the development of predictive models of bioavailability and toxicity of nickel in freshwater sediments; 3) the derivation of HC5 sediment values of nickel for the freshwater environment for different bioavailability scenarios; and, 4) the robustness of the HC5 estimate with regards to uncertainty in the sediment compartment. Laboratory toxicity data were obtained for 8 species of freshwater benthic invertebrates tested in 8 sediments with widely different Ni binding affinities. Field data were obtained for 5 of these sediments. Significant relationships between sediment parameters (namely AVS and related sediment phases such as iron and organic carbon) were observed with both laboratory and field endpoints. The relevance of this assessment to EU regulatory initiatives is discussed.

WE 171

Multi-generation exposure of the midge Chironomus riparius to three model toxicants

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In polluted ecosystems some species disappear, while others are able to maintain viable populations. Chironomids are particularly successful in persisting in extremely polluted environments, but the mechanism allowing them to survive in such hostile environments remains still unclear. The aim of the present study was therefore to investigate how *Chironomus riparius* copes with prolonged exposure to pollution, i.e. by phenotypic plasticity or genetic adaptation. To this purpose a multi-generation experiment was performed where *C. riparius* populations were exposed for nine consecutive generations to sublethal concentrations of three model toxicants, i.e. the essential metal copper, the non-essential metal cadmium and the organometal tributyltin. For each compound two exposure scenarios were designed, one where the concentration remained constant and one where after the 6th generation the exposure concentration was drastically increased for three more generations. Total emergence and delay in emergence were monitored during each generation for all populations. To assess if adaptation had taken place, 14-day toxicity tests were conducted with the corresponding toxicant after the 6th and 9th generation. We observed that all exposed populations were equally affected by the toxicants and that even when the exposure concentrations were increased after the 6th generation, the populations persisted. No signs of increased tolerance were found for any of the exposed populations in the 14-day toxicity tests. Therefore, we conclude that *C. riparius* has the ability to withstand strong selection pressure of different toxicants without immediate extinction or adaptation due its phenotypic plasticity.

WE 172

Can we expect genetic adaptation to metals at conventionally derived HC5 values?

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Under sufficiently strong selective pressures natural populations can genetically adapt and evolve an enhanced stress tolerance. The evolution of tolerance to chemical stress may have important implications for current practices in ecological risk assessment considering, for instance, possible costs of tolerance or co-tolerance with respect to other stressors. We reviewed the relevant literature and constructed a database on genetic adaptation to Cd, Zn, Cu and Pb to invertebrates. Here, we will evaluate at which concentrations genetic adaptation to Cd has been detected in freshwater invertebrates. Studies on (i) genetic adaptation in natural populations in the field and (ii) selection experiments under laboratory circumstances were considered. To distinguish between acclimation and genetic adaptation, only studies in which exposures to Cd were performed with F1 populations, i.e. progeny from from parental populations which had been reared in clean conditions, were considered. Surprisingly, only few such data were available for Cd. Concentrations at which genetic adaptation was reported, were normalized to a water hardness of 50 mg-L^{-1} and evaluated against conventionally derived effect values typically used in risk assessments, such as the HC5. For this study, a median HC5 value of 0.43 µg-L^{-1} was calculated, using quality 1 and 2 NOEC values reported in the European Union final risk assessment report on Cd and CdO. Genetic adaptation in field populations of the dipterian *Chironomus riparius* was reported in a concentration range of 6.69 µg-L^{-1} to 27.08 µg-L^{-1}, well above the HC5 value. Adaptation in evolution experiments under laboratory circumstances was detected for the dipterians *Chironomus plumosus* and *Culicoides furens* at 100 and 350 µg-L^{-1}, respectively. Different strains of the gastropod *Biomphalaria glabrata* evolved an increased Cd tolerance in the concentration range of 2.58 µg-L^{-1} to 10.33 µg-L^{-1}. Finally, genetic adaptation in *Daphnia magna* populations was observed in the range of 6.68 µg-L^{-1} to 24.6 µg-L^{-1}. In addition, a potential for genetic adaptation was predicted at 1.40 µg-L^{-1} in 6 out of 11 natural populations of *D. magna*.
It is concluded that, until now, genetic adaptation has only been detected or predicted at concentrations at least 3 times higher than the derived HC5 value.

WE 173

Sensitivity to cadmium of Carcinus maenas populations from two NW Portuguese estuaries with different levels of contamination

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Aquatic invertebrate populations from chronically contaminated sites may adapt to pollutant-induced toxicity. Carcinus maenas is an ecologically important invertebrate of European estuarine and coastal systems, and a common inhabitant of the estuaries of rivers Lima and Minho (NW Portugal). The Lima estuary has a history of chemical inputs from untreated effluents of urban and industrial origin, including a paper mill, a harbour, and a shipyard, and is contaminated by metals including Cd, among other substances. The Minho estuary is low contaminated and has been used as a reference estuary in previous ecotoxicological studies. The sensitivity to Cd of these two crab populations was investigated by performing laboratory bioassays based on biomarkers involved in key physiological functions. Crabs were captured in the studied estuaries and transported to the laboratory where they underwent a 7-day acclimation period [salinity 15 ppm, temperature 16ºC, photoperiod 14:10 (L:D)]. Crabs from both populations were then individually exposed for seven days to Cd concentrations ranging from 1.31 to 2000µg/L. At the end of the assays, biomarkers linked to moulting [epidermis and hepatopancreas chitobiase activity (NAGase)], neuromuscular function [muscle cholinesterase activity (ChE)], energy metabolism [muscle lactate dehydrogenase (LDH), and NADP+-dependent isocitrate dehydrogenase (IDH) activities], oxidative stress [hepatopancreas activities of glutathione S-transferases (GST), glutathione peroxidase (GPx) and glutathione reductase (GR), and the levels of total glutathione (TG)], and oxidative damage [lipid peroxidation (LPO), levels in the hepatopancreas] were determined. Altered biomarker responses were found following exposure to Cd, especially in crabs from the reference estuary. In particular, epidermal and hepatopancreas NAGase activity was significantly inhibited in exposed crabs from the Minho estuary, compared to controls, but not in those from Lima. The results suggest that crabs from Minho estuary are more vulnerable to Cd-induced moulting impairments than those from the Lima estuary. Moreover, the crab population from the Lima estuary appears to be less sensitive to Cd than the reference population, probably due to an adaptation induced by chronic exposure to metal contamination.

WE 174

Multi-generational exposure of Folsomia candida to Cadmium: effects on survival, reproduction and growth

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Environmental contamination can be of short duration but can also be historical or due to long term scenarios. Therefore, there has been growing concern about multigenerational exposure effects on organisms but, few studies are currently available on multi-generation experiments with soil invertebrates. In the present study, the effects of cadmium multigenerational exposure were studied in Folsomia candida. Cadmium was selected due to being a common contaminant worldwide due to human industrial activities. Organisms were exposed consecutively to two concentrations of Cd (EC50 and lower) spiked LUFA 2.2 soil, along ten generations so far. The

Collembolan test ISO guideline with adaptations was followed. Survival, reproduction and size were assessed. Results showed an increase in the number of juveniles and adults along generations, as well as size, but no major change in the EC50. At F6 generation the reproduction EC50 increased from 60mg Cd2+/kg to 82mg Cd2+/kg for the organisms consecutively exposed to 60mg Cd2+/Kg. The expression level of the metallothionein codifying gene is being analyzed using quantitative real time PCR to investigate possible explanations for the increase in organisms' performance.

WE 175

Mercury, SETAC, and Global Change: Implications for the 2013 International Mercury Treatise

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Mercury deposition and contamination is widespread and well-documented and continues to be a public-health issue of concern for certain sectors of the global human population. In early 2013 the United Nations Environment Programme's (UNEP) internationally binding treaty on the control of mercury will be signed. Documentation of the pervasiveness of this contaminant is a first step toward understanding the potential environmental health and ecological implications of mercury pollution and will be critical to the success of the UNEP program. Identifying broad scale distribution patterns of mercury bioaccumulation can convey to regulators that certain ecosystems may be degraded and require development of policies and regulations that may reduce mercury emissions, and ultimately, improve air and water quality. A more synthesized, holistic, perspective on the mechanisms related to aquatic and terrestrial biogeochemistry linkages of fate, transport, and bioavailability of mercury in aquatic ecosystems will result from long term, multi-ecosystem monitoring programs coupled with process-oriented research questions. Here we identify UNEP partnership goals, key topics, and highlight recent and relevant scientific and mercury policy advances in the field. Additionally, we outline the necessary steps for advancing and developing SETAC's contributions to the partnership and discuss methods for identifying clear and measureable objectives.

WE 176

Temporal increase in organic mercury in an endangered pelagic seabird assessed via century-old museum specimens

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Methylmercury cycling in the Pacific Ocean has garnered significant attention in recent years, especially with regard to rising mercury emissions from Asia. Uncertainty exists over the extent to which mercury in biota may have resulted from increases in anthropogenic emissions over time. To address this, we assessed historical and recent mercury exposure in an endangered seabird, the Black-footed Albatross (Phoebastria nigripes), using feather samples from museum specimens spanning the past 130 years. We additionally analyzed stable isotopes of nitrogen (δ15N) and carbon (δ13C) to control for confounding factors such as temporal change in trophic structure or diet. A significantly higher proportion of post-1940 samples contained above deleterious threshold levels (~40,000 ng/g) of methylmercury (the bioaccumulated form of mercury) relative to pre-1940 samples, and mean concentrations were significantly higher in post-1990 than in pre-1990 samples. We also found higher levels of (presumably curator-mediated) inorganic mercury in older specimens of albatross as well as two non-pelagic species lacking historical sources of natural exposure, which suggests that future studies on bioaccumulation should employ methylmercury rather than total mercury analyses on all but recently collected museum specimens. Although complementary stable isotope data suggested no historic change in albatross trophic level, there was a significant change in δ13C signature over time. After controlling for confounding factors, time showed a significantly positive association with methylmercury exposure. Changes in methylmercury levels were consistent with historic global and recent regional increases in anthropogenic mercury production, and mercury toxicity may undermine current and future reproductive efforts in the species.

WE 177

Heavy metal concentrations in internal tissues of striped dolphin (Stenella coeruleoalba) stranded on the coast of Murcia Region (SW Mediterranean)

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Many studies have been carried out concerning the monitoring of contaminants in cetaceans as an indicator of the pollution degree in marine environments. It is known heavy metals appear highly concentrated in top predators of the trophic networks such as seal, cetacean, tuna, swordfish and shark. In fact, cetaceans present several physiological particularities that cause the accumulation of these pollutants. They have great blubber that covers the whole body and effectively stored lipophilic compounds. In addition, it is necessary to add that these animals have a very low capacity to degrade some pollutants due to the lack of sebaceous and sweat glands, through which toxins are released, or gills to carry out an active exchange between blood and water. This work presents the results of a research about the levels of mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) found in the liver, kidney and brain tissues of eleven striped dolphins (Stenella coeruleoalba) stranded on the coast of Murcia Region in 2009. In all tissues, mean concentration of mercury was highest followed by cadmium, lead and arsenic. The major accumulator tissue was the kidney (69.79 ± 34.96 µg g-1 dw (dry weight) Hg; 15.53 ± 7.81 µg g-1 dw Cd; 0.21 ± 0 µg g-1 dw Pb; 3.30 ± 2.65 µg g-1 dw As), except for mercury which reaches the greatest median levels in liver (720.3 ± 573.25 µg g-1 dw Hg; 3.76 ± 2.72 µg g-1 dw Cd; 0.18 ± 0.076 µg g-1 dw Pb; 3.11 ± 1.82 µg g-1 dw As). Moreover, there was a positive correlation between metals in liver, although only was significant in mercury and cadmium concentration (Rho=0.609, p=0.047), suggesting a similar metabolism pathway. On the other hand, a significant positive relationship was found between body length and mercury levels in liver and brain samples (Rho=0.691, p=0.019; Rho=0.857, p=0.014; respectively) and between body length and cadmium in liver (Rho=0.645, p=0.032). Our metal concentrations were similar than those described in tissues of striped dolphins from others Mediterranean areas and higher than those found in remote areas. However, Hg liver levels are considered in the limit of tolerance for mammalian hepatic tissues and Cd and Pb tissue levels are not associated with toxic effects. Acknowledgements: To Fishery Service, Wildlife Recovery Center and Agricultural and Animal Health Laboratory, Department of Agriculture and Water of the Autonomous Community of Murcia Region. Thanks to MICIIN for funding (CGL2008-4318/BOS).

WE 178

Fish otoliths as potential chronological biomarkers of environmental aquatic contamination by heavy metals

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The European eel, Anguilla anguilla, as a facultative catadromic species, presents a complex life cycle. This species habitat goes from salt to freshwater grounds, including also estuarine brackish areas. It usually crosses several habitats, potentially affected by anthropogenic activities. Human activities, like industry, harbour activity or mineral extraction, or nature itself, through particular geological formations, can contaminate aquatic systems with heavy metals. Additionally, the available studies about the potential use of the chronological properties of otoliths as indicator of aquatic contamination by metals are scarce. In this study we used 180 juveniles (yellow eels) of the European eel (A. anguilla) to test the uptake of three important metals (zinc, cadmium and copper) through the water into the otoliths. Simultaneously the otolith growth during the chronic exposure (28 days) was estimated using a fluorescent otolith dye (tetracycline), important to choose the electron beam diameter (ICPMS-LA) used for otolith metal concentrations assessment. Although our results suggest that the trace metal concentration in otoliths appear to increase from the lower to higher tested concentrations on water, the obtained differences are not statistically significant. Furthermore there were no significant relationship between the otolith elemental and water concentrations for all the experiments. Our data indicates that water trace metal concentration has no significant effect on incorporation of Cu, Cd and Zn in otoliths of A. Anguilla. However these preliminary results should be interpreted with caution, since the obtained data were highly variable, and probably much of this variation is related to poor analytical precision at such low concentrations as consequence of a small ablation spot size.

WE 179

Ameliorative effect of Moringa oleifera, activated charcoal and charcoal on lead toxicity in wistar rats

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Introduction

Lead poisoning is one of the largest environmental problems. Conventional treatment of lead poisoning has been based on chelating agents which are relatively expensive for poor communities. In this present study, we evaluated the efficacy of Moringa oleifera aqueous leaf extract, activated charcoal and charcoal in treating lead induced toxicity in wister rats.

Materials and Methods

The rats were divided into 5 groups of 10 rats. An oral daily dosage of 1000 mg/kg body weight of lead acetate was administered to rats in 4 groups for 7 days. The positive control group received distilled water. After 7 days of lead acetate administration, 10 rats were sacrificed from a lead acetate group and control group. From day 8 to day 21, an oral daily dosage of 1000 mg/ kg body weight of M. oleifera, activated charcoal and charcoal were administered separately to 3 of the lead acetate treated groups while one of the groups was allowed untreated. On day 21, all the remaining rats were sacrificed. During and after the administration period, rats were examined for clinical signs, body weight changes, serum biochemistry, haematological parameters and histopathological lesions.

Results and Discussion

Lead acetate significantly decreases body weight, aspartate aminotransferase, alanine aminotransferase, and red blood cells count. Gamma glutamyl aminotransferase, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were increased. Histopathology examination revealed damages in the liver, brain, muscle and kidneys. Activated charcoal and M. oleifera showed ameliorative effects in the haematology, serum biochemistry and histopathological analysis.

Conclusion

Our results suggest that M. oleifera aqueous leaf extract and activated charcoal can treat lead induced toxicities.

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ET15 - New developments in aquatic macrophyte testing, higher tier risk assessment and ecotoxicology

WE 182

Myriophyllum spicatum toxicity test: design and first results of an interlaboratory ring test using a sediment-free test system

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Dicotyledonous macrophytes are not yet part of the initial risk assessment of plant protection products in the aquatic environment although they are an important part of the ecosystem. In this context alternative test methods with Myriophyllum sp. are under development.

The Ecotoxicological Laboratory of the German Federal Environment Agency has established a standardised sediment-free test system with the dicotyledonous water milfoil Myriophyllum spicatum using Andrew's medium with 3% sucrose following the ASTM Designation E 1913-04. A sediment-free single-phase system was chosen to get toxic threshold and effect concentrations being independent of the water-sediment-distribution of the test item. The only-water exposure of the testplants reduces time and efforts for analytics and facilitates modelling and interpretation of the results. Also, a direct comparison to results obtained with the monocotyledonous duckweed, Lemna spec. (OECD Guideline 221), is possible.

Twelve laboratories participated in an interlaboratory ring test organized by the German Federal Environment Agency from October 2010 to April 2011. The ring test aimed at 1) investigating

the practicability and reproducibility of the sediment-free test system, 2) identifying the most appropriate endpoints reflecting different modes of action of the test items, and 3) optimising and standardising the test method in order to obtain a sound test system for dicotyledonous macrophytes.
In so doing, three test items were investigated representing different modes of action: 3,5-dichlorophenol (pesticide with narcotic action, reference substance for OECD 201 and OECD 221), 2,4-dichlorophenoxyacetic acid (auxine herbicide, growth inhibitor) and isoproturon (photosynthesis inhibitor). Manifold response variables were measured such as fresh weight, dry weight, shoot length, number of whorls, number and length of lateral branches, number and length of roots. Test duration was 14 days, the plants were grown under sterile conditions, culture medium was replaced after 7 days. The temperature was adjusted to 23 + 2 °C and light intensity to 100-150 µE m-2s-1 under a light-dark cycle of 16:8 h.
To obtain statistically sound results with acceptable statistical power the test design prescribed 10 control replicates and eight treatments with five replicates, each. NOECs and EC50 values including 95% confidence limits were calculated.
First results of the interlaboratory ring test will be presented.

WE 183

Measuring shoot length of submerged aquatic plants using graph analysis

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In aquatic risk assessment procedures for herbicides within the context of EU Regulation 1109/2009/EC, tests with rooted submerged macrophytes may be required. The ecotoxicological impact of herbicides in experimental studies can be observed by measuring concentration-response relationships for the endpoint total shoot length of submerged aquatic plants, such as Myriophyllum spicatum L. and Elodea canadensis , as recommended by the HARAP workshop. Using different spieces, concentrations of the target compound and replicates ,in experimental studies, gives a lot of samples. Manual measurement of the shoot lengths takes a lot of time. The traceability of the measurements is not possible because after measurements the samples will be destroyed by analyzing the dry matter content. An ImageJ application has been developed to measure the individual shoot length of the plants taken by a digital picture. After thresholding the picture, the binary image is transferred to a skeleton and the skeleton to a weighted graph using the ImageJ plug-in Analyze-Skeleton. The nodes in the graph are the junction pixels and the edges are the slab pixels. The weight of the edges corresponds with their length. In computer science, the FloydWarshall algorithm is a graph analysis algorithm for finding shortest paths in a weighted graph. A single execution of the algorithm will find the lengths (summed weights) of the shortest paths between all pairs of nodes. The longest length can be considered as a good estimation of the total length of the plant. The warshall algorithm was implemented in the Analyze-Skeleton plug-in and used to measure the length of a large number of plants of both specimens. The lengths measured this way were compared with manual measurements. The R2 was 0.94 for E. canadensis and 0.91 for M. spicatum. From those high R2 values we can conclude that the algorithm works very well. The proposed procedure is a cost-effective approach to study concentration-response relationships of herbicides (and other relevant pesticides) on shoot length growth of submerged rooted macrophytes, as required in the test procedures underlying EU Regulation 1109/2009/EC.

WE 184

Ecotoxicity of herbicide Glyphosate used in tolerant transgenic soybean cultivation in Pampasic Region (Argentina) to aquatic macrophyte and microalgae

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Increasing use of Glyphosate in Argentina is directly related to the increase of areas cultivated with a Glyphosate tolerant transgenic variety of soybean. That has raised concern about the effects of this herbicide in the local aquatic ecosystems, as is the most used herbicide. Beside the amount of herbicide used, in most cases aerial applications is preferred due to the vast areas to be treated. In these cases the herbicide is spread directly over de aquatic systems witch are inside or near the cultivation areas. The recovery and detection of early effects from the action of this pesticide is fundamental especially on primary producers, due to be an essential trophic level of any ecosystem, because it provides the basic energy for food webs in aquatic systems. The aquatic macrophytes Lemna minor (floating) and Vallisneria spiralis (rooted) as well as four species of green microalgae were used in this assessment. Different endpoints were use (biomass, growth, physiological and anatomic parameters, oxidative stress and lipoperoxidation biomarkers). Gliphosate produced hormesis in Lemna minor and Vallisneria spiralis within its Expected Environmental Concentration (EEC) of 1,7 and 2,88 mg Gly/L. The leaf area of the fronds of Lemna minor was modified as a response to a transfer of resources against the stress caused by the herbicide, also the colony architecture was modified as the stipe was observed more often and permanent. Glyphosate increased and altered de activity of oxidative stress enzyme and lipoperoxidation of both Lemna minor and Vallisneria spiralis in the range of its EEC. A stimulation of photosynthetic rate of green algae species was observed at EEC, resulting changes that would imply effects at algal community level regarding competitiveness. The differences between macrophyte species in aquatic systems on the stimulation of growth produced by the action of low concentrations of herbicide, would alter the ability of them towards competitions mechanisms, being ones more privileged than others. This fact over time could cause a change in species composition of plant communities. The action on reproduction of the different primary producers would also be a factor to consider regarding this competition. The morphological and enzymatic biomarkers related to oxidative stress could be used as indicators of incipient changes in aquatic primary producers provoked by this herbicide widely used in our country.

WE 185

'Comet' Assay in seeds of the emergent aquatic plant Bidens laevis L. exposed to the insecticide endosulfan

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The increasing release of genotoxic chemicals into the environment is negatively affecting the ecosystems and the health of diverse organisms. Plant bioassays can be an integral part of test batteries used for the detection of genotoxic-carcinogenic environmental contamination of the aquatic environment. In fact, highly sensitive, time- and cost-effective plant models have been developed for the detection of toxic compounds arising from the use of pesticides. Plant response to mutagenic treatments can be considered on different levels of organization: the DNA molecule, the chromosome, and the genome up to the whole organism. In previous studies, we identified the emergent aquatic macrophyte Bidens laevis as a sensitive species able to show increased frequencies of chromosomal aberrations under exposure to endosulfan. However, for detecting genotoxicity at the molecular level, the "comet"assay is widely used. The goals of this work were, in seeds of Bidens laevis: 1) to find a suitable concentration of methyl methanesulfonate (MMS) to be used as positive control for the "comet"assay and 2) to study the potential clastogenic effect of endosulfan. A sample of seeds were exposed to 0; 10; 20; 100; 500 and 1000 mg/L MMS during 48 h (n=8) whereas another sample was exposed to 0; 0,02; 0,5; 5; 10; 50 and 100 ug/L endosulfan (n=10) at room temperature in the darkness; using a negative control and 20 mg/L MMS as positive control. The alkaline technique of the plant "comet"assay was used. At 10 and 20 mg/L MMS, normal germinative power was obtained while higher concentrations caused inhibition of germination and necrosis of the seed. Notwithstanding, a significant increase of the damage index (DI) with respect to the negative control was found at 20 mg/L MMS ($p<0.05$). Radicles from seeds exposed to endosulfan did not differ in their DI from the negative control at any concentration ($p>0.05$), indicating that this insecticide is aneugenic instead of clastogenic in B. laevis as we have previously demonstrated working with roots.

WE 186

Are additional macrophyte tests necessary for a safe risk assessment of auxins and fatty acid synthesis inhibitors?

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In the current risk assessment the toxicity of herbicides on aquatic macrophytes is based on laboratory tests with the monocot *Lemna* (duckweed species). However, this floating macrophyte is not sensitive to auxins and fatty acid synthesis inhibitors. For that reason, an additional test with the submerged dicot *Myriophyllum* sp. has been proposed, which is known to be sensitive to auxins (Maltby et al. 2010). Furthermore and in contrast to *Myriophyllum* and *Lemna*, grasses like the emerse swamp species *Glyceria maxima* seem to respond negatively to fatty acid synthesis inhibitors.
Microcosm experiments were conducted to further elucidate these differences in sensitivity of the three macrophyte species. 18 microcosms were filled with sand and water, and established with *Landoltia* sp. (duckweed species), *G. maxima* and *M. spicatum*. Three microcosms served as controls. Five microcosms were dosed with five different concentrations of the herbicides fluroxypyr (auxin), clodinafop-propargyl (fatty acid synthesis inhibitor) and isoproturon (photosynthesis inhibitor), respectively. The experiment lasted 10 weeks from the middle of May to July. Besides endpoints such as fresh weight, shoot length, and number of leaves, pictures were taken to document and rate the growth deformities of *M. spicatum*.
It was confirmed, that there are huge differences in the sensitivity of the tested species towards the herbicides with different modes of actions. As expected, *M. spicatum* was most sensitive to fluroxypyr with fresh weight of roots (FWR) being the most sensitive endpoint. *Glyceria maxima* was strongly inhibited by clodinafop-propargyl (EC_{50} total length of leaves, 3 weeks: 48,73 $\mu g*L^{-1}$, 95% CI 6,00$\mu g*L^{-1}$ to 395,37 $\mu g*L^{-1}$), while no negative effects were measured for *M. spicatum* and *Landoltia* sp.. Regarding isoproturon, the EC50 of *M. spicatum* after 8 weeks for FWR was 34, 96 $\mu g*L^{-1}$ (95% CI 15,14 $\mu g*L^{-1}$ to 80,72 $\mu g*L^{-1}$), which is similar to the EC_{50} as reported in the literature for duckweed. However, dry weight of *Landoltia* sp. showed no dose-response relationship, but more sensitive endpoints have yet to be analyzed. Overall, the results support the suggestion of Maltby et al. (2010) to introduce additional macrophyte tests.

ET17 - Tropical ecotoxicology

WE 189

Benthic macroinvertebrate community responses to metal leaching and ultraviolet radiation in high altitude Andean streams

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High altitude Andean streams drain metal-rich bedrock and are subjected to particularly high ultraviolet-B (UV-B) radiation. The combination of these factors may create highly selective conditions that challenge the survival and persistence of biota. The aim of the present study was therefore to evaluate the effect of environmental drivers on benthic macroinvertebrate community composition in Andean streams in Peru. At the high altitude sites (4000 meters above sea level, m a.s.l.), UV-B radiation was more than two-fold higher than at lower sites (3000 m a.s.l.). At leaching sites metal concentrations in the water were up to 590 fold higher than at reference sites, with Cumulative Criterion Units predicting altered community composition. Principal Component Analysis of physical and chemical variables showed that the reference sites were influenced mostly by UV-B radiation and the polluted sites by metals. Canonical Correspondence Analysis indicated a strong influence of UV-B in structuring communities at reference sites, and of metals at polluted sites. Under the most extreme metal and UV-B conditions only mites and chironomids persisted, while under less extreme conditions a replacement of sensitive taxa by tolerant ones sustained a high faunal diversity. It is concluded that igneous rock leaching high up in the Andes together with the high UV-B radiation regime modulate benthic macroinvertebrate community composition.

WE 190

Age and toxicant exposure modify the energy budget in Daphnia schodleri (Anomopoda: Daphniidae)

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Daphnia schodleri is a cladoceran which belongs to D. pulicaria complex and its wide distribution includes Mexico. This is an important organism because its ecology and possible use as experimental model due to its relatively large size. Recent studies have been focused on searching and linking biomarkers, such as enzymatic pathways, with high ecological relevance organization levels. Nevertheless, some of them do not offer a general overview of toxic effects, as it has been referred to the organisms´ energy content, which could be modified during toxicants exposure

and promote changes in their fitness and decreasing its reproductive performance, besides energy reserves could be consumed in order to deal with the energetic requirements for detoxification. With the aim of assess how age specific requirements are modified by a toxicant, seven age groups (0, 3, 5, 7, 14, 21 and 28 d) were exposed during 24 h to sublethal concentrations of hexavalent chromium in two experimental designs: a) 6.4 and 32 µg L^{-1}; and b) two fractions (1/5 and 1/25) of previously determined LC_{50} for every age group. After exposure, three energy reserve macromolecules were quantified (proteins, carbohydrates and lipids), and obtained results transformed into energetic equivalents using their respective energy of combustion (mcal daphnid-1). Results in the first experimental design showed that organisms younger than 7 days consumed higher amounts of their energy budget than older. In the second design, several changes were recorded in all age groups, with higher differences in organisms at both extremes of their life cycle. Although Cr (VI) concentrations for 7-14 day old daphnids were higher than for the rest, their caloric content did not decrease at the same level than other ages. Whereas enhanced tolerance to chromium was observed in the middle age organisms, according to their higher LC_{50} values, major energetic requirements were found in organisms at both sides of this cladoceran life cycle, which mean higher susceptibility to toxicant exposure. If stress conditions persist, their normal growth rate and reproductive behavior could be modified. Thereafter, their population structure in contaminated environments may be altered.

WE 191

Anthropogenic organic contaminants in water, sediments and benthic organisms in the mangrove-fringed Segara Anakan Lagoon, Java, Indonesia

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Segara Anakan in Central Java, Indonesia, is a mangrove-fringed coastal lagoon with a high diversity of macrobenthic invertebrates. The ecosystem provides vital services to the local population whilst simultaneously it is increasingly affected by anthropogenic activities. Organic pollution of the lagoon is expected to originate from municipal sewage, shipping traffic and from Indonesia's largest oil refinery, releasing effluents into the lagoon.
In order to characterize chemical contamination, water, sediments, mangrove invertebrates and fish were sampled at 5 stations of varying distance to the oil refinery, including peanut worms (Phascolosoma arcuatum), clams (Polymesoda erosa), oysters (Crassostrea sp.), snails (Telescopium telescopium), crabs (Neoepisesarma versicolor) and mullets (Mugil cephalus). Non-target GC/MS screening analysis was performed on concentrated extracts of all samples.
Most of the 51 identified contaminants in both abiotic and biotic samples belonged to the class of polycyclic aromatic hydrocarbons (PAHs). The characteristics of the PAH assemblages pointed to emissions from the oil refinery or oil spills as the main source of contamination. PAHs mainly consisted of alkylated PAHs which are more abundant in crude oil and more persistent in the environment than the better-known parent PAHs. Highest total PAH concentrations were 42 µg/l in water and 122 mg/kg dry weight in sediment. Apart from petrochemicals, other contaminants e.g. from municipal sewage or agriculture had a patchy distribution and were detected in only a few samples, which presumably is caused by the lagoon's hydrodynamics or by low input levels. Organic contaminants were detected in all tissue samples. Different benthic species from the same stations stored different numbers of contaminants depending on their microhabitat and their feeding mode. The number of contaminants in T. telescopium decreased with increasing distance to the oil refinery suggesting that it is a potential sentinel species for sediment pollution. This study clearly showed high contaminant concentrations in the vicinity of the oil refinery and presence of contaminants in all investigated aquatic organisms. The health risk for benthic organisms, fish and humans remains to be determined. In particular, environmental fate and toxicity of alkylated PAHs which did not receive much attention in the past require further investigation.

WE 192

The use of mysids and copepods bioassays to assess the environmental status of a tropical estuary

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The dynamics of contaminants in sediments is a complex and poor explored phenomenon, in which the availability to benthic communities is quite difficult to estimate. In this context, toxicity bioassays have been applied worldwide to contribute to the assessment and monitoring of sediment quality, once they provide information on how the living systems respond to the influence of contaminants. In this view, the purpose of this work was to assess the toxicity of sediment collected at Ceará River at northeast of Brazil, using bioassays with the mysid *Mysisdopsis juniae* and the copepod *Nitokra* sp. Two campaigns were conducted (November/10; February/11), and sediments were collected from the exposed estuarine banks, during low tides at three sampling stations in the Ceará River. Mysid tests were carried out in polypropylene vessels (4 replicates/sample), with approximately 200 ml of sediment and 700 ml of filtered seawater 24 hours prior to adding the mysids (n= 5 juveniles ≤ 24h old). The chambers were kept under gentle aeration during ten days, and then the surviving mysids had the length and dry weight measured. Copepod toxicity tests were performed in chambers (4 replicates/sample) prepared 24 hours before exposure of ovigerous females (n=10) with nearly 2 g of homogenized sediment and 5 ml of seawater. The containers were kept without aeration for 10 days. After exposure, a solution of formalin 10 % and the vital dye Rose bengal were added in order to preserve and identify the animals, that were classified in adults and young individuals (copepodits and nauplii), and counted to evaluate the reproduction. It is important emphasize that there are a few sediment bioassays standardized with tropical species and this study will contribute to improve biomonitoring programs in tropical coastal areas.

WE 193

Biomarkers responses in Crassostrea rhizophorae (Bivalvia: Ostreidae) in a tropical estuary collected in the dry and rainy seasons

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Estuaries are very important ecosystems, which present high biological productivity and economical value, associated to intensive human activities that can have a negative impact on the environmental health. In this view the aim of this study was to evaluate the responses of biomarkers in *Crassostrea rhizophorae* collected at Ceará River at northeast of Brazil, during the dry and rainy seasons. The Ceará River Basin is one of the three major water sources of the Metropolitan Region of Fortaleza, Ceará State. As an urban river, it receives domestic and industrial effluents, but the impacts of such discharges were not properly investigated. Oysters were collected at three sites along the estuary in November/10 and February/11, dry and rainy seasons, respectively. Tissues were dissected and frozen until analyzes of acetylcholinesterase, carboxylesterase, glutathione-S-transferase, catalase, lipid peroxidation, energy reserves and condition index. Tropical areas are stressed by constant range of high temperatures throughout all year even during the rainy season. Some studies have shown that this period contribute to increase the toxicity in waterbodies by the leaching of superficial residues, along with domestic and industrial sewages that are drained to the estuary, carrying contaminants and a great organic load.

WE 194

Genotoxic effects of the herbicide Roundup Transorb® and its active ingredient glyphosate on a Neotropical fish species

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In Brazil, glyphosate-based herbicides are the most often utilized and high concentrations of glyphosate have already been detected in water near to intense cultivation areas in southern Brazil. Despite its extensive use, little is known about the genotoxic effects of this herbicide to Neotropical fish species. Roundup Transorb® (RT), an example of an herbicide that uses glyphosate as the active principle, is classified as harmful to the environment and the main differential of this product is its rapid translocation allowed by the surfactant used in this formulation. In this work, genotoxic effects of glyphosate and RT in erythrocytes of *Prochilodus lineatus* were evaluated in vivo tests (6, 24 and 96 h) using the Comet Assay. Juvenile fish were exposed, for 6, 24 and 96 h, to 1 mg.L^{-1} and 5 mg.L^{-1} of glyphosate or RT, or only to clean water (negative control or NC). Fish of the positive control group (PC) were injected with cyclophosphamide (40mg.kg^{-1}). After each experimental period blood cells solution were resuspended in agarose (low melting point), placed on slides, submersed on a lyse solution, subjected to electrophoresis, neutralized, fixed in ethanol, stained with GelRed and analyzed under a fluorescence microscope. DNA damage was visually scored by the length of DNA migration in four classes (0: undamaged; 1: minimum damage; 2: medium damage; 3: maximum damage) and each comet assigned a value of 0 to 3 and the total score varied from 0 to 300. The results showed that the score of DNA damage (mean ± SE, n = 6) in the erythrocytes of fish exposed to glyphosate was significantly higher than CN after 6 h (NC: 18.8 ± 4.8, 1 mg.L^{-1}: 33.8 ± 6.6 and 5 mg.L^{-1}: 43 ± 2.6; PC: 74.6 ± 24.0) and 96 h (NC: 35 ± 2.0, 1 mg.L^{-1}: 60.8 ± 7.4 and 5 mg.L^{-1}: 65.1 ± 6.0, PC: 79.5 ± 2.1). After 24 h exposure to glyphosate significant difference were not found (NC: 20 ± 4, 1 mg.L^{-1}: 33 ± 7 and 5 mg.L^{-1}: 27 ± 6; PC: 88.5 ± 1.8). Moreover, the scores of DNA damage found after RT exposure were higher than the NC after 24 h (NC: 26.6 ± 2.0, 1 mg.L^{-1}: 59.8 ± 7.4 and 5 mg.L^{-1}: 94.1 ± 4.1, PC: 88.5 ± 1.8) and 96 h (NC: 33.6 ± 2.0, 1 mg.L^{-1}: 52.1 ± 3.3 and 5 mg.L^{-1}: 69.1 ± 7.0; PC: 79.5 ± 2.1). These results show that both glyphosate and the formulate herbicide RT can produce DNA damage on red blood cells of P. lineatus, contradicting the idea that the active principle is less genotoxic than the formulate product.

WE 195

Biochemical and genotoxic effects of natural blooms of cyanobacteria and Microcystis aeruginosa, containing microcystin, on the freshwater fish Prochilodus lineatus

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Microcystin is a toxic substance released by cyanobacteria, among which stands out *Microcystis aeruginosa* (MA). In the natural environment, blooms of cyanobacteria (FC) where MA is present alone will rarely happen. Normally, we can find in cyanobacteria blooms different cyanotoxins and substances that together to microcystins may increase or decrease the effects of this toxin on the aquatic organisms. In this study we evaluate whether microcystin-LR (MC-LR), present in lyophilized FC and MA, is able interfere on detoxification enzymes (glutathione S-transferase-GST) and on antioxidant defenses (superoxide dismutase-SOD, catalase-CAT, glutathione peroxidase-GPx and reduced glutathione, GSH) and cause DNA damage. Juveniles of *Prochilodus lineatus* were exposed to solutions containing 1 mg.L^{-1} of lyophilized FC or MA or only water (control) for 24 and 96 h. After exposure, aliquots of water were collected for quantification of MC-LR and the fish were anesthetized for blood sampling, and then killed to remove the gills and liver. Biochemical analyses were run in gills and liver samples and the comet assay (CA) was performed using red blood cells. The analysis of water containing FC and MA showed 0.9 µg.L-1 MC-LR. When compared to controls, fish exposed to FC for 24 h showed a significant increase on liver SOD, and after MA exposure there was an increase on SOD, CAT and GPx in the gills and GST, CAT and GSH in the liver. After 96 h exposure, the exposure to FC lead to an activation of SOD and GPx in gills and GSH in the liver, while after MA exposure, there was an inhibition of GSH in gills and CAT in liver. DNA damage was detected only after 96 h exposure to MA. In summary, these results show that after 96 h exposure to MA there was inhibition of antioxidant defenses leading to oxidative stress and DNA damage of erythrocytes. Thus, it appears that exposure to lyophilized MA was more toxic to the P. lineatus than the exposure to lyophilized FC, although the two materials have the same concentration of MC-LR. This variation is a sign that, in addition to MC-LR, there are other cyanotoxins and/or other substances in lyophilized FC that inhibited the effect of MC-LR on the fish species studied in this work.

HM03 - Integrated long term monitoring as a tool for the global assessment of POPs

WE 198

Determination and risk of POPs in forest soils of Taurus Mountains

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Persistent organic pollutants (POPs) are global concern due to their ubiquitous presence and toxicity. These substances can be transported long-range and have been dispersed world-wide. The United Nations Environmental Programme(UNEP) initiated work on the Stockholm Convention on POP's with the intension of reducing and ultimately eliminating these pollutants. Forest and their soils are important sink for POP's in the environment. For example , the study area , Taurus mountains in Turkey, are geographical and meteorological traps for atmospheric pollutants and organic forest soil is an important sink for pollutants deposited on needless and leaves during their lifespan, so the sampling locations for forest soils were selected up to 1881 m height above the sea level and the samples were collected, extracted and analysed. The concentration of

polychlorinated dibenzo-p-dioxin(PCDDs), dibenzofuran(PCDFs), caplanar-biphenyls(PCBs), hexachlorocyclohexanes(HCH), dichlorodiphenyltrichloroethanes(DDT) and organochlorine pesticides. The concentration of PCDD in soils were detected slightly lower for in comparison than European studies. The level of PCB was higher than PCDD and PCDF but the TEQ values were similar. The DDT concentration was observed really high because of the heavily agriculture and may effect from neighbour countries.

WE 199

The determination and source of DDT pollution in Turkish soils

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The concentration and impact of DDT on the environment was expected to decrease after its ban in the mid 1980's. Unfortunately, DDT contamination via its presence as an impurity in dicofol has led to a new source of contamination. This is particularly true especially in cotton production in Soke Plain, Turkey, where difocol-based pesticides are being used. The aim of this research was to investigate the extent and source of DDT contamination in cotton soils.
Soke Plain soil samples were collected from 0-30 cm, 30-60 cm, and 60-90 cm depth and analyzed by GC/MS/MS. o,p'-DDT and p, p'-DDE were detected at 16.2% and 17.6% of the sites in the 0-30 cm depth of soils. In the 20-60 cm, p, p'-DDT (14.9%), o, p'-DDE (8.1%) and p, p'-DDE (2.7 %) were found in soil samples and p, p'-DDT was the most prevalent with 9.5% of the sampling sites.
The dominant source of DDT particularly in the 60-90 cm depth was due to historic use of DDT. The presence of p, p-DDE, o, p-DDE and p,p-DDT in the topsoil was attributed to recent dicofol applications.

WE 200

Chemical assessment of organoclorine pesticides and polychloro biphenyls residues in sediment and pomatoschistus microps tissues from Ria de Aveiro, Portugal

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In the European Union (EU) about 3000 different substances are used in human medicine such as analgesics and antiinflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds and many others. Also a large number of pharmaceuticals are used in veterinary medicine, among them antibiotics and anti-inflammatory chemicals. In the last few years, knowledge about the marine and coastal environmental occurrence of pharmaceuticals and other pollutants has increased to a large extent due to new analytical techniques able to determine polar compounds at trace quantities. The assessment of endocrine disrupting activity in complex environmental mixtures requires application of integrative procedures combining chemical analysis and specific bioassays. This approach was focused on health and environmentally relevant compounds and based on the toxicity identification and bioaccumulation evaluation, to find a correlation between organochlorinated compounds and chlorobiophenyls congener concentrations in sediments and fish tissues of Ria de Aveiro The fish species Pomatoschistus microps was chosen because they might possibly be effective pollution indicators. They are very common and live in marine transitional and coastal environments which are continually exposed to garbage dumping, untreated sewage inflow, land and river runoff, atmospheric fallout from heavy traffic and various small-scale industries. In this work we observed the potential of fishes to provide valuable new insights into ecotoxicological effects and to make the functional link between environmental effects and human health level disturbances. Data for bioaccumulation on fishes were obtained in Portuguese coastal ecosystem Results indicated the involvement of this species in the bioaccumulation of a large spectrum of POPs, primary organochlorine contaminants such as PCBs, DDTs, and other pesticides, as well as exposure to metals and metal-containing organics such as methylmercury and organotins, with concentrations varying between 0.131-4.508 μg/g dry weights.

WE 201

Differential accumulation of metals and changes in Cholinesterases and d15N levels in the sea Anemone Anemonia sulcata (Cnidaria) as a function of aquaculture effluent gradient exposition

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Aquaculture technically is a process akin to the fermentation process where it has to be seeded and it also produces an effluent discharge, which must be regarded as a potential pollutant. Pollution, destruction of sensitive coastal habitats, threats to aquatic biodiversity and significant socioeconomic costs are the more important aquaculture impacts on environment and can involve health risks. The assessment of aquaculture effluents effects in complex environmental mixtures requires application of integrative procedures combining chemical analysis and specific bioassays. This approach was focused on health and environmentally relevant compounds, and based on the toxicity identification on water and sediments and bioaccumulation evaluation Information on aquaculture-related environmental impact was assessed using the stable nitrogen isotopic composition (δ15N) and metal analysis of benthic sessile sea anemone Anemonia sulcata tissues in the exposed areas compared to two unpolluted reference sites. A map of the δ15N values and metal bioaccumulation in organisms was created, which could be used for tracing the dispersion of 15N loading generated by aquaculture and sewage in the receiving environment, and their transfer into biota in the adjacent coastal ecosystem. The use of biomarkers to diagnose the contamination of ecosystems and the exposure of wild populations has several advantages relatively to other approaches. Biomarkers can be related to exposure to or toxic effects of environmental chemicals. Sea anemones known as non-selective suspension feeders, but hosting abundant bacterial populations, may have δ15N values lower than bioaculated metals, but still reflecting the overall enrichment of the environment in 15N due to the presence of enriched effluents from aquaculture discharges. The integration of the relevant results obtained are presented and discussed to address the central question of the work.

WE 202

Talitrid amphipods as biomonitors of PCBs and PBDEs contamination of supralittoral of sandy shores

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Coastal areas are characterized by a great input of environmental contaminants that can reach these zones by land based sources (including wastes from industrial, urban and agriculture activities, aquaculture, tourism), from sea based sources (oil spills, oil exploration and production) as well as atmospheric depositions. The contaminants of main concern include persistent organic pollutants (POPs), oils, fertilizers, heavy metals and pathogens. Talitrid amphipods constitute one of the main animal components (in terms of biomass) of sandy beaches representing, moreover, a key species in the food web of these ecosystems. In the last two decades, many species of sandhoppers and beachfleas have been successfully employed as biomonitor of trace metals contamination. In fact, studies carried out along European sandy shores have shown the ability of one of the most common Mediterranean sandhopper species (*Talitrus saltator*) to accumulate many trace elements (e.g. Hg, Cu, Cd and Zn).
To date, no studies have been reported about POPs contamination of supralittoral of sandy shores and their presence in talitrid amphipods. In this study we analysed supralittoral amphipod *T. saltator* and sand samples collected from different coastal areas of Tuscany (Italy) looking for PBDEs and PCBs in order to verify if this species could represent a suitable biomonitor of POPs contamination of supralittoral of sandy shores. 20 PCBs and 9 PBDEs were identified in both sand and amphipods samples and their concentration in *T. saltator* was, for most part of PCBs and PBDEs congeners, higher than in the sand. Furthermore, results showed significant differences in bioaccumulation between amphipods form different localities. Therefore *T. saltator* could represent a promising biomonitor of PCBs and PBDEs contamination of the supralittoral band of sandy shores.

WE 203

Global Aquatic Passive Sampling (AQUA-GAPS): using passive samplers to monitor POPs in the waters of the world

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We believe there are major benefits in starting global aquatic passive sampling (AQUA-GAPS) and propose the phased development of an AQUA-GAPS network of stations. The program could be started by initiating monitoring with PE samplers at key locations which are accessible and relatively well studied already e.g., the Great Lakes, US East coast, Baltic or North Seas, Mediterranean, and South China Sea. Investigators with access to ship time and buoys would be invited to participate to locate samplers and if capable, to also analyze them. Although analyzing samples at on central lab, as in GAPS would be desirable, we believe multiple labs could be involved so long as there was an interlaboratory quality assurance and training program. The list of POPs to be analyzed would be those readily analyzed by low resolution GC-mass spectrometry and for which analytical standards are readily available (the legacy chlorinated pesticides, lindane, endosulfan, selected PCB congeners, chlorobenzenes, tetra- and penta-BDEs). The program would also encourage deployment of different passive devices for comparison with PE samplers. Some of the Stockholm Convention regional and subregional centres for POPs that are located near deployment sites might want to be involved in their roles of capacity building and technology transfer. We seek volunteer investigators to help conduct the initial monitoring phase including the associated costs for passive sampler deployment and analysis.

WE 204

Persistent organic pollutants and stable isotopes in vegetation from King George Island, Antarctica

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Vegetation samples from King George Island, Antarctica (62º 05' S, 058º 23' W) were collected in the austral summer of 2004-05. Lichens (*Usnea aurantiaco-atra* and *Usnea antarctica*), mosses (*Sanionia uncinata*, *Syntrichia princeps* and *Brachytecium* sp), and one angiosperm (*Colobanthus quitensis*) species were analyzed for persistent organic pollutants (PCBs, DDTs, HCHs, HCB, PBDEs, mirex, aldrin, dieldrin, endrin and chlordane-related compounds) as well as $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. The following contaminants were found above the method detection limit (MDL): HCB (0.14 to 1.06 ng g^{-1} dry weight), HCHs (< MDL to 1.20 ng g^{-1} dw), DDTs (< MDL to 1.73 ng g^{-1} dw), PCBs (7.76 to 18.6 ng g^{-1} dw) and PBDEs (146 to 811 pg g^{-1} dw). In all cases, levels in mosses were higher than in lichens , suggesting similar biogeochemical processes involved, mainly due to chemical similarity and long range transport characteristics. Carbon stable isotope ratios ($\delta^{13}C$) showed clearly different ranges for lichens (-21.13 to -18.43"ð) and mosses (-25.99 to -21.64). The only angiosperm species investigated exhibited ^{13}C signature within the moss range. A large range of $\delta^{15}N$ was found (-7.67 to 20.75"ð) and seemed to be related to nitrogen uptake from different animal-derived sources. Pearson's correlation showed significant results between some historically linked contaminant groups and suggested the influence of the origin of both nitrogen and pollutants, notably taking secondary sources (animal derived nitrogen) in consideration.

WE 205

Specific effects of air contaminants from passive sampling campaign in Africa

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Polluted air has been associated mainly with lung and hearth diseases until recently. Recent studies indicate that the air pollutants could act also as endocrine disruptors (ED). Evaluation of this type of effects cannot be based only on data form chemical analyses. That shows the importance of incorporation of specific bioassays into evaluation part of the air pollution monitoring programs. The utility of these methods in monitoring of atmospheric pollution levels has

been shown previously. In this work, we show usage of passive sampling in evaluation of specific biological effects in vitro. Passive air sampling presents an interesting alternative to relatively expensive active sampling. It allows relatively long-term sample collection even in areas without developed infrastructure. Samples in this study came from localities all over African continent. The localities were chosen to describe background level of pollution as well as some hot spots. The sample extracts were assessed for priority chemical pollutants and specific effects using in vitro bioassays with reporter genes linked with aryl hydrocarbon receptor (AhR) and estrogen receptor, the receptors that are traditionally linked with ED. The results have confirmed that the endocrine disruptive potential of the air samples could be closely related to type of pollution and that endocrine disruptive effects seem to be connected both with particulate and gas fraction of the air. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

WE 206

Sampling rates when measuring gas phase POPs air concentration with passive air samplers.

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The Stockholm Convention was adopted globally in 2004 for reducing or eliminating the release of Persistent Organic Pollutants (POPs) since the exposure to these chemicals supposes a risk for human and environmental health. As essential part of this Convention is the Global Monitoring Plan which aims to control and evaluate its effectiveness. Air has been selected in this Plan for assessing spatial trends and the regional and global transport of POPs. For this purpose Passive Air Samplers (PASs) with polyurethane foam (PUF) disks as sorbent media has being used. PASs are advantageous against high-volume air samplers (HVSs) because of their low cost, simple construction, and electricity-free operation. However, high uncertainties over PASs sampling rate (R) have been addressed for these devices. Sampling rates are calculated by the quantification of loss of depuration compounds added to the PUF disk prior to deployment. Several calibration studies have calculated these rates for gas phase POPs, reporting a typically value of ~ 3 to5 m3/day. Nevertheless, sampling rates out of this range have been observed. This variation has been mainly attributed to diverse climatic or environmental conditions such as wind speed or temperature.
To evaluate the potential influence of temperature on sampling rates, a literature review was performed. A total of 288 site-specific R were collected from scientific studies conducted worldwide and calculated under a wide range of temperatures (from -27 to 36ºC). The study of this database showed that approximately 66% of data were within the range of 3 to 5 m3/day, while the rest of the data (34%) were located outside it. This revealed the need to calculate specific sampling rates when using PASs for monitoring gas phase POPs. Statistical analysis was performed to evaluate correlations in the database. A weak relationship was obtained between temperature and R ($p<0.01$; $r = -0.24$). This result indicates that estimations of sampling rates from temperature are not trustworthy and suggests that other parameters like wind effects should be considered.

WE 207

Monitoring the continental and intercontinental background of persistent organic pollutants in Africa

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To facilitate the evaluation of the effectiveness of the Stockholm Convention on Persistent Organic Pollutants (POPs), long-term monitoring of these substances in the environment is required. As Africa is one of the least studied regions and almost no data on POPs exist here, an effective monitoring network has been designed. The results of a pilot passive air sampling programme in January - July 2008 conducted at 26 stations in 15 countries and meteorological and climatological data from the 1961-2007 period were used to apply objective criteria for the selection of a small but sufficient number of stations for the long-term monitoring network.
The character of the sampling sites (urban, industrial, rural, background) was determined by discussing the sampling results and diagnostic ratios of polycyclic aromatic hydrocarbons were checked, too. The stations for the long-term monitoring network were selected from the list of the pilot phase sampling sites according to the following criteria: (i) absence of source influence and adequate geographic distribution, (ii) suitability to address long-range transport (avoidance of unsuitable local meteorological features) and (iii) long-term stability of air flow.
Nine stations remained after applying criteria (i) and (ii): Tunis, Khartoum (Sudan), Tombouctou (Mali), Sheda (Nigeria), Mt. Kenya, Asala (Ethiopia), Lusaka (Zambia), Reduit (Mauritius) and Molopo (SAF). With minor exemptions, the advection during the pilot sampling phase in 2008 was found to be typical for recent climate a these sites.The analysis of air flow patterns indicated a low variability between years, however, on-going changes in air flow were indicated.
The functionality of the monitoring network is not threatened by on-going long-term changes of the advection to the selected stations, as these do affect the coverage of target areas only to a minimal extent. The network does not cover several regions, e.g. the densely populated areas of Southern Cameroon, parts of the Abessinian plateau and most of the Great Lakes area. Also, the outflows of western and south-western Europe were considered important to be covered by the future monitoring network. Therefore, a monitoring station at the Canary Islands was added to the network, which now consists of this additional station and a slightly changed (due to practical reasons) list of the proposed stations. Regular long-term monitoring started in the beginning of 2010.

WE 208

Are the Mt. Qomolangma regions in Himalayas (Nepal) really remote areas for persistent organic pollutant contamination?

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The transport and fate of these semi/volatile persistent toxic substances (PTS) in remote areas has received increasing attention during the past decade. PAHs, polybromodiphenyl ethers (PBDEs) and organochlorine compounds (OC, as DDT, hexachlorobenzene - HCB, hexachlorocyclohexanes - HCHs and PCBs) were measured in water and sediment samples from several high altitude mountain lakes and in soil samples collected in 2007 near the same lakes and along the ascent in Sagarmatha National Park, located in Nepal and included in the Himalaya ridge. The study represents the first published research in the Nepal Himalayn region.
Results showed that HCH levels in all the matrices considered were always below detection limits. In water samples, low-level substituted PCBs and PBDEs, along with more volatile PAHs, were the most common contaminants, confirming the hypothesis that lighter compounds were more prone to Long Range Atmospheric Transport (LRAT). Moreover, in sediment and soil samples, the PCB profile was mainly composed of medium-level chlorinated congeners, consistent with the theory of OC accumulation in cold, high altitude regions driven by the process of cold condensation. The overall estimation of anthropogenic organic micropollutant presence in water samples showed a strong positive correlation between concentrations of PCBs and PAHs and the altitude of the considered sites, even if the altitudinal gradient for water samples was very moderate. In contrast, a significant negative correlation was found between PAHs and altitude in soil samples, considering a wider altitudinal gradient. This inverse relation was likely due to nearby local PAH emission sources. In addition, PCB normalised concentration on organic carbon content in soil samples showed a positive significant correlation with altitude, confirming the tendency of less chlorinated congeners to be preferentially transported at higher altitudes in cold regions. The PAH profile for water and soil samples showed the main contribution of pyrogenic PAHs due to emissions of solid combustion (wood, grass, coal), whereas the profile for sediments indicated the main contribution of pyrogenic PAHs from gasoline emissions (e.g., kerosene). Results of the analyses showed that PAH levels measured in Himalayan samples must be considered as low to medium contamination, whereas the regarded Himalayan stations can be considered undisturbed remote areas concerning PCB, PBDE and OC compounds.

WE 209

Long-term trends of POPs in human milk in Czech Republic

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Human biomonitoring of POPs concentrations in breast milk in Czech Republic is carried out since 1994. More then 4600 breast milk samples were collected from 9 sites in the years 1994 - 2009 (about 80 samples/region/year). The human milk samples are analyzed for a number of chlorinated organic chemicals including polychlorinated biphenyls (PCBs) and selected chlorinated pesticides (OCPs). Exposure factors that may influence these levels were investigated using a questionnaire according to the WHO protocol (age of mothers, BMI - body mass index, medication, occupational exposure and lifestyle habits, above all smoking). The main aim of this study was to evaluate long-term trends of selected POPs in human milk in Czech Republic - as background levels for central Europe (as part of ArcRisk project: http://www.arcrisk.eu/). The data indicates a continuation of a long - term decreasing trend of selected OCPs over 16 years. These dangerous organic substances (neurotoxicity, carcinogenicity and endocrine disrupting effects) are widespread throughout the environment, persisting for decades. They accumulate in the fatty tissue, entering the human body through the food-chain. Human biomonitoring is a useful tool to evaluate internal exposure of humans with different chemical substances. The next aim of this project is to predict of human health risks by using of slope factor and Rfd (reference doses) approach with a spatial GIS visualisation. Relationships between exposure factors from questionnaires will be also investigated.

WE 210

Temporal trends of PBDEs in gannet (Morus bassanus) eggs from two UK colonies

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Within Europe, varying trends in Polybrominated Diphenyl Ether (PBDE) concentrations have been observed over time across different regions and in different organisms. This variation is likely to be due, at least in part, to differences between species in routes and rates of exposure and metabolism. Although trends in PBDEs in birds have been observed elsewhere in Europe, there is little information for UK birds and no published long term time series of PBDE concentrations in fauna generally for the UK. We determined trends of PBDE concentrations in the eggs of gannets from two UK colonies off the coast of Scotland over a 30 year period (1977 - 2007). A suite of PBDE congeners, including Tri through Octa BDE homologues, was analysed for using a GC-MS in EI mode and employed C13-labelled spikes and internal standards for quality control. Fourteen separate BDE congeners were detected and all eggs throughout the time series contained BDE congeners 35, 47, 49, 99, 100, 153 & 154. BDE47 and other congeners associated with the PentaBDE technical formula dominated the profile.
No significant differences were found between ΣPBDE concentrations at the two colonies (Ailsa Craig and Bass Rock). Trends appeared to be strongly influenced by direct changes in PBDE production and emissions and also coincided with the abatement of sewage disposal at sea. At both colonies, BDEs 47, 49, 99, 100, 153 & 154 exhibited the same temporal pattern as ΣPBDE concentrations, increasing from the late 1970s, peaking in 1994 and from then on declining significantly up until the end of our time series. Levels of BDEs 47, 49 and 100 were significantly higher at Bass Rock than Ailsa Craig as were levels of BDE35 although this congener exhibited a linear decline over time at both colonies.
Throughout the time series, the contribution of the more highly brominated congeners (hexa and hepta homologues) to ΣPBDE concentrations increased relative to that of lower brominated congeners (tri-penta homologues), consistent with the hypothesis that there may be an increasing contribution to ΣPBDEs of higher brominated congeners derived from the debromination of decaBDE.

HM04 - Sorption and bioavailability of organic chemicals: mechanisms relevant for toxicity and bioremediation

WE 213

Influence of biosurfactants on microbial availability of polycyclic aromatic hydrocarbons sorbed to solid-phase organic matter and black carbon

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Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils is often limited due to the low bioavailability of these hydrophobic organic compounds. In particular, natural organic matter (NOM) and black carbon (BC) are geochemical soil components responsible for strong PAHs sorption, resulting in a very low bioavailability and long-term persistence of PAHs in the environment. The use of biosurfactants is a promising option for enhancing the bioavailability of soil-sorbed PAHs. It is known that biosurfactants, at levels above their critical micelle concentration (CMC), can promote the dissolution of solid PAHs. However, not much is known about the effects of biosurfactants on solid-water distribution and microbial uptake of PAHs in systems containing solid-phase NOM and BC. In a well-controlled batch system, we studied the

sorption behaviour of four different PAHs (pyrene, phenanthrene, fluoranthene and fluorene) with solid-phase NOM and BC and the effect of rhamnolipid biosurfactants on sorption equilibrium. Biosurfactants sorption was also determined. Biodegradation of sorbed PAHs in the absence and presence of biosurfactants was also followed using a representative PAH-degrading soil bacterium (*Mycobacterium gilvum* VM552). Experimental conditions included different sources of NOM and BC and different solid-aqueous phase ratios. The results showed that biosurfactants, especially when they were present at levels above CMC, often promoted desorption and biodegradation of PAHs.

WE 214

Modeling experimental findings on sorption and biodegradation of PAHs

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Polycyclic aromatic hydrocarbons (PAHs) in the environment are to a major degree bound to organic matter or soot particles. It is disputed whether and to which extent the adsorbed fraction of PAH in soil and sediment can be attacked by microbial enzymes, or whether dissolution in aqueous media is required. In that case, the degradation depends critically on the bioavailability, or better the bioaccessibility of PAH for microbes. The increasing non-accessibility of PAH with time ('aging') may explain the formation of a residual fraction, which has been observed in remediation projects.

A unified model for sorption, sequestration and degradation of neutral organic compounds in soils and sediments has been developed and tested (see also Trapp et al. session J01). Adsorption was considered as a two-phase process, with rapid and slow adsorption rates. Calculated or experimental sorption rates and kinetic data for growth and metabolism of PAH-degrading bacteria were obtained as input parameters. The model simulations were compared to existing solutions (such as the Best equation) and to experimental results. With this new model approach, a range of experimental observations available in literature could be simulated, encompassing various soil types and PAHs, and different bacterial strains. Own experiments are currently performed on phenantrene, fluoranthene and other PAHs and on ad/desorption as well as on biodegradation. The results shall be used to calibrate and verify the new model approach. The model was also used to simulate typical scenarios of adsorption (aging) and microbial degradation, in order to identify sensitive parameters and processes. Furthermore, the impact of dissolved organic matter and various types of amendments was studied, which potentially enhance diffusive mass transfer and biodegradation performance. The final goal is to optimize remediation options.

WE 215

Sorption and desorption characteristics of two insecticides (chlordecone and cadusafos) previously used in banana plantations of the French West Indies

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Chlordecone (CLD) is a highly persistent organochlorine pesticide that was used for a wide range of pests around the world, among them, the banana weevil *Cosmopolites sordidus* in plantations of the French West Indies. It was banned in France in 1993. One of the molecules that replaced it was cadusafos, an organophosphate that has since then been removed from the market in the European Union in 2008. Nearly 20 years after its prohibition, CLD is still detected in every compartment of the environment, i.e. in soils, surface and groundwaters and some agricultural products and it is suspected to increase the prevalence of prostate cancers in exposed populations. The physico-chemical characteristics of this molecule along with its high stability due to the numerous chlorinated groups that protect the molecule from degradation, make sorption one of the most important processes that govern the fate of chlordecone in soils. However, the literature in the environmental fate of this molecule is scarce and mainly from the 80's or earlier. The aim of this work is to improve the knowledge in the sorption and desorption of CLD and cadusafos in tropical volcanic soils of the French West Indies. Sorption-desorption isotherms were carried out in batch experiments in 7 different soils and fitted to the Freundlich model. Additionally, the nature of the soil organic matter, known to be one of the determinants of the adsorption of pesticides, was examined by ^{13}C-CP MAS NMR. Sorption of CLD in soils was very high (26< K_f< 102 L kg^{-1}) with a high hysteresis effect in 5 of the soils studied and no desorption at all in the two remaining soils. Freundlich's K_f parameter of cadusafos was ranging between 0.8 and 21 L kg^{-1} and contrarily to CLD, desorption was not showing a high degree of hysteresis. The K_f values were correlated to the OC content (P<0.01) for both molecules and derived K_{oc} were ranging between 1200 and 2500 L kg^{-1} for CLD and between 67 and 135 L kg^{-1} for cadusafos. Despite the different nature of the soils, NMR spectra for soil organic matter were very similar between the 7 studied soils. Thus, the Koc heterogeneity is not only influenced by the chemistry of the organic matter, but must also be attributed to other soil properties. In the literature, values on the K_{oc} of CLD were missing, they were sparse for cadusafos and they represent a necessary piece of information for the use of models to predict the transfer of these molecules in surface and groundwaters.

WE 216

On the challenge to remediate a soil contaminated with crystalline DDT by activated charcoal

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The production and distribution of the insecticide dichlorodiphenyltrichlorethane (DDT) resulted in a polluted area of about 85 hectares at Nowshera, Khyber Pakhtunkhwa (KPK), Pakistan. At the factory's place the contamination is up to 800 g/kg DDT in dry soil. To reduce DDT exposure of the environment and humans, this contaminated site has to be remediated. Therefore, the aim of this joint research project is to test a remediation strategy with activated charcoal (AC) added to substantially reduce the bioavailable fraction of the aged DDT in the soil. To the best of our knowledge, AC has been used for remediation purposes in a concentration range of ppb to ppm in soils and sediments, but never in the percent range. Under these circumstances, limitations are set at extremes and are manifold. Results from this study will give important information on the performance of AC materials in a naturally contaminated soil and reveal the influence of soil treatment on the immobilization of DDT.

Pots with five kg of field soil were placed in the laboratory. Then, 2 % and 10 % GAC and 0.1 % and 2 % PAC were added. To test the influence of different soil treatments on the binding quality of the AC, the amended soil was then treated in three different ways. Some pots were watered during a month to simulate monsoon season, but left untreated for the following five months (treatment 0). Others were mixed fortnightly to simulate ploughing (treatment P) and a third group was subjected to regularly watering (treatment W) and watering and ploughing (treatment PW) over the whole period of time. After certain time periods, bioavailable DDT and metabolites were assessed by six hours Tenax® extractions with two grams of Tenax® beads and one gram of dry soil.

Results from this experiment were surprising. The Tenax® extractable DDT decreased drastically over time not only in all AC amendments and treatments, but also in the control. After 150 days, the concentration of the DDT extracted by Tenax® was very low with a few per milles. Overall, two main questions arise under this situation with extremely high DDT concentrations: 1) is Tenax® the appropriate tool to assess the bioavailability, and 2) is AC amendment capable to substantially reduce DDT exposure. These important issues will be addressed in this presentation.

WE 217

Distribution of aged 14C-atrazine residues in soil following 22 years of environmental exposure.

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Distribution and localisation of pesticide residues into the different physical fractions of soil may reveal information on processes taking place in soil. Soils amended with ^{14}C-atrazine, were aged for 22 years under environmental conditions in a lysimeter in Germany. The soil was sampled and subjected to physical and chemical fractionation before and after incubation with the earthworm Apporectodea caliginosa for 7 days. No significant change in the soil physical and chemical fractionation of the ^{14}C-atrazine residues and organic carbon was observed in this study as a consequence of the activity of the A. caliginosa. The low organic carbon of the soil, the absence of relatively fresh organic matter and the long ageing time might explain the limited bioavailability of the ^{14}C-atrazine to the earthworm. Approximately 9% of the applied 14C-atrazine associated activity was found to be present in the top soil layer 0-10 cm of the lysimeter. This amount represented approximately 36% of the total amount of 14C-atrazine associated activity presented in the soil after 22 years of environmental ageing. Soil half lives calculated in this study taking into account the non-extractable 14C-atrazine residues were much greater than those reported from previous studies. This finding is of particular importance given that the soil used here was aged under natural environmental conditions compared to laboratory studies.

WE 218

Use of solid-phase microextraction and toxicity tests to determine the bioavailability and toxicity of hetero-PAH with the oligochaete Lumbriculus variegatus in sediment-pore water systems

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Polyaromatic heterocycles (hetero-PAH) are N-, S-, O- substituted polyaromatic hydrocarbons (PAH). They occur together with their unsubstituted analoga e.g. at creosote-contaminated sites. Their acute, chronic and reproduction toxicity as well as their higher water solubility compared to their unsubstituted parent compounds account for their ecotoxicolgical relevance.

In the present study the chronic toxicity of two hetero-PAH acridine (AC) and xanthene (XA) in a sediment-pore water system of a spiked natural river sediment (Altrip, Rhein, Worms; AC 6.3-146.2 µg/g DW; XA 0.1-270 µg/g DW) on the reproduction of sediment dwelling endobenthic oligochaetes *Lumbriculus variegatus* was determined according to OECD guideline 225. In parallel to chronic toxicity, the freely dissolved pore water concentrations (c_{free}) of the substances were determined by solid-phase micro extraction (SPME) and high performance liquid chromatography.

Determined effect concentrations (ECx) for the reproduction of *L. variegatus* were based on the total sediment concentration (EC_{50} AC 37.8 µg/g DW, EC_{50} XA 2.4 µg/g DW), the tissue concentration (EC_{50} AC 0.0052 µg/mg DW tissue) and c_{free} (EC_{50} AC 0.034 µg/ml). Hormesis was observed for the reproduction of *L.variegatus* at the lowest tested concentrations of both substances. Furthermore, with the SPME technique it was possible to determine cfree of both substances in sediment pore water at higher sediment concentrations (AC ≥ 30.5 µg/g DW and XA ≥90 µg/g DW) and to prove metabolic degradation of xanthene to xanthone in the sediment. In contrary, no degradation of acridine to acridone was detected in both, the sediment and *L. variegatus*.

The results emphasize, that for sediment contact tests, i.e., testing the ecotoxicity of hydrophobic organic chemicals (log K_{OW} < 5.7) on endobenthic organisms, it is important to simultaneously determine c_{free} of the substances to be able to link the observed ecotoxic effects to the real exposure.

WE 219

Characterizing the bioavailability of cationic surfactant using passive sampler

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Cationic surfactants are ubiquitous contaminants that are extensively detected in sediment, sewage sludge or surface water due to their wide applications in industrial and domestic products. However, the understanding of the impact of this class of compounds on the environment is still limited. The freely dissolved concentration of an organic compound is an important parameter for accessing the environmental fate and bioavailability of the chemical. The free concentration of cationic surfactants, are difficult to determine because of their high adsorptive capacity to lab apparatus and because the difficulty to separate the bound particles from the aqueous phase.

In the current study, a solid phase microextraction (SPME) method was optimized and applied to characterize the bioavailability of two cationic surfactants, hexadecyl trimethyl ammonium chloride (IV-16) and benzyl dimethyl dodecyl ammonium chloride (C12-BAC). The effect of solution chemistry on the sorption isotherm to SPME was first examined. Sorption experiments of the two cationics to Pahokee peat and humic acid were carried out. The influence of pH and the presence of inorganic cations on the sorption behavior were examined within the range of relevant environmental concentrations. The results suggest both ionic and nonionic interactions could play a role in the sorption process for cationic surfactants.

WE 220

Sorption of cationic organic compounds: how important is clay compared to organic mat-

ter?

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Many emerging environmental compounds of concern are (partially) positively charged under typical environmental conditions. Sorption to organic matter (OM) in soils, sediments and dissolved particles occurs mainly through an ion-exchange process at negatively charged groups in OM. Besides OM, most types of common clay minerals also contain negatively charged surfaces to which these organic cations may sorb. Since sorption strongly controls the bioavailability and mobility of these compounds in the environment, and in soil toxicity tests, insight is needed for which compounds the clay fraction forms a considerable sorption phase relative to the fraction of OM. In a dynamic HPLC set-up, retention of organic cations was measured on columns partially filled either with OM (micronized Pahokee Peat) or clay mineral (illite), mixed in a certain percentage with inert packing material. Clear differences between sorption affinity of quaternary amines and primary amines to OM and clay exist. For quaternary amines, retention time on a column packed with 1% illite is stronger than when packed with 1% OM, whereas for primary amines the opposite is observed. Clearly, clay minerals form a significant sorption phase for many organic cations. It will be a matter of the fraction size of OM and clay, e.g. in a river sediment, which type of substrate dominates the sorption. Besides the type of charged amine moiety, also functional groups in the nonionic part of the organic cation determine the specific affinity to either OM or clay.

WE 221

Chlordecone degradation and impact on soil respiration

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Chlordecone (CLD) is a persistent organochlorine pesticide included in the Annex A of the Stockholm Convention. Despite its prohibition in France since 1993, it was still significantly detected in 2001 in the French West Indies. The aim of this work was to study the persistence of CLD in soils, to assess its likely toxic effect in soil microbial activity and to monitor the appearance of possible metabolites. Degradation was studied in soil microcosms under controlled conditions in two horizons (NEH-A and NEH-B) from the same tropical andosol. Uniform ^{14}C labelled CLD was used. ^{14}C-CLD was applied at normal field dosage on both soils. Different application rates were also tested (NEH-A with twice the field dosage, NEH-B with half of it). At time intervals of 0, 32, 88, 150 and 214 days, the distribution of ^{14}C-CLD in different compartments of the experimental system (soluble, extractable, bound and mineralised fractions) was determined. The impact of CLD in soil respiration was assessed by the Substrate Induced Response (SIR). The presence of CLD metabolites was studied using Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectra (GC-MS). The results were similar between the different soil field application rates. Due to the low solubility of CLD, the desorbed fraction was low and constant during the incubation (2% and 8% in NEH-A and NEH-B, respectively). The bound residues fraction was similar and almost constant in both soils during the experiment (≈12%). Mineralization was higher in the NEH-A (4.9%) than the NEH-B (3.3%). This was attributed to the higher basal respiration of NEH-A. CLD did not affect SIR, although it decreased soil respiration response at longer periods of time. The TLC analysis of the standards solutions of ^{14}C-CLD showed up to 3 different regions of radioactivity (corresponding 90% of the activity to CLD region and 9 and 1% to the other regions). GC-MS identified four different chlorinated compounds in these regions: CLD, mono-chlordecone, mirex and an unknown highly chlorinated compound. In the TLC analysis of the extractable fractions of soil NEH-A (214d) it was not possible to observe the same regions of radioactivity than for the standard. This extractable fraction was measured through GC-MS and only mirex and CLD were identified. In summary, CLD degradation is extremely low and the majority of CLD is in an extractable form, suggesting that it is potentially available for transfer into the soil solution.

WE 222

Impact of ageing on bioaccessibility of pentachlorophenole in the baterial contact assay with Arthrobacter globiformis

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Background and aim The miniaturised bacterial contact assay is a fast and sensitive whole sediment contact test. Toxicity is determined by measuring the inhibition of bacterial dehydrogenase activity (DA). Transformation of resazurine to the fluorescent dye resorufine through DA in the samples is compared to controls using a direct fluorescence measurement. The assay can be used for aqueous and solid samples.

This project investigated the impact of short-term ageing in the bacterial contact assay. An artificial and a natural sediment were spiked with pentachlorophenol and tested at regular intervals. Based on previous studies with other specimens a fast decrease of the effect was expected. Furthermore, effects were assumed to decrease faster in the artificial sediment.

Materials and methods Test bacteria were incubated in 24-wellplates in an aqueous suspension of test sediment. Artificial sediment was prepared according to OECD-guideline 218 (modified). Natural sediment was sampled at Altrip, a back water of the River Rhine. Both sediments were spiked with 500 mg PCP/kg dry weight in three independent replicates. The first test was carried out immediately after spiking, subsequent tests were performed at increasing intervals of time.

Results Inhibition of DA fluctuated between 60 % and 100 %, thus exceeding the toxicity threshold defined for this test. No significant change occurred after 27 weeks of ageing. Furthermore, no differences between both sediment types could be determined.

Discussion Ageing sediments for 26 weeks did not affect the DA of *Arthrobacter globiformis* through pentachlorophenol. This observation could result from a higher bioaccessibility of the contaminant for bacteria, in contrast to organisms with different pathways of exposure. Enhanced accessibility to bacteria may also explain that no differences between sediment types were found, though both have distinctly different characteristics in terms of pH, TOC etc. This is shown especially in comparison to organisms of higher complexity and lower access to sediment-bound compounds, where such differences have been shown.

Conclusion The results of this study show that the size of the exposed organisms plays an important role in ageing-related changes of the biological effects observed in whole sediment contact tests. Further experiments are necessary and currently on-going in order to increase the understanding of how ageing impacts biotests with organisms of higher complexity.

WE 223

Interactions of chemicals with engineered nanoparticles (quantum dots) and combined effects on bacteria

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The toxicity of chemicals as well as particles has become an area of intense research during the last decades in the environmental sciences. However, only few studies did address a possible modification of effects through interactions of chemicals with particles. In principle, sorption of chemicals can decrease their toxicity as their bioavailability is reduced. On the other hand, engineered nanoparticles have been shown to affect living cells in different ways. Consequently, in a ternary system of bacteria, engineered nanoparticles, and chemicals, the sorption of chemicals on such particles must not necessarily reduce the toxicity towards bacteria.

In this study, toluene and quantum dots (QDs) were selected as representatives for well-investigated organic pollutants and engineered nanoparticles, respectively. The QDs used in this study consisted of a CdSe-core that was passivated by a ZnS-shell. Finally, the surface of the QDs was coated with polyethylene glycol and functionalized with amino-groups leading to hydrophilic surface properties. The diameter of the QDs was around 9 nm, and in aqueous salt solutions they showed a tendency to form aggregates as revealed by transmission electron microscopy. First results based on sorption studies in batch experiments showed that in physiological salt solution, toluene interacted with QDs leading to reduced freely dissolved concentrations of toluene in the presence of QDs. Ongoing experiments investigate the effect of interactions between toluene and QDs on dose-response relationships.

LC04 - Life Cycle Inventory modelling and attributional/consequential issues (LCI)

WE 226

Inclusion of land use changes in LCA on bioenergy: parameterization of land use change patterns

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Bioenergy is considered as an important option for the mitigation of climate change. However, the impact of land use change on soil and vegetation carbon pools may jeopardize the potentially achievable savings of greenhouse gas (GHG) emissions by the replacement of fossil fuels. In recent literature different economic and geographic model approaches are discussed in order to assess not only direct but also indirect land use changes. To develop a novel approach to consequential LCA, we consider coupling of LCA with a land use model. Economic models calculate the interdependence between the demands for raw agricultural materials, the agricultural land needed for their production and the price correlations. Examples applied for analyzing land-use changes include GTAP (Global Trade Analysis Project, Purdue University), CAPRI (Common Agricultural Policy Regional Impact Analysis, University of Bonn) and IMPACT (International Food Policy Research Institute, IFPRI). Due to their relatively low spatial resolution (e.g. world regions, countries or districts) these models are not suitable for detailed spatially explicit environmental impact analysis. In order to overcome this limitation, we have chosen to apply the geographic model LandSHIFT which accounts for global and continental level land use change and has been developed as a new approach for spatially explicit simulations of large-scale land-use change dynamics and their environmental impacts. The model input is a set of exogenous drivers such as population and production of agricultural commodities, commonly derived from the aforementioned large-scale economic models. The model output is a time series of high resolution raster maps of the changing land use pattern. These land use maps can be processed as input for LCA respectively. The research investigation focuses on the approach of coupling the two models of interest. Using the example of different scenarios according to multiple prospective bioenergy targets the objective of our study is to assess the impact on land use change dynamics and the consequent GHG along the entire process chain in LCA especially due to land use change. Furthermore the research aim is to investigate whether land use can be modelled as a linear or nonlinear function of agricultural or resource output with increasing bioenergy production targets within the framework of LCA.

WE 227

Partial and General equilibrium modelling to integrate consequential effects of indirect land use changes (ILUC) in LCA of biogas

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The promotion of renewable energies, such as biomass, is at the heart of the European Community's policy actions since a decade. Policies for supporting biofuels require life cycle carbon reporting to ensure that biofuels achieve greenhouse gas reductions relative to fossil fuels. However, the use of agricultural crops for energy purposes is responsible also for several environmental impacts not related to climate change, such as eutrophication and acidification due to fertilizer runoff, and biodiversity loss. The most consensual methodology to evaluate the overall environmental impact associated with the production of energy from agricultural crops is Life Cycle Assessment (LCA). In the field of bioenergy, comparative LCAs of bioenergy production are usually of the "attributional"type. In attributional LCA (ALCA) average LCI data are used and it is generally assumed that the studied lifecycle does not affect other systems. This means that the relationships between the lifecycle and other economic systems (i.e. the market on which it operates) are disregarded. For instance in the bioenergy sector, ALCA disregards the (marginal) effects of increasing production of a specific crop in a certain area. However ALCA is a recognized simplification in the study of bioenergy systems, for which it is particularly relevant to consider the consequences of the increased use of bioenergy on other economic systems. To ameliorate this problem another approach, named Consequential LCA (CLCA), is being developed. It aims at generating pertinent (market based) information on the consequences of actions. In other words, CLCA aims at describing how the environmentally relevant physical flows to and from the technosphere will change in response to possible changes made within the life cycle, including the indirect effects on land use change and the effects on other economic systems, based on actual market conditions.

After a review of the most widespread PEMs and GEMs applied to agriculture and trade policies, the paper describes the first results of a project led by the Resource Centre for Environmental Technologies (CRTE) of the Public Research Centre Henri Tudor (Luxembourg). The main scientific objective of the project is to provide an operational methodology for the inclusion of indirect effects of land use changes into CLCA of bioenergy and then to apply the methodology to the case of biomethane production from energy maize in Luxembourg.

WE 228

A constraint-based method for performing life cycle inventory analysis using the matrix model

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In a decision-making support process based upon environmental criteria the analyst might be interested in the definition of the system configuration(s) (different process combinations, different features of the processes input materials, etc.) consistent with a set of imposed constraints on the maximum allowed environmental burdens (and consequent environmental impacts) and natural resources consumption. While the classical matrix method for Life Cycle Inventory (LCI) compilation is the tool by which the environmental impacts linked to the studied system can be calculated, it is not very practical when one wants to automatically solve the above mentioned constraint satisfaction problem. Although optimisation methods have been proposed in connection with Life Cycle Assessment (LCA) problems, based on Linear Programming (LP) models, this has not been done in a way that is consistent with the matrix model. Nor would this have been possible with these approaches.

In this work, we present an approach based on constraint programming (CP) for a case study concerning the energy valorisation of grape marc by the production of grape marc pellets. Basically, we take the ordinary matrix model and calculate backward from a given set of burdens to the (best) possible set of inputs from the technosphere compliant with those pre-imposed burdens. Further constraints are imposed in the model in order to assure that the solutions found (if any) are also physically feasible. This optimization process could provide significant added value in decision making or ecodesign processes based on LCA, since it allows the LCA analyst to identify (if they exist) those system configurations that meet certain environmental goals, thus avoiding a time consuming and sometimes unproductive search for optimal solutions based on a conventional trial and error approach.

The technology matrix comprises over 1'600 entries and the inventory vector has over 1'500 elements, of which only a few have been selected to test the optimization algorithm. The results of the study showed that the water content in fresh grape marc entering the dehydration phase the fresh marc undergoes has a significant influence on the computed impacts. The applied CP-based approach allowed to infer the ranges of water content in fresh grape marc that were consistent with the imposed constraints at the inventory table level (environmental burdens) as well as at the midpoint indicators level (environmental impacts).

WE 229

End-of-Life vehicles management: evaluation of recycling and recovery potentials in a dynamic approach

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Since its birth in 1990s, LCA has shown great potential for support decision-making, and that rapidly contributed to rise and wide the use of this methodology. Decision-making to be successful needs to identify consequences and future impacts of choices depending on marginal mechanisms and that may affect environment. In this sense, a formal distinction is now adopt between two types of LCA approaches: attributional/status-quo LCA, in which future developments and marginal effects are not considered, and consequential/dynamic LCA, which instead assumes the influence of the time-dependent parameters and changes on the system investigated. These parametersare used in consequential/dynamic LCA for modelling future systems and assess how they may affect the results [Finnveden et al., 2009].

In a previous study [Ciacci et al., 2010], a comparison among different ASR management strategies was carried out aiming at proposing alternatives to the current scenario of landfill disposal in terms of environmental benefits and reduction of waste disposal. Such ASR management strategies (landfilling, incinerating, mechanical recycling and gasification) are here adopted as a starting point for a dynamic approach of LCA methodology, according to Pehnt (2006). In particular, this study focuses on changes expected in both the composition variation in vehicle production, and for background system parameters, such as the increasing for renewable energy sources in electricity production mix. Starting from 1 ton ASR obtained from a dedicated shredding campaign, the present functional unit has been changed according to two different previsions: the first related to a reliable ASR composition in 2015, following the current increasing trend of plastic and non-ferrous metals (especially aluminum) and aimed to decrease car weight; the second is based on the application of eco-design principles towards dismantling and collecting practices. Finally, previsions on potential benefits from recycling aluminum and polyolefins (PP/PE) from ELVs are discussed. Results show benefits from mechanical recycling of plastics and aluminum particularly if eco-design application would allow a reduction of the different kinds of plastics used in cars, and then to improve polymer and non ferrous metals separation and recycling.

WE 230

Combining technical optimisation in early R&D with Life Cycle Assessment - the case of micro process technology

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New technologies in the field of decentralised small and/or mobile applications (e.g. consumer electronics) are characterised by a shorter lifetime, utilisation of multiple rare earth metals and huge production numbers. Due to the awarness of environmental impacts of these technologies during their life cycle, the optimisation of their design seems absolutly essential to obtain a more sustainable technology. Hence, the question arises how to combine technical optimisation with Life Cycle Assessment?

Micro process technology is based on chemical and physical phenomena occuring inside volumina of less than 1 mm in diameter, usually carried out in continuous flow mode. General advantages are enhanced material yield and energy efficiency in chemical processes by improved heat and mass transfer. The field of micro process technology covers developments which might substitute existing processes as well as enabling new technologies which will lead to ample changes in markets or even societal conditions.

Using the example of a micro structured reactor for gas to liquid fuel conversion in early R&D, a new methodology for combining technical optimisation with Life Cycle Impact Assessment was developed. As result, different possible micro reactor designs can be compaired due to their ecological impact using Life Cycle Assessment. Further advantages of this methodology are the identification of improvable and not-improvable technical parameters in terms of lowering environmental impact, the easy integration in other LCAs and the transfer of this methodology to other micro process technology applications.

WE 231

Models of chemical inventories based on statistical analysis of on-site production data

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Chemicals are involved in the production of almost all industrial and consumer goods, therefore the improvement of the environmental performance of chemical production is a very important issue for assuring sustainability in modern life. For this purpose Life Cycle Assessment (LCA) is a suitable evaluation method. However, there is a lack of inventory data of production processes in the chemical industry, being this a significant problem for many LCA practitioners.

To overcome this problem, generic models of chemical production processes based on statistical analysis of on-site production data are being developed. These generic models will be on the level of unit processes, this means all operations that are required to achieve a change in the chemical structure (e.g. the actual chemical reaction and subsequent separation and purification steps) and will be not product specific, but reaction type specific. The main output prameters of these models include reaction yield, use of raw materials and utilities, as well as emissions to air, water and soil. These models will take the form of generic data ranges with an empirical average, minimum and maximum values, resulting in empirical confidence intervals for each process parameter. The data sources represent different chemical industry sectors, with different purposes, from basic chemicals to pharmaceuticals and pesticides. This allows a parameterization independent of the industry sector, and thus the development of generic gate to gate process models.

Using this approach, a complete synthesis can be modeled by combining individual process models. These models can be also used to fill data gaps in existing process documentation. In this way these models will allow a better assessment of the sustainability of chemical production.

WE 232

An improved model for estimating pesticide emissions for agricultural LCA

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Credible quantification of chemical emissions in the inventory phase of Life Cycle Assessment (LCA) is crucial since chemicals are the dominating cause of the human and ecotoxicity-related environmental impacts in Life Cycle Impact Assessment (LCIA). When applying LCA for assessment of agricultural products, off-target pesticide emissions need to be quantified as accurately as possible because of the considerable toxicity effects associated with chemicals designed to have a high impact on biological organisms like for example insects or weed plants.

PestLCI was developed to estimate the fractions of the applied pesticide that is emitted from a field to the surrounding environmental compartments: air, surface water, and ground water. However, the applicability of the model has been limited to 1 typical Danish soil type and 1 climatic profile obtained from the national Danish meteorological station.

To overcome these limitations, a reworked and updated version of PestLCI is presented here. The new model includes 16 European climate types and 6 mean European soil characteristic profiles covering all dominant European soil types to widen the geographical scope and to allow contemporary (varying site and or climate condition) and future (change climate condition of a location) differentiation.

In addition, the tillage frequency is now incorporated as an input parameter. The tillage frequency has an impact on the soil permeability through its relation to the occurring frequency of macro pores in the top soil, and thus the initial leaching rate of pesticide through preferential flow.

A third improvement of the updated model is a simplified user interface which makes the model easier to evaluate and operate.

The updated PestLCI model is demonstrated on cases involving different climatic circumstances and locations presenting the resulting variations in pesticide emission patterns.

WE 233

Current lack and needs for better life cycle inventory data for water footprinting

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Water is a growing concern in life cycle assessment. Several impact assessment methods have been developed or are being developed to assess the impacts on water. However, current inventory databases (Gabi, Ecoinvent, etc) provide only limited data on water, mainly on withdrawal, and are all mis-matching the elementary flows requirement of the methods developed recently to address the issue of water impact assessment (methods such as Pfister et al. 2010, Water Footprint Network, Motoshita et al. 2010). Indeed, most of these methods address specific water use, at inventory level, that are not included in existing life cycle databases or are difficult to extrapolate from existing data. Furthermore, a lack of sufficient and relevant data is in many cases the dominant factor limiting the ability of corporate water accounting and impact assessment (CEO Water Mandate, 2010).

In light of this issue, a project was launched by a consortium of companies - and leaded by Quantis - to create an exhaustive "water"life cycle inventory database. This water database is based on the ecoinvent (www.ecoinvent.ch), for approximately 4'000 processes and will include:

- A full balanced water accounting taking into account water flows that are addressed in the recently developed accounting and impacts methods
- Different regionalization possibilities at the level of the country, the level of the watershed or using an archetype approach
- A preset choice of impact assessment methods applied to the inventory flows

The applications in life cycle inventory and impact assessment ranges from assessing a large number of products, assessing the supply chain and indirect water consumption of materials and energy for corporate reporting, water management and risk assessment. The use of this database will also make possible for researchers to apply and develop further methods to assess the different types of environmental impacts related to water. The availability of inventory data will make it possible to widen the scope of actual "water footprint"(at inventory and impact levels) studies and include in-stream and off-stream water uses, consumptive, non-consumptive and degradation water uses in a consistent way. Result on a case study will be presented.

WE 234

Modeling global crop water demand using a life cycle approach

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Global water resource demand is expected to increase by as much as 50 percent in the next 40 years as the human population tops nine billion. Agricultural water use accounts for as much as

70 percent of total water use. Increasing demand for food, feed, fiber and fuel will increase demand for irrigation water. Global climate change will shift rainfall patterns, resulting in potential increase in water stress in certain regions. We developed a crop water demand simulation process incorporating the CERES 4.0 Maize model in the Decisions Support System for Agrotechnology Transfer (DSSAT) program. The goal of this project was to simulate global corn yield based on a range of input parameters at the highest geospatial resolution supported by the data. A customized user-interface in the MATrix LABoratory program (MATLAB) was created to allow for the input of global or regional datasets within DSSAT. Preliminary objectives of this project were to calibrate the CERES-Maize model for the Untied States using corn yield data from National Agricultural Statistics Service over a 10 year period and apply the calibrated model to future climate scenarios.
Once applied, water use impacts related to maize production were assessed based on future irrigation demands. Both water use efficiency and evapotranspiration were also calculated for each pixel. There values, along with predicted irrigation withdrawals were overlaid with water scarcity indices to forecast areas of high risk under future climate conditions. The model will help present policy makers plan for the agricultural water demands of tomorrow. Future studies will involve adapting the previously described methodology to a global scale and extend crop coverage to soybean, rice, and wheat. After future water demands for all crops are found, this information can be applied to environmental impact assessments that are lacking data for agricultural water use.

WE 235
Assessing water quality compensation treatments and related inventory in LCA
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Impacts from a lack of functional water for human uses in Life Cycle Assessment can be generated directly on Human Health, if they occur in a region affected by water-related diseases or malnutrition, or through the use of compensation strategies when the socio-economic context allows it. Compensation refers to the adaptation by the human users to a change in the water availability in order to still meet their needs. These strategies can refer to compensation to achieve functional/good quality water from non-functional/poor quality water, by water treatment, or to compensate for a physical lack of water, by other scenarios like importation for example. Both these types of strategies will generate impacts which should be included when evaluating impacts from water use in LCA. The water treatments required to compensate water quality will vary according to the available water and desired final quality, and these treatments need to be identified in order to properly assess the impacts from this compensation.
The work presented here proposes a methodology to evaluate the sequence of different treatment steps required, as well as the related additional inventory required for the compensation of a degraded water quality, based on fixed input and output quality. Seven water categories describing the quality and users for which each category is functional are first presented. Decision trees created to generate relevant treatment sequences for moving from one category to another are then described and used to evaluate the 25 different possibilities of water quality compensation, based on the water categories presented. Five different treatment sequences result and the inventory from each is assessed. This method is then used to assess impacts from a pulp and paper plant releasing different water qualities in a traditional LCA framework.

WE 236
Life Cycle Assessment of urban water cycle in Mediterranean cities
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Given the increasing demand of urban water for both potable and non-potable applications, in many countries urban water authorities are struggling to satisfy the demand meanwhile improving the environmental profile of the urban water system.
The main goal of this study is the environmental analysis of the whole urban water cycle, taking into account all its elements: potable water treatment plants, water distribution system, water use, wastewater treatment plants (WWTP) and sewage systems. Based on the methodology of Life Cycle Assessment (LCA), we assessed the current urban water systems on the Spanish coast of the Mediterranean Sea and proposed possible scenarios for improving their environmental performance in order to find out the best scenario for the urban water management.
Urban Water Cycle here considered includes potabilization, distribution, collection, treatment and optional wastewater reclamation. Inventory analysis was performed based on local operation data complemented with Ecoinvent Database whenever the local data were missing. The environmental profiles of the different water system scenarios were analysed based on CML 2001 and Eco-Eficiency indicators. The results show that Global Warming Potential, GWP impact in the considered water system without water reclamation is divided in 44% potabilization, 38% distribution and collection and 18% water treatment. When water reuse is included, the GWP of the water system remains practically unchanged because the inclusion of an obligatory tertiary treatment is balanced by the credits obtained by the fertilizers saving in agricultural uses. Nevertheless water reclaiming provides water that can be used for non-drinking uses and consequently a net reduction on freshwater consumption indicator is obtained. The study also considered a hypothetical situation of drought conditions, when a new desalination plant has to be included in order to cover the water requirements. Results show that this solution would imply an increase of about 30% of the environmental impact of the global water system and therefore, it should be used only to complement the purification process already existing. This study demonstrate the usefulness if LCA for the assessment of the urban water systems enabling the identification of critical processes and the potential improvements of the system.

WE 237
Life cycle assessment of decentralized combined heat and power plants based on difficult fuels
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In combined heat and power (CHP) plants waste heat can be used for district heating or process steam. By this, the efficiency of the power plant can be increased to about 90 %, which saves primary energy and reduces ecological impacts.
There is a broad range of fuels which can be used in a CHP plant. A research project at Karlsruhe Institute of Technology (KIT) aims to enlarge the scope of fuels to so-called 'difficult' fuels: these are materials with various disadvantages like low heating value or low ash melting temperature and may be renewable (e.g. straw, forest residues) or non renewable materials (e.g. refuse derived fuels, litter). Advantages of these fuels are that they are so far mostly unused and that they are available on a regional scale (within a radius of 100 km around the CHP plant). This keeps the transportation costs low and reduces environmental burdens. Regional fuels also result in lower dependency in imported fuels.
For LCA of this new type of CHP concept, a modular model has been developed which may be used in future studies of consequentional LCA of regional energy provision. This model enables to compare different fuels and types of heat use. It consists of transporting, drying and crushing the fuels as wells as of modules for the CHP plant itself. The data for combustion is generated in laboratory scale and pilot plants. After combustion, the flue gas runs over a steam generator, which produces high pressure steam. The whole water-steam cycle, including steam turbine and heat exchangers is simulated. After the steam generator, the cooled flue gas is cleaned by state of the art technology. That means, depending on the kind of fuel, SO2 and NOx reduction as well as a filtration of fly ash.
The LCI model itself as well as first assessment results will be presented.

WE 238
Energy production from microalgae - a contribution to a sustainable energy supply?
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By 2020, 20% of the energy consumption in Europe should come from renewable energy sources. This is set as a mandatory target by the renewable Energy Directive of the European Union. Microalgae have the potential to serve as a renewable and sustainable energy source in the future. Microalgae, such as green algae and cyanobacteria generate biomass by photosynthesis. The biomass is a form of chemical energy which can be further converted into hydrogen, biodiesel or other forms of liquid or gaseous fuels. The production of energy with microalgae can be more sustainable than its alternatives concerning some important criteria: algae can be grown in closed systems on non-arable land, need CO2 as only carbon source and can also grow in salt or wastewater. However, up to now (based on lab and small pilot plant experiences) net energy generation from microalgae is negative and there is no functioning system of energy production with microalgae in use, yet.
Until now, microalgae have been used to produce high value chemicals, such as proteins or colorants for food and chemical industry. When algae should contribute to a sustainable energy supply, the production process should be energy-efficient as well as competitive concerning economic and ecologic criteria.
To account for this, progress in the development of bioreactors will have to ensure a positive energy balance. In addition to that, new concepts of energy production with microalgae are arising, integrating biomass and energy production into a larger system to achieve synergy effects and an overall positive net energy generation.
In this work, a first assessment of different processes of energy production with microalgae has been carried out with a detailed life cycle model. Within this model, different methods and process combinations can be compared to each other and to fossil based systems. Benefits and drawbacks of different systems can be discovered. LCA results will be used to support developers of a novel bioreactor and to give suggestions for further research.

WE 239
Life Cycle Assessment of the production of Succinic Acid: a contribution to consequentional LCA of biorefinery
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In recent years there has been increasing interest in the concept of biorefinery which denotes the idea of a future broad use of biomass substituting present day petrol-based chemical production. The core of chemical industry production of bulk and fine chemicals is only one area within concepts of biorefinery, but is of high interest because it is based on carbon chemistry and therefore will always be dependent from carbon feedstock. LCA on future biobased chemical production has to account for the fact, that - like in present day chemistry - also processes of "green"chemical production will be highly interconnected: therefore not simply one petrol-based product will be substituted by one biobased product, but major changes in production routes and their interlinkages will be introduced.
As an example for the comparison between petrol- and bio-based products an LCA of the production process of succinic acid has been carried out.
Regarding the wide range of possible applications, LCA of production processes for succinic acid seems interesting in it self, but also of high interest as a first step for a modular approach to Consequentional LCA of future biorefinery concepts.
Succinic acid is currently produced also from a petroleum-based process. However, it was found that bio-based versus petroleum-based succinic acid production is not an adequate comparison. Currently, petroleum-based maleic acid, instead of succinic acid, is used in industry as a building blockplatform chemical for the same products for which bio-based succinic acid will be used within the concept of biorefinery. As a functional unit 1000 kg was adopted also for maleic acid. One important conclusion is, that comparison of the same compound from biomass and from fossil resources may not be the approrioate approach. Instead, function within production chains of petrochemistry compared to future production chains of biomass has to be taken into account. This will lead to comprehensive picture of the interlinked processes of today's chemical industry compared to biorefinery as a basis for assessment of possible benefits from bio-based production as a whole. To elucidate this, further building blocks derived from sugar platform chemicals shall be investigated including assessment of the respective petroleum-based counterparts.

NM02 - Fate and effects of nanoparticles

WE 244
Research needs for nanosafety - activities of the German Federal Environment Agency
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The Federal Environment Agency (UBA) - for human and environment - is Germany's central federal authority on environmental matters. The aims of the UBA in terms of Nanotechnology are:
- to evaluate the chances and risks of nanotechnology,
- to further develop and/or adapt the existing strategies for testing and evaluating nanomaterials,
- to close gaps of information concerning risks of nanomaterials,
- to adapt the existing laws of the Chemical`s Act, and
- to strengthen national and international activities for coordinated risk analysis.
Therefore UBA detects and supports the need of research to evaluate the chances and risks of nanotechnology. For this aim it participates at the OECD Working Group for Manufactured Nanomaterials (WPMN) and coordinates the German Activities in the OECD Sponsorship

Programme for the safety testing of a representative set of manufactured nanomaterials. Within the Sponsorship Programme UBA is responsible for the testing of TiO2 nanomaterials and partly responsible for nano silver, as well as it contributes data to four additional nanomaterials.
At our poster we will present the aims and activities of the UBA in terms of chances and risks of nanotechnologies and will especially focus on currently running research projects.

WE 245

Malformations and toxicity mechanism of silver nanoparticles in the medaka fish embryo model

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Silver nanomaterials are one of the major components of healthcare products. This study explores the toxic mechanisms of silver nano-sized colloidal particles (AgNPs; average particle size 3.6 diameter nm, purity 99.99%, pure water solution) using the medaka fish embryo model. Three different developmental stages of medaka embryos (stage 11, blastula stage; stage 21, brain regionalization and otic vesicle formation; and stage 30, blood vessel development) were exposed to several concentrations of SNC. SNC produced morphological changes in the embryos such as blood clots, percardiovascular edema, tubular hearts, and spinal deformities at 0.5 and 1.0 mg/L. Reduced and delayed hatching were observed as well. Stage 21 embryos were most susceptible among all embryo stages tested. To determine the distribution of SNC medaka egg embryos were exposed to 0.5 mg/L for 6 days, and embryo, chorion and embryonic fluid were isolated and subjected to ICP-OES analyses. Silver was detected in a single medaka embryo and chorion at levels of 16.6 ± 9.3 pg and 720 ± 29 pg, respectively. Furthermore, TEM analyses showed SNC adhered onto the surface of the chorion and inside the chorion layer. To investigate oxidative mechanisms of injury, reactive oxygen species scavengers GSH (0.5 mM) and NAC (0.05 mM) were evaluated for their potential to rescue embryos exposed to 10 mg/L SNC. NAC rescued all embryos by 96 hr following treatment while GSH did not. NAC was able to block the process of lipid peroxidation by inducing a decrease in MDA levels, whereas SNC did not affect CAT and SOD activities. To elucidate AgNPs' effects on gene expressions, medaka embryos exposed from stage 21 for 48 hours were subjected to medaka embryo microarray. Two important genes relative to phenotypic development were influenced; HoxB6B gene was up-regulated and retinol-binding protein gene was down-regulated, respectively. Disruptions of those genes were well known to induce malformation in organisms. The two genes may have important roles to cause embryonic malformation through AgNPs exposure. Here, there has been a problem regarding AgNPs' toxic mode of action whether AgNP or Ag ion cause toxic effects. A new tool for Ag ions released from AgNPs is developed and medaka embryos are subjected to the assay to test toxicity of Ag ion only.

WE 246

Silver Nanoparticles effects on diatom Thalassiosira pseudonana and cyanobacterium Synechococcus sp.

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Engineered nanoparticles are used in a wide range of industrial applications including electronics and commercial products. Because of their large surface area and high reactivity, nanosized metal particles display remarkable physical, chemical and biological properties (Maynard, Aitken et al. 2006; Nowack and Bucheli 2007) .
Silver nanoparticles (AgNP) are one of the most frequently used nanomaterials due to their antibacterial properties and it is likely that they will be released in the aquatic environment (Navarro, Piccapietra et al. 2008) , therefore it is critical to assess the potential toxic effects of AgNP on the aquatic organisms. We studied the toxicity of AgNP towards two selected organisms, the marine diatoms Thalassiosira pseudonana and the freshwater cyanobacterium Synecocchocus sp., and link those effects to the size of nanoparticles.
Furthermore, we developed a procedure to measure the kinetic release of silver ions from nanoparticles in marine and fresh water system to simulate the habitat of the diatoms and cyanobacteria, respectively.
The poster will show the growth inhibition of diatoms and cyanobacteria upon exposure to three different size of AgNP. The analysis of silver ions release into their respective media suggest that the observed effects of toxicity are due to the silver nanoparticles.
References
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WE 247

Toxic effects of silver nanoparticles to natural freshwater microbial communities

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The toxicity of silver nanoparticles (AgNPs) to aquatic natural freshwater microbial communities (biofilms composed of algae and bacteria) was determined. Those organism groups might be especially at risk when exposed to AgNPs since silver is known to possess specific antibacterial properties. The aim of the presented study was to evaluate the toxic effect of two sizes of AgNPs to natural freshwater microbial communities and to compare the toxicity of AgNPs with the bulk material, AgNO3. The study is part of the Nanosphere research environment, in which one overall aim is to investigate whether conventional testing approaches are valid for assessing the impact of engineered nanoparticles on aquatic ecosystems or whether (and which) adaptations are necessary.
The biofilms were exposed in natural water to a concentration series of 10-1000 nmol/L AgNP10 (total silver content, 10 nm nominal particle size, no coating) and 5-1000 nmol/L AgNP40 (total silver content, 40 nm nominal particle size, citrate coating) for four days under semi-static conditions. The toxicity of $AgNO_3$ treatments with the corresponding total silver concentrations was analysed additionally. Effects on the algal community fraction of the biofilm were studied by HPLC-based pigment profiling: growth was measured as chlorophyll α content, species composition and interferences with specific physiological processes were estimated as changes in pigment patterns. The activity and composition of the bacterial community in the biofilm was analysed by metabolic profiling in so-called ecolog-plates. 1000nmol/L AgNP40 completely inhibited algae as well as bacteria. 100 nmol/L AgNP40 caused only 40% algal growth inhibition, the same concentration of $AgNO_3$, however, still caused a 90% effect. The uncoated 10nm AgNPs caused no measureable growth inhibition at concentrations up to 1000 nmol/L. All experiments were backed up by analytical measurements, measuring total silver content (ICP-MS), dissolved Ag+ (ICP-MS after ultrafiltration or centrifuge-aided membrane filtration) and particle size distribution (NanoSight).

WE 248

Assessment of silver toxicity to aquatic organisms: effect comparison of silver nanoparticles with ionic silver

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Silver nanoparticles (Ag NPs) are used in about one quarter of the commercialized products made from engineered nanomaterials. Due to the higher surface area per mass of silver, Ag NPs have an higher release capability than bulk silver metal. The main goal of this work is to present the comparison of the toxicity of Ag NPs and Ag^+ to *Pseudokirchneriella subcapitata* and evaluation of the toxicity of Ag NPs to *P. subcapitata* and *Daphnia magna*. Algae were exposed to Ag NPs or $AgNO_3$ in medium TM2 and allowed to grow during 72h at 25ºC.
The growth inhibition rate was determined according to OECD Guideline 201. Acute tests with D. magna followed OECD Guideline 202 (acute immobilization tests) and were carried out in ASTM hard water. Neonates were exposed for 48 h to 0-250 µg/L of Ag NPs (experiment without food) and 175-400 ug/L Ag NPs (experiment with food: $3x10^5$ cells/ml/daphnid). Feeding inhibition tests with D. magna were conducted with juvenile daphnids (4th instar) exposed to 175-800 µg/L Ag NPs in the presence of food ($3x10^5$ cells/ml/daphnia) and allowed to feed during 6h in the dark. Feeding rates were determined using standard procedures.
Ag NPs are toxic to algae and daphnids at low concentrations: 348 µg/L. Tests with algae show that AgNPs are less toxic than the ionic form in a mass basis. Altough growth curves of *Daphnia* exposed to NP's in water and in algae do not exhibit differences between treatments, the size-specific fecundity is affected suggesting that energetics of daphnids is affected resulting in changes of relative allocation of resources to growth and reproduction.

WE 249

Fate and effects of engineered silver nanoparticles in a terrestrial environment

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Silver nanoparticles (nano-Ag) are engineered nanoparticles with the highest number of commercial applications due to its anti-microbial activity. Many nano-Ag products may be directly applied to soil (e.g., pesticide applications) or disposed in traditional landfills, resulting in possible leaching into surrounding soil. There is a paucity of information regarding the environmental fate and effects of nano-Ag, as well as guidance concerning the disposal of engineered nanoparticles. Thus, the aim of this study was to study the leachability of nano-Ag in soil, as well as the potential effects on terrestrial invertebrates (the earthworm Eisenia fetida). Soil column studies were conducted with field soil spiked with nano-Ag, micron (µm)-sized Ag, and polyvinylpyrrolidone (PVP)-coated nano-Ag (35 nm, 1.5-2.5 µm, and 20 nm, respectively, at 0.01 and 100 mg/kg) as a function of particle size, coatings, and/or leachate solution pH. Leachate samples were collected every hour for 48 hours and were analyzed for total Ag by inductively coupled plasma-mass spectrometry. The analysis revealed that for 100 mg/kg spiked soils, nano-Ag, micron-sized Ag, and PVP nano-Ag were not mobilized through the soil when leached with ultrapure water, however, silver mobility did increase when leached with a 1% nitric acid solution. The nano-Ag soil leached less Ag when compared to micron-sized Ag and PVP nano-Ag soil, yet Ag was detected earlier in the leachate series when the soil was spiked with PVP nano-Ag. The use of artificial rain water (pH 4.2 for eastern U.S., pH 5 for western U.S.) resulted in no Ag detected in leachate from all three spiked soils. Behavioral tests using E. fetida demonstrated that the earthworms did not avoid soil containing micron-sized Ag (NOAEL > 100 mg/kg), but earthworms did significantly avoid soils containing nano-Ag in a dose-dependent manner (NOAEL = 10 mg/kg, LOAEL = 100 mg/kg). However, PVP-coating of nano-Ag resulted in no soil avoidance similar to micron-sized Ag. The present experiments indicated that it is unlikely that Ag from engineered nano-Ag will leach from a soil system except under extremely acidic conditions, resulting in an increased potential for exposure to terrestrial organisms (e.g., epigeic and endogeic earthworms). However, terrestrial invertebrates will begin to avoid nano-Ag laden soil at sublethal concentrations, resulting in a reduced risk to terrestrial fauna.

WE 250

Effects of titanium dioxide nanoparticles on aquatic invertebrates

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Photocatalytically active titanium dioxide nanoparticles ($nTiO_2$) are produced for large scale applications as component of desinfectants, self-cleaning glass, solar cells, electric devices, food additives, pharmaceuticals and cosmetic products. Due to its increasing use, it is most likely that $nTiO_2$ will enter the aquatic environment as a result of accidental spills during manufacturing, transport or its presence in waste and sewage.
Information on the ecotoxicity of nanoparticles in aquatic systems is rare. There are only few studies - by the majority on acute effects - on the toxicity of $nTiO_2$ using aquatic species as test organisms. Aquatic invertebrates are able to accumulate particles by means of water filtration and surface contact with the risk of consequent biomagnification along the food chain. *Daphnia magna* is a primary consumer in aquatic environments and an important food source for secondary consumers. Therefore we consider it important to understand biological responses of *D. magna* to $nTiO_2$.
In this study we investigated the impact of $nTiO_2$ (P25) with a nominal particle size of 21 nm in different concentrations on mortality and reproduction of *D. magna*. To analyse potential differences between different exposure routes, chronic reproduction tests were performed at two exposure systems: water and food. The size distribution and shape of P25 particles in aqueous media were assessed by dynamic light scattering (DLS) and scanning electron microscopy (SEM).
First results indicate that the chronic exposure of P25 to *D. magna* leads to an increased mortality and a reduced reproduction and growth. Feeding *D. magna* with exposed algae (*Scenedesmus obliquus*) even resulted in higher toxicity of $nTiO_2$ in comparison with the water exposure. Long-term exposure to P25 may affect populations of aquatic organisms and may accumulate along the food chain. The findings lead to the conclusion that food chain studies with *D. magna* appropriate to study potential environmental pathways of $nTiO_2$.

WE 251

Evaluation of TiO2 manufactured nanoparticles toxicity toward bacterial model in Seine freshwater

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Despite the increased production and use of engineered nanoparticles (NPs), environmental concerns still remain owing to the potential toxicity of these components on human health and natural environment.

In the framework of two actions, a regional research network "C'Nano - En Seine"and the international Gdr I'CEINT, we are studying ecotoxicological impact of different NPs. Our main objective is to gain understanding at the microscopic scale of the interaction between micro-organisms and manufactured NPs [1,2,3] with a focus on the use of natural freshwater as the medium of contact. In the present work, the effects of TiO_2-NPs toward *Escherichia coli* have been assessed in the freshwater from the Seine river (Paris). The two components (bacteria and NPs) have been considered on an equal footing of importance. First, a complete characterization of NPs morphological and physico-chemical state during the contact has been carried out using Small Angle X-ray Scattering, AFM, cryo-TEM, zeta and DLS. This allows the set up of reproducible and standardized experimental protocols of contact. Then, their cytotoxicity toward E.coli (impacts on morphology, metabolism or survival) has been evaluated using both traditional methods (optical density and CFU counting) and new reliable microplate techniques. In order to understand the toxicity mechanisms, the interaction between bacteria wall and NPs has been observed at the nano-scale thanks to accurate imagery techniques such as Transmission Electron Microscopy (TEM) or Atomic Force Microscopy (AFM).

The correlations between the state of dispersion of the NPs, their concentration, chemical nature and the toxicity will be presented.

WE 252

Impact of nano-TiO2 to marine organisms: Phaeodactylum tricornutum and Artemia franciscana

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Nano-TiO2 is employed in different categories of products for its antibacterial and depollutant properties (i.e. in air and water depuration systems, as road and pavement additive for NOx abatement or on self-cleaning surfaces) due to its ability to generate hydrossic radicals. Anyway, it is massively use (about 70%) in most common consumer products like cosmetics and sun-screens. Thus, it has been recognised that it enters directly or indirectly into the marine environment where salinity and ionic strength may act on the stability of nano-TiO2 dispersions. In particular, it may be affected the size of their aggregates, potentially speeding up nano-particles sedimentation processes. As a result nano-TiO2 bioavailability is continuously changed, making the analysis and interpretation of results really hard. The purpose of this study is to evaluate the in vivo toxicity of nano-TiO2 via the growth inhibition test with the unicellular green alga *Phaeodactylum tricornutum* and the anostracan crustacean *Artemia franciscana* following the relative standardised protocols. In the case of *A. franciscana* besides the traditional exposure conditions, two alternative scenarios were considered: "no light, but feeding"to observe potential photocatalytic effects and "no feeding, but light"to verify effects on starvation. Results evidenced an EC50 in the range 25-64 mg L-1 to *P. tricornutum*, while toxic effects were observed to A. franciscana after 24 h between 25-67 mg L-1. The scenario "no light, but feeding", highlighted that the absence of light reduced the nano-TiO2 toxicity, leading to the observation of very slight toxic effects after 24-96 h exposure (maximum affect at 96 h = 47% at 16 mg L-1). The scenario "no feeding, but light"showed increased toxicity level at all exposure times, in particular the maximum effect was identified after 96 h.

WE 253

The effect factor for nano TiO2: preliminary toxicity tests on Daphnia magna and future developments

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Nanotechnologies are being developed for application in a large variety of sectors, from food industry to environmental remediation technology. However, numerous uncertainties exist regarding their possible impacts on the environment and human health. The Life Cycle Assessment (LCA) methodology could be a tool to evaluate, analyze and manage the environmental and health effects for the emerging technologies. The evaluation of the environmental performance of nanotechnologies trough LCA is not always possible due to the lack of data regarding the environmental pathway of NPs. The focus of the present research is to evaluate the freshwater ecotoxicological Effect Factor (EF) for TiO2 nanoparticles (which are widely used in different applications as sunscreens, solar cells, ecc.), following the framework of the USEtox model. This model is used for the Life Cycle Impact Assessment (LCIA) phase and is aimed to provide the Characterization Factor (CF) for the ecotoxicity impact category. The CF is quantified trough the evaluation of the Fate Factor (FF) and Effect Factor (EF). The latter is based on chronic or acute toxicity value as EC50. In order to establish the acute EC50 for nano TiO2, two preliminary toxicity tests on Daphnia magna (72h) have been performed. The particles were prepared following two different treatments. In the first case the nano TiO2 suspensions were stirred for 24 h only before the test; in the second case the particles were maintained in agitation also for the whole duration of the bioassay. No adverse effects have been observed for the concentration applied, this contrasts with the findings of other studies that reported effects of nano TiO2 on D. magna. The future developments of our research are to conduct other toxicity tests on D. magna, following different treatments of the sample (example: irradiation with UV-A, filtration etc), and to conduct toxicity test on different trophic level (algae, crustaceans, fish) as suggested by USEtox framework. The research is intended to identify suitable treatments for nanoparticles to be used in toxicity test, taking into account their tendency to aggregate and precipitate and that this could influence the results of the tests. The suitable treatment should also reproduce the actual exposure modality of the organisms in the field.

WE 254

Environmental fate of aquatic nanoparticles: case study TiO2 NP

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Titanium dioxide nanoparticles were synthesized using a wet-chemical method based on the hydrolysis of TiCl4. Stable suspensions of positively-charged TiO2 were obtained at pH 2.5. One batch of this product was quickly shifted above of the point of zero charge to pH 12. These stock dispersions were used in batch adsorption experiments with three different types of well characterized macromolecules, Sodium alginate, Fulvic acid and Humic acid, and evaluated in terms of final surface charge (Zeta potential), final size and adsorption efficiencies.

The stability of nanoparticle dispersions with and without sorbed molecules was investigated by monitoring the aggregation behaviour of TiO2 nanoparticles in different electrolyte environments (NaCl, CaCl2 and Na2SO4) and at varying pH. these aggregation rates were used to determine the attachement efficiencies as a function of pH, counterion valence and macromolecule concentration. These attachement efficiencies can be related to more complex mathematical models in order to evaluate and predict the effects of environmental macromolecules on the fate of aquatic nanoparticles.

WE 255

Interactions between TiO2 nanoparticles and cadmium: consequences for uptake and ecotoxicity

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As a consequence of the increasing use of engineered nanoparticles in e.g. industrial applications and consumer products, these particles will be released into the aquatic environment. They will be present in the water phase where they are likely to form aggregates/agglomerates. Through sedimentation, the sediments are expected to be a sink for nanoparticles. Both in the water phase and in sediments they will mix and interact with other environmental pollutants, including heavy metals. In this study the toxicity of cadmium to three relevant freshwater species, green algae Pseudokirchneriella subcapitata, crustacean Daphnia magna and sediment organism Lumbriculus variegatus, was investigated both in the absence and presence of TiO2 nanoparticles. Also uptake of cadmium in D. magna and L. variegatus was investigated in tests where organisms were exposed to cadmium in sublethal concentrations in the absence and presence of 2mg/L TiO2 nanoparticles (P25 Evonic, d: 30 nm). Mass balances for cadmium in the test systems were determined. A high degree of sorption of cadmium onto TiO2 particles was found, which makes TiO2 nanoparticles potential carriers for cadmium. The observed toxicity was higher than expected based on water phase cadmium concentrations, and results shows that adsorbed fraction of cadmium is bioavailable.

WE 256

Fate and behaviour of three different TiO2 nanomaterials in soils

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Titanium dioxide nanomaterials are commonly used in many everyday life products (e.g. paints, cosmetics). First studies indicated that nanomaterials can be released from products during their life cycle (Kaegi et al. 2008) and can reach the environment.

In the presented study laboratory experiments were performed to investigate the fate and behaviour of different (functionalised and non-functionalised) TiO2 materials (P25, PC105, UVTitanM262) in soil compartments. More specifically adsorption / desorption tests and the mobility studies in soil columns in a laboratory scale were conducted (according to OECD Guidelines No 312 and 106). Different soil types were used to identify and assess the effect of different soil characteristics like clay content, pH-value, organic matter, grain size or cation exchange capacity on the adsorption and mobility behaviour.

Preliminary results showed no mobility of the titanium dioxide nanomaterial in soil columns, if dry powder of the titanium dioxide material were applied to the soil before percolation with water. Therefore titanium dioxide powders suspended in water containing smaller agglomerates (compared to the powder) were used in the following investigations, for which a higher mobility of TiO2 was expected. A Standard Operating Procedure (SOP) for maintaining stable nanomaterial suspensions with respect to a constant particle size distribution and zeta potential for a minimum of 24h, essential for such experiments, is currently evaluated in a round-robin test. Detailed results on the mobility of nanomaterials in soils under such conditions will be presented and discussed.

This study was sponsored by the Federal Environment Agency (UBA) within the framework of the "Umweltforschungsplan" - FKZ 3709 65 417: " Environmental risk of nanomaterials: Environmental fate of selected nanomaterials in relation to shape, size and surface properties ".

References:

Fang, J. et al. (2009), Environmental Pollution 157(4), 1101-1109.
Kaegi, R. et al. (2008), Environmental Pollution 156(2), 233-239.

WE 257

Behaviour of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303A

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Titanium dioxides are used in huge amounts as white pigment or UV-filter in consumer products such as paints, paper coatings, plastics, and sunscreens. Industrial applications include its use in self-cleaning coatings as well as a photocatalyst in advanced wastewater treatment. The use of nanoscale TiO_2 is predicted to increase in the near future. While the clearance efficiency of microscale TiO_2 (> 0.7 µm) in sewage treatment plants (STP) has been determined to be in the order of 70-85% (Kiser et al. 2009), no data on the behaviour of nanoscale TiO_2 in STP are available so far.

In the presented study the fate and behaviour of TiO_2 nanomaterials (P25 with an average

particle size in suspension < 250 nm) in the laboratory sewage treatment plants simulation test (LSTP) according to OECD Guideline 303A has been investigated. The nanoscale suspensions were stabilised with sodium metaphosphate (SMP) and added continuously in a ten-fold concentrated suspension to reach a final concentration of 1, 5, and 10 mg L^{-1} TiO_2 and 1 g L^{-1} SMP. The organic synthetic wastewater (final concentration 50 mg L^{-1} DOC) was added as a concentrate to matrix of synthetic drinking water. After one week acclimatisation the test was run for one week per concentration. Sampling for TiO2 measurement was carried out daily in the outflow and in the surplus sludge for enabling a TiO_2 balance.
The biodegradation and nitrification of the synthetic wastewater in the test LSTW was comparable to that of the reference LSTP (DOC-Elimination 98%-100%, NH_4-N < 0.2 mg L^{-1}).
In the outflow of the test LSTP an increase of suspended solids (activated sludge flocs) was observed which was explained with the use of the dispersant SMP. Preliminary results of the TiO_2 measurements indicate that more than 90% of the nanoscale TiO_2 introduced was bound to the activated sludge.
This study was sponsored by the Federal Environment Agency (UBA) within the framework of the "Umweltforschungsplan" - FKZ 3709 65 417: " Environmental risk of nanomaterials: Environmental fate of selected nanomaterials in relation to shape, size and surface properties.
Reference
Kiser, M. A. et al. 2009. Nanomaterial Removal and Release from Wastewater Treatment Plants. Environ. Sci. Technol., 43 (17), pp 6757-6763

WE 258

Ecotoxicity of photocatalytically active titanium dioxide nanoparticles: the impact of UV-exposure on toxicity to Caenorhabditis elegans

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Titanium dioxide nanoparticles (nTiO2) are used in a variety of consumer products like cosmetics, paints and self-cleaning surfaces. The detection of nTiO2 in facade runoff and surface waters nearby indicates that at least part of the nTiO2 is released into the aquatic environment (Kaegi et al. 2008). With the number of applications still rising, it has been suggested, that also the flux to the environment will increase significantly within the next years. The knowledge concerning the ecotoxicological relevance of TiO2, however, is still very fragmentary and insufficient for reliable risk analysis.
In this study we will try to elucidate the impact of light exposure on the toxicity of TiO2 doted sediment in the presence of photosensitive contaminants.
As test organisms, the nematode *Caenorhabditis elegans*, a multicellular model organism living in soil was chosen because of its ecological relevance. Within the range of TiO2-nanoparticles, P25, a photocatalytically active titanium dioxide with a nominal particle size of 21 nm was applied. In nematode testing without light exposure, P25 only showed a significant effect at relatively high concentrations (35 % reduction in progeny at 100 mg nTiO2/L). As nTiO2 is a photocatalyst and induces the formation of reactive oxygen species in cells when exposed to UV irradiation we are currently testing the following hypotheses:
a) (Prolonged) irradiation leads to elevated toxicities of nTiO2 and indicates an elevated risk for those ecosystems exposed to both, sunlight and nTiO2 at the same time.
b) As a photocatalyst, TiO2 influences the toxicity of co-contaminants via photo modification or photosensitization processes.
First results of the ongoing exposure experiments will be presented in this poster.
Kaegi et al. 2008. Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. Environmental Pollution 156(2), 233-239.

WE 259

CdSe quantum dots in seawater: chemical stability and effects on a marine microalga

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QDs are nanocrystals with a heavy metal-containing reactive core, exhibiting unique optical properties, which make them one great promise for biomedical and microelectronic technologies. Because of their increasing use, it is expected that they end up in natural water bodies, from freshwater to the sea. Lack of knowledge about the stability and bioavailability of these newly synthesized particles in the aquatic systems represents a novel problem for the environment. Only very recently, some papers report data on the transformations of QDs in aquatic environments and toxicity in living organisms. The few studies regard freshwater systems and so far, to our knowledge, no study has been carried out in seawater. In the present work we report preliminary results on: 1) the characterization of the chemical behaviour of QDs in seawater; 2) the uptake and response of the marine diatom Phaeodactylum tricornutum to QD exposure. To this purpose, CdSe QDs (Sigma) were solubilised in water by encapsulating with the amphiphilic polymer PSMA (poly(styrene-co-maleic anhydride)) and ethanolamine (Lees et al., 2009. ACSNano 3: 1121-1128). After dispersion in natural seawater, the optical properties were followed as a function of time and salinity. Absorption spectra of water-soluble QDs exhibited a steady increase in absorbance as the wavelength decreased from 600 nm towards the UV-region, with a characteristic peak at 540 nm. The time course of the absorption spectra after re-suspension in seawater exhibited a progressive flatting of the peak at 540 nm, suggesting a partial degradation of the nanoparticles. This effect decreased at lower salinity. The interactions between the microalga and QDs were tested by measuring: 1) the cellular concentration of Cd, 2) the synthesis of phytochelatins (PCs) as a biomarker of the bioavailability of heavy metals, 3) the activity of antioxidant enzymes as an indication of oxidative stress. Short-term experiments (5 h) showed that P. tricornutum responded to QDs exposure by synthesizing PCs and by increasing the intracellular pool of glutathione. The presence of PCs suggests the occurring of bioavailable Cd species, derived from a partial degradation of QDs. Since glutathione is a main cellular antioxidant, the concomitant increase of glutathione seem to suggest the occurring of oxidative stress. Experiments are in progress to assess the enzymatic antioxidant response of P. tricornutum.

WE 260

Stability and uptake by daphnia of QDs with three different coatings in synthetic fresh water

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CdSe/ZnS quantum dot (QD) is widely used for biomedical purpose as a diagnostic tool. Its exposure into environment is being a threat against the ecosystem by releasing toxic metals such as Cd and Se as well as nanoparticle itself. CdSe/ZnS coated with three different polymers (amphiphilic polymer, amphiphilic polymer/PEG, and amphiphilic polymer/PEI) were purchased and introduced into synthetic surface water (hard, moderately hard, and soft water). Optical properties were measured with time and sedimentation was observed with the change of Cd concentration at the upper layer of suspension by ICP-AES. Different coatings had significant role in the sedimentation. PEG coated QDs which had neutral surface charge significantly sedimented in all synthetic waters and loosed optical property measured by fluorescence. Highly positive charge on PEI QDs remained stable for 48h. Interestingly, fluorescence of PEI QDs significantly increased with time in all three synthetic waters. All QDs did not release Cd and Zn for 48 h. Daphnia uptakes and depurations were monitored in moderately hard water by measuring Cd concentrations in daphnia. Three QDs were well accumulated into daphnia for 24 h. During uptake, dissolved Cd concentrations increased significantly implying in-vivo dissolution of QD by daphnia. QDs with neutral and negative charge at surface depurated quickly from daphnia while significant amount of positively charged QDs remained in daphnia after 48 h depuration. The study concluded that surface coatings played significant role in bioaccumulation by daphnia as well as stability in fresh water.

WE 261

Effects of nano sized CuO and ionic Cu: Toxicity to Daphnia magna and tight epithelial cells

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Nanoparticles (NPs) have unique chemical and physical properties caused by their small size and high surface area to volume ratio. This means that the NPs can be more reactive and potentially more toxic than their bulk counterparts. This study focuses on toxicity of two CuO NPs; monodispersed spherical 7 nm and highly polydispersed polyhedral 10-100 nm, respectively, compared to copper ions (Cu2+) to the crustacean Daphnia magna and epithelial kidney cells from the aquatic toad Xenopus laevis (A6 cell line) as a model for tight ion-transporting epithelia. D. magna was exposed to both CuO NPs and Cu2+ for 48 h according to OECD Guideline 202. In addition, epithelial cells (dividing and differentiated cells) were also exposed to CuO NPs and Cu2+ in an in vitro study for up to 7 days. The study demonstrated similarity in the toxicity of CuO NPs (7 nm) and Cu2+ to D. magna, whereas toxicity of polydispersed CuO NPs occurred at much higher concentrations. For epithelial cells, exposure to both sizes of CuO NPs and Cu2+ caused significant increases in cell death (most likely via apoptosis), and morphological changes, but after different times of exposure. For cells exposed to polydispersed CuO NPs the increase in apoptotic bodies occurred within 48 h of exposure, whereas the effects of both CuO NPs (7 nm) and Cu2+ occurred much later (after 3 and 5 days of exposure, respectively). The mechanisms by which the copper forms exhibit toxic effects differ such that Cu2+ and CuO NPs (7 nm) decrease cell proliferation and influence cell cycle progression, whereas polydispersed CuO NPs cause the formation of apoptotic bodies almost instantaneously. Furthermore, the effects of the different forms of copper on cells seem to depend on cell cycle stage. The implications from the study are that the toxic effects of copper depend on the form and size of the metal oxide particles.

WE 262

Copper oxide nanoparticles induce oxidative stress, DNA strand breaks and laccase activity in aquatic fungi

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The extensive use of nano metal-based products increases the chance of their release into aquatic environments and may put biota and associated ecological processes at risk. Fungi play a key role in organic matter turnover in freshwaters while they transfer carbon and energy to higher trophic levels. Although aquatic fungi are common in clean and well-aerated freshwaters, they also occur at hyperpolluted sites. We investigated the effects of nano CuO (4 levels, ≤ 100 mg L^{-1}) on fungal biomass production, extracellular laccase activity, accumulation of reactive oxygen species (ROS), plasma membrane integrity and occurrence of DNA strand breaks, after 5 and 12 days, in 4 fungal isolates: *Phoma* sp. UHH 5-1-03 (Ph) and *Articulospora tetracladia* UMB-072.01 (At72) collected at clean sites, and *A. tetracladia* UMB-061.01 (At61) and *Heliscus lugdunensis* H-4-2-4 (H4) collected at polluted sites. Fungal biomass was inhibited by the increase in nano CuO concentration and incubation time. After 12 days, the strongest inhibition in biomass production was found in At72 (95.5%) and Ph (81.2%) exposed to 100 mg L^{-1} nano CuO, while the lowest effects were observed in At61 (59.4%). Laccase activity was induced by all nano CuO concentrations after 12 days, except in At61; maximum activities were found in Ph followed by At72 and H4 (2823, 474 and 166 U g^{-1}, respectively) when exposed to 100 mg L^{-1} nano CuO. ROS accumulation increased with increasing nano CuO concentration at both times in all fungi. After 12 days, severe plasma membrane disruption was found in At72 exposed to all nano CuO concentrations and in Ph exposed to 100 mg L^{-1} nano CuO. Severe DNA strand breaks were observed in At72 and Ph exposed longer to 100 mg L^{-1} of nano CuO. The increase in nano CuO concentration and exposure time led to an increase in DNA strand breaks, which is a signature of an apoptotic process. Overall results suggest that nano CuO is toxic to aquatic fungi, particularly to those isolated from clean streams.
Acknowledgements: This work was supported by the DAAD-FCT project (Micro)analysis of nanoparticles on aquatic fungi and A. Pradhan received the FCT grant SFRH/BD/45614/2008.

WE 263

Effects of copper on Enchytraeus crypticus (Oligochatea): comparison of exposure to Cu-NP, Cu-salt and historical contamination, including in depth characterisation

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The aim of this study was to compare the effects on Enchytraeids of copper-nanoparticles (Cu-NP) laboratory spiked soil, with copper-salt (Cu(NO3)2) laboratory spiked soil, and with soil contaminated in the field. Enchytraeids are small soil inhabiting oligochaetes, which actively contribute to the acceleration of organic matter decomposition and nutrient recycling processes. In the study we used a broad series of different characterization techniques including both direct and indirect measures of nanomaterials, including possibly presence of ions in the soils pore

water. The measures were attempted to link with the survival and reproduction of Enchytraeus crypticus. Results showed clear reproductive effect of all forms of Cu, the effects were more pronounced by exposure to the Cu-salt form, than the Cu-NP, and with field soils causing least effects. The physical chemical characterization displayed differences between all exposures regimes, although free ions measures were not able to explain differences in toxicity. The physical chemical characterization could to some extend explain toxicity observed.

WE 264

Nano copper oxide is a threat to an endemic shredder of the Iberian Peninsula

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The increased usage of nano metal-based products enhances the possibility of its release into freshwater ecosystems and this may put aquatic biota and ecological processes at risk. Invertebrate shredders mostly use leaf litter as a food resource and play a key role in the energy and nutrient transfer in aquatic detritus foodwebs. In this study, we assessed the sublethal toxicity of nano CuO on the growth and feeding behaviour of larvae of the shredder *Allogamus ligonifer* (Thricoptera, Limnepilidae), an endemic species in streams of the Iberian Peninsula. The experiment was carried out in glass containers with sterile stream water, each housing a larva (15 replicates). The invertebrates were allowed to feed for 10 days on microbially colonized alder leaves according to the following treatments: (1) leaves previously treated for 5 days with 25 or 75 mg L^{-1} nano CuO and stream water, (2) untreated leaves and stream water with 25 or 75 mg L^{-1} nano CuO, and (3) untreated leaves and untreated stream water (control). The experiment was replicated in the absence of invertebrates to discriminate the contribution of microbes to leaf litter decomposition. Microbes contributed with 23% to total leaf mass loss in control, and microbial decomposition decreased with the increase of nano CuO concentration. Leaf consumption rate by the invertebrate was 0.27 mg leaf DM day^{-1} mg^{-1} animal DM. The highest inhibition of leaf consumption (48 %) was found in the treatment in which the animals were exposed to water with 75 mg L^{-1} nano CuO, followed by the treatment in which the animals were fed on leaves previously exposed to 75 mg L^{-1} nano CuO (26%). A similar response pattern was found for the growth rate of invertebrates when exposed to nano CuO via stream water or leaves (56 in control vs 30 and 41 µg individual DM mg^{-1} individual DM day^{-1} in treatments). Results will be interpreted based on the adsorption and accumulation of nano and/or ionic copper in invertebrates (body and case) and leaves.

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WE 265

ZnO nanoparticles in soil: testing different spiking procedures

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Due to complex behaviour of nanoparticles in soil, realising realistic exposure in ecotoxicity testing poses major challenges. Soil by definition is heterogeneous, which requires conscientious introduction of any test compound and oftentimes sub-samples are analyzed to ensure a homogeneous distribution of the compound. In general, manufactured nanoparticles are insoluble in water, making it a difficult task to obtain homogeneity of nanoparticle distribution in spiked soil. Depending on their physical and chemical properties as well as soil properties, nanoparticles tend to form aggregates and are likely to settle within a relatively short time. The present study investigates the spiking of natural soil with two sizes of zinc oxide particles (30 and 200 nm) using two different spiking procedures, namely as dry powder and as suspension in soil extract.
Five samples per treatment were randomly taken from the batches of spiked soil and analysed for total zinc concentration by flame Atomic Absorption Spectrometry. Scanning electron microscopy (SEM) was applied in an attempt to visualize zinc oxide nanoparticles in our test soil. SEM did not allow to see whether particles were present as such or as aggregates or agglomerates.
Both spiking procedures show a good recovery (> 85%) of the metal zinc and based on total zinc concentrations no difference was found between the two spiking methods. Both spiking procedures resulted in a fairly homogeneous distribution of the zinc oxide nanoparticles in soil, as evidenced by the low variation between replicate samples (<10% in most cases). We conclude that spiking with dry powder or suspension does not influence zinc distribution in the soil.

WE 266

Evaluation of the bioavailability of nanosized and bulk ZnO in soils and solutions using two recombinant sensor bacteria

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The environmental hazard of metal-containing nanoparticles (NPs) in soils depends to a large extent on their bioavailability. The approach used in this study enables the determination of bioavailable fractions of metals from metal-containing NPs in a combined approach involving chemical analysis and recombinant metal-specific microbial sensors. Bioavailability of Zn was evaluated in aqueous solutions and soil suspensions spiked with nano ZnO, bulk ZnO and $ZnCl_2$ and compared with the bioavailability of Zn in the soil pore water from freshly spiked and 3 months aged soils using the recombinant zinc sensor bacteria *Pseudomonas fluorescens* (OS8::KnzntRPzntAlux) and *Escherichia coli* MC1061 (pSLzntR/pDNPzntAlux). The soil used was Lufa 2.2. standard soil. Bulk ZnO and the respective soluble salt ($ZnCl_2$) were used as controls for size-dependent and solubility effects. Response of the sensor bacteria to bioavailable Zn was detected by an increase in luminescence and expressed as increased induction (folds) of luminescence compared to the background values. Zn^{2+} from $ZnCl_2$ was considered 100% bioavailable and used as standard. Luminescent control strains *P. fluorescens* (OS8::Knlux) and *E. coli* MC1061 (pDNlux), not induced by zinc, were used to correct for potential reduction in luminescence by turbidity/color of samples. Zn concentrations in all solutions were measured by atomic absorption spectroscopy.
At Zn concentrations up to 10 mg l^{-1}, ZnO (nano and bulk) was fully dissolved and bioavailable in Milli Q water. However, when spiked to soil extracts, only 30 % and 15 % of Zn proved bioavailable for bulk ZnO and ZnO NP, respectively. Chemical analysis showed that both nano and bulk ZnO yielded similar dissolved Zn concentrations with only 1 to 5 % of the total Zn extracted from the soil into the soil pore water. Higher Zn concentrations were measured in soil pore water after 3 months ageing under laboratory conditions. The bioavailability of ZnO (bulk and nano) measured with the biosensor bacteria, ranged from 1% to 5% in freshly spiked soils and 5% and 11% in aged soils.

WE 267

Comparative investigation of zinc oxide nanoparticles and ionic zinc toxicity towards soil organisms

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The huge advances of nanotechnologies pose an urgent need in the development of analytical tools to correctly assess the related potential risks for the environment and human health. A high priority is given to the toxicity assessment of ZnO nanoparticles (NPs) which potential toxicological impact is still largely unexplored with the exception of freshwater systems and some studies on humans [1,2,3]. In most of these cases, soluble Zn^{2+} from ZnO seems to be the main source for the (eco)toxicity [1]. In comparison to freshwater, much less data were reported on the NPs effects in soils although the soil solid phase is a primary, large sink for any human waste.
In this work the impact of ZnO NPs on terrestrial organisms was comparatively investigated with the one produced by the same amount of ionic zinc, together with the evaluation of the contribution of the soluble fraction of ZnO NPs, as Zn^{2+}. At this aim, a battery of toxicity contact tests with different organisms: *L. sativum*, *H. incongruens*, *F. candida* and a genotoxicity test with *V. faba* were performed. The OECD soil samples spiked with ZnO NPs and $ZnCl_2$ were extensively characterized to verify the actual dimension of the dispersed nanoparticles and the oxide solubility.
An overall higher toxic effect of ZnO NPs in its insoluble, nanostructured form with respect to the one exerted by the same amount of ionic zinc was observed. The ostracods were the most sensitive organisms showing a clear link to stress/stimuli caused by the peculiar characteristics of the nanostate. *L. sativum* was also mainly affected by ZnO NPs, while collembolan reproduction test produced similar results for both the zinc forms. Slight genotoxic effects with *V. faba* micronucleus test were observed with both soils.

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WE 268

Do nanoparticles create super-stress for all growth stages in isopods?

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In a field situation, populations of organisms exposed to a stressor generally consist of organisms representing large ranges of age, growth or moulting stage and gender ratio. To reduce variation in responses, laboratory toxicity tests usually use age-synchronized animals of pre-determined age, size and gender ratio.
Over the last years several ecotoxicity studies have been focused on the comparison of several endpoints (e.g. survival, reproduction) when organisms are exposed to stressors, but no information has been presented considering effects within different life stages and related to life-stage specific sensitivities or detoxification capacities.
In this study, terrestrial isopods (Porcellionides pruinosus) from three life stages (juveniles, pre-adults and adults) were exposed during a 7-day period to bulk zinc oxide (ZnO) and nano zinc oxide (nano- ZnO) particles using a concentration below the EC50 for all stages.
Biomarkers (e.g. AChE, LPO, GST, CAT, etc.), energy reserves content (lipids, carbohydrates and proteins), energy consumption and cellular energy allocation (CEA) were determined after exposure. Results showed a correlation between responses and life stages. As expected, oxidative stress was observed upon exposure to both bulk ZnO and nano-ZnO and the physiological adaptation to these stressors was not equal for all stages. Biomarkers did not respond or evolve in the same way when considering the different age ranges.

WE 269

Comparing ecotoxicity of nano and bulk forms of CeO2

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The development and use of engineered nanomaterials (NMs) is increasing at an ever accelerating rate even if little is known about their likely inputs, fate, behaviour and effects in the environment. Moreover, concerns are being expressed whether NMs would not pose a serious environmental threat.
Ecotoxicological data concerning nanomaterials are also generally lacking even if the investigation of the environmental effects on NMs is of utmost importance, especially in the aquatic environment since it will ultimately receive the run-off and wastewater from domestic and industrial sources and has been targeted for some nano-scale environmental remediation techniques. Already published ecotoxicity studies have shown that in many cases, NMs may pose an increased health and environmental risk if compared to bulk forms of the same substances.
The aim of this work was to evaluate acute aquatic toxicity of CeO_2, in both nano and bulk forms. The nano CeO_2 is being used in ceramics, optics, as a cleansing agent and has been tested as a gasoline additive to enhance combustion. We have tested several types of nano CeO_2 that differed in particle size and method of preparation. The toxicity was evaluated using a soil worm *Enchytraeus crypticus*. The tests confirmed differences in toxicity between the nano and bulk form and between the different forms of nano CeO_2.

WE 270

On the controversial biological response of a model aquatic organism exposed to single walled-carbon nanotubes

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This study investigates the biological response of P. subcapitata exposed to single-walled carbon nanotubes (SWNTs). The effects of SWNTs and surfactant types and concentrations on P. subcapitata were determined. Changes in algal biomass and cell morphology associated with specific SWNT-treatments and types were monitored, and the mechanisms of the different biological responses investigated through a combination of biochemical and spectroscopic methods. Results show either growth inhibition or stimulation depending on the type of surfactant used, and the speciation of SWNTs into metallic versus semi-conducting fractions. Trends of glutathione concentrations determined in culture media and the presence of high levels iron-based impurities in SWNTs used suggested the presence of reactive oxygen species and the resulting induced

oxidative stress as main cause of observed growth inhibition. TEM observations of control and treated algal cells and the determination of SWNT aggregation states in culture media through spectroscopic techniques were used to investigate other potential toxicity mechanisms

WE 271

Assessment of the toxicity of organic and inorganic nanomaterials on two species of freshwater green algae

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Nanotechnology refers to the manipulation and study of structures and devices with size scales in the range of 1-100 nanometers, in at least one dimension. The research of nanotechnology has grown exponentially in the last decades, contributing to the release of these newly produced materials to the environment, namely to the aquatic ecosystems. Algae are essential in aquatic ecosystems since they are the first trophic level in the food chains and the group providing oxygen and organic substances to other forms of life, guaranteeing the sustainability of freshwater ecosystems. Accordingly, this study aimed at investigating the effects of aqueous media contaminated with seven nanomaterials (NM) (vesicles of sodium dodecylsulfate-dodecyldimethylammonium bromide SDS/DDAB, vesicles of monoline/sodium oleate Mo/NaO, Fe/Co, nanogold rods, quatum dot LumidotTM CdSe/ZnS 530, TiO2, and TiSiO4) in the growth rate of two species of green algae: Pseudokirchneriella subcapitata and Chlorella vulgaris. The toxicity of NM contaminated media for these two species of green algae was studied by conducting 72-h growth inhibition tests. Each species was exposed to a series of ten dilutions of each NM-contaminated media. The zeta potential, hydrodynamic diameter and surface charge of the NM-suspensions were characterized using the methodology of dynamic light scattering. The obtained results showed that SDS/DDAB, gold nanorods and quantum dots significantly inhibited the growth of freshwater green alga . These results emphasize the importance of systematic studies of nanoecotoxicological effects of different nanoparticles.

RA03 - Developments in assessing the bioconcentration of chemicals and its ecological relevance

WE 274

Bioaccumulation in fish - revision of the OECD-testguideline

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Bioaccumulation is a key element within the environmental hazard and risk assessment of substances. The OECD test guideline 305 addressing determination of bioconcentration is currently under revision within the OECD test guideline programme.
The test measures a chemical's bioconcentration factor (BCF; the ratio of the concentration in the fish to the dissolved concentration in the water) in fish ;by exposing fish to the chemical dissolved in the water. This particular method is used in regulations for industrial chemicals, plant protection products, biocides and pharmaceuticals, for risk assessment, Classification and Labelling and in PBT assessment.
The guideline will be expanded with a new possible method, exposing test animals to food spiked with the test substance, which results in a biomagnification factor (the ratio of the substance in the animal's body to the concentration in its food) rather than a BCF. Validation testing needs to be conducted to demonstrate reproducibility of results and provide information on interlaboratory variation for this new method.
A number of laboratories have volunteered to conduct studies for this validation exercise, using rainbow trout and carp. Five test substances will be tested in each study, with the substances being concurrently "spiked"to fish food on which the test fish are fed daily during a set exposure period. This exposure period is followed by a depuration phase, in which the fish are fed a clean diet for a set period. Chemical analysis of fish tissue during the depuration phase, coupled with set test substance concentrations in the food and feeding rate, and known fish weights, and fish and food lipid contents allows the calculation, for each test substance, of:

- a depuration rate constant
- the assimilation efficiency
- the growth rate constant
- the biomagnification factor

It is also possible to produce a kinetic BCF (range) from the data, if an uptake rate constant can be estimated.
In the poster, the envisaged changes and first ringtest results will be described.

WE 275

Saving test animals, time, and costs in fish bioconcentration tests

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The OECD 305 protocol for determining the bioconcentration factor (BCF) in fish is long, costly, and test animal intensive. Commonly an uptake phase of 28 days is followed by a depuration phase of 56 days, and a minimum of 40 fish are required. In an effort to reduce the costs and test animal requirements, a revised protocol was tested. The key elements of the revised protocol included:

- Shortened uptake and elimination phases (4/10 days)
- Reduced number of fish (8)
- Simultaneous study of several chemicals
- Use of internal benchmarking to increase accuracy and precision

The revised protocol and the OECD 305 protocol were run for 10 chemicals using rainbow trout. Good agreement was found between the BCFs measured with the two methods. This supports the feasibility of reducing the cost and animal requirements of fish bioconcentration experiments. The revised protocol should be particularly useful in assessing whether chemicals lie above or below regulatory thresholds, as the internal benchmarking facilitates the determination of relative bioaccumulation behaviour.

WE 276

A partition based dosing method for fish bioconcentration experiments

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Creating and maintaining a constant freely dissolved concentration of test chemicals in water is a requirement for laboratory bioconcentration experiments. This can be difficult, particularly for poorly soluble chemicals. Generator columns have been used, but they are limited to dosing at the aqueous solubility limit. We developed a new partition based dosing method using a silicone membrane module consisting of 15 000 silicone microtubes through which water is pumped. The silicone was loaded with the chemical by pumping a methanol solution into the tubes and stepwise displacing the methanol with water. Water was then pumped through the tubes into an aquarium. The system was tested using a mixture of 10 organic chemicals with log KOW ranging from 3.95 to 6.60. The concentrations in the aquarium water were measured using both solid phase extraction and passive sampling. The concentrations of most chemicals were maintained within _10 % over a 4 week period. A decline in concentration of some of the low KOW chemicals was observed. The concentrations in the water could be easily controlled by regulating the water flow through the passive dosing module. The reproducibility of the water concentrations obtained using different passive dosing modules was also investigated.

WE 277

Comparison of in-tissue passive sampling and whole fish extraction to measure elimination kinetics of organic chemicals in fish

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Fish bioconcentration and bioaccumulation tests such as OECD 305 measure the change in chemical concentration in the fish over time, i.e. during chemical uptake and depuration experiments. This requires that fish be sacrificed at a number of time points during the experiments, and it means that the bioaccumulation behaviour is not being measured in individual fish, but rather using a collection of data points, each from a different fish. In an effort to reduce the animal requirements for these tests and to enable the study of bioaccumulation kinetics in individual organisms, a passive sampling method was developed that could be employed in-vivo in fish. The passive sampler consisted of an acupuncture needle with a sleeve of silicon tubing. Rainbow trout were simultaneously exposed to 10 organic chemicals for 25 days and then allowed to depurate for 56 days. The depuration kinetics were studied using both the novel in-tissue passive samplers and ii) by the traditional sacrifice/extraction/analysis of whole fish. There was good qualitative agreement between the kinetics measured with the two methods. However, the variability was considerably higher using the passive samplers, and some chemicals could not be quantified. The results suggest that in-tissue passive sampling method has the potential to be useful in laboratory bioconcentration experiments, lowering the costs for sample preparation and helping to reduce the number of test animals required. However, it also has several disadvantages in this context including the need to use large fish to allow deployment of the samplers and the need to expose the fish to high chemical concentrations due to the higher LOQ of the passive sampling method compared to whole fish extraction.

WE 278

Comparison of liquid-liquid-extraction (LLE) and solid-phase microextraction (SPME) to determine aqueous analyte concentrations in fish bioconcentration studies according to OECD TG 305

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Measured bioconcentration factors (BCF) and n-octanol-water partition coefficients (Kow) of hydrophobic organic chemicals (HOC) show a curvilinear relationship up to log KOW 5-6. BCF values of more lipophilic substances tend to level of or decline (hydrophobicity cutoff). Debated reasons are steric effects, but also measurement artefacts. Jonker and van der Heijden (2007; Environ Sci Technol 41:7363) suggested that the overestimation of bioavailable aqueous HOC by the presence of non-bioavailable HOC bound to dissolved organic matter, might lead to an underestimation of the true uptake. This was supported by the determination of freely dissolved aqueous concentrations by solid-phase microextraction (SPME).
The suitability of SPME to measure highly hydrophobic substances is described in the literature. However, the standard method for sample preparation in BCF-tests according to OECD TG 305 is liquid-liquid-extraction (LLE). The aim of this study was to investigate the potential of SPME with regard to the determination of aqueous analyte concentrations in fish BCF studies with special reference to different contents of organic matter (OM).
Water with different concentrations of organic matter was spiked with five test substances (log KOW 5.3 - 8.1) and analyzed at equilibrium conditions. Analysis of samples with SPME was compared to conventional sample preparation with LLE. With SPME, the detection of freely dissolved analyte concentrations is possible, as is the determination of sorption. Extraction of samples with LLE yields total analyte concentrations. Results show, that the freely dissolved concentration of highly hydrophobic analytes (which corresponds to the bioavailable fraction) is significantly reduced due to sorption to OM - already beneath the permitted concentration of 2 mg L-1 TOC in the dilution water according to OECD TG 305.
In a further approach, the sorption potential of different types of OM was investigated. Generally, sorption to Aldrich humic acid was stronger than sorption to all natural OM tested.
Results are discussed with reference to BCF-tests. The BCF is defined as the ratio of concentration (fish) to concentration (water) at steady-state. SPME measurements should not replace LLE procedures to keep the results of BCF studies comparable, however, SPME can give important information on the ratio between bound and freely dissolved compounds and help to estimate suitable TOC for highly lipophilic substances prior to a BCF study.

WE 279

Can high concentrations of perfluorinated organic compounds in fish be explained by dietary accumulation?

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Perfluorinated organic compounds (PFC) are a large group of chemicals which have been used since the 1950s because of their unique properties such as chemical inertness, resistance to heat and their ability to repel water and oil. We are only now beginning to realize that these

environmental contaminants may have serious environmental and health effects. PFCs have been detected in aquatic and terrestrial organisms with the highest concentrations found in top predators. High concentrations of PFC (i.e. PFOS and PFOA) were found in fish collected from different German rivers, although the analytical results were variable between species and the different tissues analysed (www.lfu.bayern.de). Apart of a few studies on bioaccumulation in fish, a sound evaluation of the trophic transfer of PFC in the food chain is missing. Martin et al. (Env. Tox. Chem. 22, 189-195, 2003) investigated the dietary accumulation of PFC in juvenile rainbow trout but dietary exposure did not result in biomagnification. The authors emphasized that extrapolation of the estimated bioaccumulation factors to larger fish should not be performed because the half life, especially of the more hydrophobic compounds, can be higher in large or mature fish than in smaller animals of the same species. The aim of this study was to compare the dietary accumulation potential of different perfluorosulfonates (PFOS, PFHxS, PFBS) and perfluorocarboxylates (PFOA, PFNA) in small and large rainbow trout. A dietary accumulation study was carried out on juvenile rainbow trout (2g) according to the draft document of the revised OECD TG 305. Biomagnification factors (BMF) were calculated for each of the five PFCs tested. In a further experiment large rainbow trout (400g) were fed for four weeks an experimental diet spiked with the same compounds followed by a depuration period lasting four weeks. The trajectories of tissue concentrations were analysed. Kinetic BMF factors were calculated and compared with the results obtained for juvenile animals. The results of both studies are summarized and discussed with regard to the accumulation of PFC in the aquatic food web and the potential impact on human health.

WE 280

Exploring a new in vivo screening technique for assessing the bioconcentration potential of pharmaceuticals in fish

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Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. Such studies are typically considered appropriate for pharmaceuticals having a logD value > 3 and required, as per EMEA Guidelines, for pharmaceuticals having logD values > 4.5.

The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to ½ the duration of the uptake phase. Based on the sampling schedule outlined in the guideline, >100 fish per study may be required to determine a kinetic BCF value. Based on the resources required to conduct the full bioconcentration study and the potential for fish to metabolize xenobiotics, alternatives to the current bioconcentration tests as a first tier have been explored and employed for the last several years. A new approach was used explore the possibility of developing a new screening tool to assess the bioconcentration potential in fish. Results of this research will be presented.

WE 281

Extrapolation of BCF from the in vitro rainbow trout (Oncorhynchus mykiss) liver S9 metabolic assay results

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In many bioconcentration models, the bioconcentration factor BCF is assumed to be driven by hydrophobicity; therefore, the octanol-water partition coefficient log Kow is the main and sometimes only parameter used for estimation in these models. However, bioconcentration in reality integrates all processes related to absorption, distribution, metabolism and excretion (ADME). Internationally accepted extrapolation models for BCF, which include the metabolism parameter, have been available for selected mammalian species. However, this type of model did not previously exist for fish. This need to address metabolism in fish in bioaccumulation predictions and the need for a standard approach for measuring metabolism led to the development of an in vitro rainbow trout liver S9 assay. This S9 metabolism assay has been successfully applied to nine chemicals in several laboratories. A previous presentation provided a summary of the data obtained to-date. This presentation will focus on describing the structure of an extrapolation model that can be used to estimate the BCF in trout from these S9 assay data and the improved parameterization using new insights into fish physiology and measured protein binding parameters. The extrapolation model predictions of BCF are compared to measured values of BCF to assess the validity of the S9 assay and extrapolation model and demonstrate the validity of this approach

WE 282

The development and evaluation of a mass balance bioaccumulation model for ionogenic chemicals in fish

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Approximately 33% of the tens of thousands of organic chemicals listed on Canada's Domestic Substances List (DSL) and the European Union chemical management program (REACH) are ionogenic and are expected to dissociate under environmentally relevant pH conditions. Measured bioaccumulation data are limited for legislated hazard and risk evaluations in general, and for ionogenic chemicals in particular. Quantitative Structure-Activity Relationships (QSARs) and mass balance models have been developed to quantify bioaccumulation processes for neutral organic chemicals (e.g., uptake by gill respiration and diet; elimination by gill respiration, fecal egestion, metabolic biotransformation and growth dilution). There is a need to develop and evaluate mass balance models for ionogenic chemicals that synthesize the available kinetic and chemical partitioning information into a holistic bioaccumulation framework. Such a model can then be used to identify key processes contributing to bioaccumulation, to develop hypotheses for strategic laboratory testing, and to provide screening level estimates for chemical assessment programs. A new mechanistic bioaccumulation model in fish is presented that integrates the available information on ionogenic chemicals. This model includes a mechanistic approach to address dissociation in the water and its influence on mass transfer and chemical uptake at the gills. It also quantifies the partitioning of the ionic and neutral forms of a chemical between the internal compartments of a fish, such as water, protein, storage lipid and membrane lipid. The new model's predictions are compared with measured rate constants and bioconcentration factors for ionogenic chemicals and with existing regression-based BCF and mass balance models. Future research needs to reduce uncertainty in the model predictions are highlighted.

WE 283

Using molecular dimensions to evaluate bioaccumulation potential

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In the REACH Regulation, PBT and vPvB assessment is compulsory for all chemicals produced or imported above 10 t/year. Although the main regulatory criterion for B or vB attribution is the aquatic bioconcentration factor (BCF) (REACh Annex XIII), an evaluation of all available information is necessary and screening criteria, as log Kow, can be used. So, when direct comparison with criterion lay down in REACh Annex XIII is not possible, other indicators are to be considered in a weight of evidence approach. Among these, the molecular size and molecular weight (MW) can indicate a low bioaccumulation potential. Several descriptors can reflect molecular size but at present, the most reliable is the "average maximum diameter"(Dmax aver) developed by Mekenyan and Dimitrov . These authors showed that a chemical may be considered as not bioaccumulative if Dmax aver > 1.7 nm and MW > 1100 g/mol because very bulky molecules pass the cell membranes with difficulty . To calculate Dmax aver, conformational analysis is necessary and OASIS is the reference software to obtain energetically stable conformers representing conformational space of the molecules. As OASIS is a very expensive program, our aim was to find an alternative and equivalent tool. Basing our work on the review of Brooke and Cronin , we examined and tested different modeling softwares giving descriptors related to molecular dimensions. By this way, we led to the combination of two softwares : Spartan was used for modeling calculations whereas the BBX descriptor from Mol2Mol furnished a surrogate for Dmax aver. This new procedure was tested on a set of 18 molecules reflecting a wide structural diversity and results were compared with data obtained with OASIS. In this communication we will so present this work and discuss the scope and limitation of our method in the context of PBT assessment.

WE 284

The CEFIC ECO16 project: critical body residue validation for aquatic organisms exposed to chemicals causing toxicity by baseline narcosis.

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Biomembrane homeostasis can be disturbed by partitioning of any organic chemical into the membrane structure, which may ultimately lead to narcosis. Due to the non-specific nature of this mechanism, the membrane-disrupting potential of one molecule is generally considered equal among all chemicals. As a result, chemicals that primarily exert toxicity via a narcotic mode of action may be expected to display equal critical body residues (CBRs). Although this theory could greatly facilitate environmental risk assessment for these chemicals, a considerable variation is observed in CBRs reported in the literature. The main cause for this variation is thought to be related to the disregard of the process of internal distribution. This oversimplification leads to the faulty assumptions that (a) the quantity of a narcotic chemical, which caused lethality in an organism, is present in lipids, while in fact it may be divided among other compartments (e.g. proteins, carbohydrates), and (b) the concentration in the storage lipid phase is equal to the one in membrane lipids, though the phases may show differential sorption behavior. Until now, few attempts have been made to accurately determine the concentration of a narcotic toxicant actually present in the membrane phase of an exposed organism at the time of death. The objectives of the current CEFIC ECO16 project are to get more insight into the variation in CBRs of different chemicals and in different organisms by (1) compiling a comprehensive CBR database, (2) developing a sophisticated model to describe the process of internal distribution of narcotic toxicants and implementing it in the gathered data, and (3) acquiring experimentally derived data to provide the model with appropriate partitioning parameters and accompanying CBR data of a number of benchmark chemicals.

WE 285

PBT assessment and trophic dilution - Regulatory consequences

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The identification of substances which are persistent, bioaccumulative and toxic, so-called PBT substances, is important in the prioritization for regulatory action in the EU as well as globally. The bioaccumulation potential of a substance gives an indication that higher concentrations of a compound are found higher in the food chain and this may become harmful to ecosystems and humans. As a measure for accumulation the bioconcentration factor (BCF) of a compound is used. This is defined as the ratio between the uptake rate of a compound from water into the organism and its elimination rate to water. In the evaluation of the bioaccumulation potential of substances, the European REACH guidance on PBT assessment makes in principle no difference in which species the bioaccumulation has been measured. In general, other regulations focuses on higher trophic levels, <*ie.* fish, where data on invertebrates are only considered when data on fish are not available. This may lead to a bias due to unbalanced weighing of available data. It also raises the question on how to consider substances that will accumulate in lower trophic levels, like mussels, but do not fulfil the bioaccumulation criterion in fish and do not biomagnify through the food chain (*e.g.* PAHs). This so-called trophic dilution in food webs is the result of efficient metabolic transformation at higher trophic levels. For compounds that are not easily metabolized (*e.g.* PCBs) it can be hypothesized that bioaccumulation will occur in all trophic levels, resulting in biomagnification in the food chain. Consequences for the assessment of bioaccumulation in a regulatory context, caused by data gaps or data availability for certain trophic levels, are discussed.

WE 286

Simulated trophic magnification factors: sensitivity analysis and comparison to human exposure modelling

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In this study two ecosystem level bioaccumulation screening metrics are simulated and compared.

Trophic magnification factor (TMF) is a metric applied to chemical measurements collected from a range of biota in a localized food web, and is interpreted as an approximation of the average biomagnification factor (BMF) for the chemical in the analyzed food web. Many confounding factors such as species-specific metabolic elimination and food web structure mean that the TMF of a chemical measured in one food web cannot be used to assess the bioaccumulation of the chemical in another food web. Another metric for evaluating bioaccumulation potential is the human emission ratio (HER) which relates the amount of chemical in the physical environment directly to the human end-point. HER is also strongly dependent on the structure of the food web and other factors. A linked chemical fate and bioaccumulation model (CoZMoMAN) is applied to a set of environmentally relevant chemicals, and the model results are used to calculate TMF and HER values in several different ecosystems. Comparison to experimentally measured TMF values and human monitoring data are made, when possible, to test the validity of the modelling outcomes. Subsequently, a set of hypothetical chemicals is used to perform a model sensitivity analysis to test the relative influence of chemical properties (partitioning, biotransformation) and ecosystem properties (food-web structure, physical environmental) on the two different bioaccumulation screening metrics.

WE 287

Applying multimedia models to calculate trophic magnification factors (TMFs): exploring basic assumptions and the role of the physical environment

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The trophic magnification factor (TMF) is considered to be a useful metric for assessing the bioaccumulation potential of organic chemicals in food webs. Fugacity is an equilibrium criterion and thus reflects the relative thermodynamic status of a chemical in the physical environment (air, water, soil and sediment) and in aquatic, terrestrial, and human food webs. For most neutral organic chemicals the TMF is calculated using lipid normalized concentrations measured in organisms of a food web and their relative trophic position. This lipid normalized TMF calculation is analogous to calculating the change in chemical fugacities in organisms of a food web with respect to their relative trophic position. Fugacity based fate and food web bioaccumulation models have been used to estimate the fate and bioaccumulation of organic chemicals in the environment. The Risk Assessment IDentification And Ranking (RAIDAR) model is a fugacity based mass balance model that combines environmental fate and food web calculations to assess exposures and potential risks. RAIDAR includes representative species comprising aquatic, terrestrial, agricultural and human food webs. The RAIDAR model was used to calculate fugacity ratios in representative food webs (i.e. TMFs) and to illustrate the range of TMFs as a function of chemical partitioning space. Screening-level model predicted TMFs are in good agreement with measured TMFs for many well studied chemicals (e.g. PCBs, HCB, PBDEs, HCHs, pyrene). Model sensitivity and uncertainty analyses provide guidance to improve the model calculations. The model was used to show how disequilibrium conditions in the physical environment (air, water, soil and sediment) influence steady state TMFs. These results highlight the need to clearly define the TMF with respect to the analyzed components of the food web and the need to consider holistic methods that include environmental fate and food web bioaccumulation for screening chemicals and for chemical exposure assessment.

RA06 - Monitoring and risk-assessment of organic compounds in developing countries

WE 290

Monitoring of organic pollutants in the South African marine environment - which are better mussels or artificial devices?

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The developed global community is increasingly aware of man's activities on environmental sustainability; however the developing world has yet to realise its toxicological and ecological impact due to the increased discharges of effluents containing pollutants such as organic contaminants. A distinct lack of historical and current data on the status of organic pollutant contaminants within the South African marine environment has been acknowledged. The last published assessment of dissolved hydrocarbons conducted in Table Bay Harbour was in the 1980s. As a consequence there is increased awareness to conduct more current organic pollutant assessments. Brown mussels (P. perna) and artificial devices, semi-permeable membrane devices (SPMDs) were transplanted at five South African harbours for 10 weeks to assess organic bioaccumulation. The SPMDs are well documented in literature for their efficiency at indicating trace organic contaminants in the water column as well as their ability to provide standardised results between varying localities worldwide. Spatial patterns of PAH and PCB contaminants were determined by GC-MS and GC-ECD after appropriate sample preparation according to methods. Significant ($p < 0.05$) spatial differences were identified between the five harbour sites, and as observed, contaminants measured in the mussel tissue deviated from those represented by the SPMDs. Results indicated no positive correlations between the SPMDs and the transplanted mussels, however the SPMDs provided complementary information which may be of toxicological significance. They were able to positively identify petrogenic contaminants from a known oil spill event and their ability to accumulate dioxin-like PCBs which were not detected in the mussels. Differences occurring between the uptake profiles of the SPMDs and mussels can be attributed to the passive device monitoring contaminants in the dissolved phase from the water column whereas concentrations reflected by the mussels are a net result of dietary exposure and passive diffusion. The results indicate that information provided by both the mussels and SPMDs allow for a more in depth scrutiny of environmental conditions as a result of anthropogenic influence. They further provide resource managers with an effective tool and methodology to identify and correct issues before they become problematic.

WE 291

Evaluation of the genotoxicity of 17a-methyltestosterone on Oreochromis niloticus widely used in pisciculture to induce monosex males

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The hormone 17 α - methyltestosterone (MT) is used in fish farms through animal feed to produce monosex males. In Brazil, this substance is still used without registration. The purpose of this study is to investigate the genotoxic effects of this hormone on Oreochromis niloticus. Tilapia is grown in fish farms due to its strong commercial interest. Evaluation of the genotoxicity was carried out through micronucleus test (MN), comet assay (CA) and nuclear abnormalities (NAs) in individuals with reproductive and non reproductive maturity. Fish were purchased from the Federal District Government fish farm. The animals were exposed to the substance diluted in water for 96 hours at concentrations of 0.01, 0.1 and 1mg/L, not exceeding 0.04% of ethyl alcohol in the solution. Then, reproductively mature individuals, measuring 09-12 cm, were kept for 28 days in fish tanks in our laboratory and fed the same diet used to produce monosex male larvae. Finally, O. niloticus were collected at the end of 28 days of feed administration containing MT in a batch of larvae produced in the pisciculture (in situ). Our results showed that MT was genotoxic after exposure to 96 hours when compared with the negative control and control with alcohol. After 28-day of exposure to MT in the diet, no genotoxicity was observed. Results from in situ exposure study showed an increased level of MN. According to the literature, 17 α - methyltestosterone is degraded in the liver of animals, so it can be suggested that the route of exposure can influences on the genotoxicity of the MT. Regarding the results obtained from the in situ study, we can observed that in the early developmental stages MT present genotoxic risks.

Key words: genotoxicity, methyltestosterone, Oreochromis niloticus
Support: Brazilian Reseaarch Council (CNPq) and University of Brasília.

WE 292

Identification and quantification of organic contaminants in fish from the Tonlé Sap watershed, central Cambodia

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The Tonlé Sap watershed is a unique freshwater lake-river system in central Cambodia. The lake, which undergoes dramatic changes in size between wet and dry season, supports a highly productive inland fishery and is home to several floating villages. The objective of the present study was to investigate the occurrence and levels of organic contaminants in fish from this important watershed. In November, 2010, several samples of fish and prawns originating from the Tonlé Sap were obtained from local markets near Phnom Pehn, Cambodia. Fish were both from culture and natural origin. Fish species included tilapia, snakehead, catfish, and silver barb. Both fish muscle and liver were collected. Fish and prawn tissues were solvent extracted and analyzed by gas chromatography - mass spectrometry (GC-MS) or Liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) techniques. Target analytes included legacy persistent organic pollutants (POPs) but also emerging contaminants such as brominated flame retardants (BFRs), perfluoroalkyl chemicals (PFCs), synthetic musks and plasticizers. QA/QC included blank measurements, recovery tests with spiked 13C mass-labeled compounds and replicate analyses. Based on the measured residue concentrations, daily intake of the various contaminants were assessed for the population of this region of Cambodia and compared with similar data from elsewhere. The results from this pilot study highlight the need for a larger scale study of the fish stocks in the Tonlé Sap.

WE 293

Water-related complex pollution in Central Asia

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We studied pollutant contents in upper, ground, and drinking waters - in the worst polluted areas of CA states (Tadjikistan, Uzbekistan, Kyrgyzstan, Kazakhstan) in 2000-2009 years. The pollutants are: a) hydrocarbons aromatic, pesticides mostly - ground, drinking water and vegetables; b) bio-pollutants (abdominal typhus, coli bacteria, cholera, 4 helminthes). We have created 5 ranks maps of each impact by human toxicity. And then we created common (complex) map by superposition methods, which shows scale of water-polluted areas of CA. We have referred created ranks by analysis of base human health markers (immunity level 6 tests, genetic disorder 3 tests). Results of both studies were correlated each other. The first consequence of studies is detection of most vulnerable areas in CA that need immediately rescuing. It has been offered/improved the water-toxicants alleviation methods: a) twice prolongation of water staying in sediment basin (in water-purified stations); b) protecting 5 tailings from rivers (we did project for protecting genetic of vulnerable groups (install 17 updated drinking water filters in schools, nursery, hospitals); c) there are 3 common projects for gathering obsolete pesticides and keep in contemporary warehouses, we created filter system for chromium purify; d) bio-pollutants is a results of human and animals fertilize utilization and toilets absent, we installed composting toilets in rice-land and in Alpine area.

WE 294

Risk assessment in the jungle: the use of glyphosate to control the production of coca in Colombia

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Conducting risk assessments in remote locations is logistically challenging but can be very useful as an educational tool is scientists from the region can use the exercise a learning tool. To address identified data needs, laboratory studies with 8 species of tadpoles of frogs from Colombia were conducted. This involved using local facilities as well as residue analysis with ELISA in pace of the more widely used LC-MS. These studies showed that under laboratory conditions LC50 values ranged from 1,200 to 2,780 µg glyphosate a.e./L. However, under realistic field conditions in sediment-bottomed microcosms, LC50s ranged from 5,963 to 7,303 µg glyphosate a.e./L when treated with an overspray to mimic aerial application. Bioassays with terrestrial stages of frogs (juveniles and adults) were conducted with simple equipment obtained from local farm and garden suppliers. Spraying was conducted with a modified atomizer to apply consistent volumes of spray solution. These studies, carried out under conditions that mimicked the field, produced LC50 values between 4.5 and 22.8 kg a.e./ha. A human health-effect study was carried out in volunteers from 5 Colombian regions. These regions were characterized by different exposure to glyphosate and other pesticides and the study was to assess the effects of exposure to eradication and other sprays on the frequency of binucleated lymphocytes with micronuclei (BNMN). This study was conducted through the use of local medical health workers from regional clinics who

were familiar to the volunteers and would be trusted as impartial. Blood was collected before spraying, 4-5 days and 4 months post-spraying. The samples were processed in a local laboratory and prepared for assessing Italy. These results showed that the highest frequency of BNMN was in Boyacá where no aerial eradication spraying of glyphosate was carried out and Valle del Cauca where glyphosate was used for maturation of sugar cane. There was no significant association between self-reported direct contact with eradication sprays and frequency of BNMN. Overall, these results suggest that genotoxic damage associated with glyphosate spraying, as evidenced by the MN test, is small and transient. These data were used in a risk assessment which concluded that risks to sensitive wildlife and human health from the use of glyphosate in the control of coca production in Colombia are small to negligible.

WE 295

Fuzzy approach for risk assessment of brominated flame retardants in aquatic ecosystems of Latin America

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Brominated flame retardants (BFR) are pollutants that represent a threat to human health and the environment due to their industrial use, their persistence and their ability to bioaccumulate and biomagnify in food chains, especially in the aquatic one. For the last ten years contamination levels for this type of compounds have been reported for European, North American and Asian human tissue, sediments and biota samples [1-3]. However, monitoring efforts on the assessment of BFR contamination levels in Latin America are scarce.

Contamination levels, bioaccumulation and biomagnification in the aquatic trophic chain of two groups of BFR compounds (polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCD)) are currently being evaluated in two Latin American ecosystems, one from Chile (San Vicente bay and Lenga estuary) and another one from Colombia (delta-estuary of the Magdalena river) under the framework of an international research project titled BROMACUA. Within this framework, a methodology based on fuzzy logic has been developed to evaluate the risk due to contamination in aquatic organisms by using experimentally determined levels of contamination and data from the literature. Fuzzy logic is characterized to be conceptually easy to understand and is based on natural language. It has been used successfully to model non-linear functions, to establish inference systems on top of the experience of experts and to deal with imprecise data. Fuzzy approaches can be very useful in the environmental field due to the uncertainty associated to this type of data.

A description of the fuzzy model proposed is given in this work, including a summary of the main outcomes of a questionnaire (replied by 38 international experts) that has been created to obtain information on different topics relevant for the model development. Results associated to the behavior and the sensitivity of the proposed model are also presented.

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WE 296

Good quality of groundwater sources as the challenge for science and sustainability

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Ground water sources are strategic resource for every country and population. It's availability and sanitary safety is strongly connected with human health, protection of environment and economy status. Good quality of groundwater sources, providing safe drinking water for population, became the priority, and, at the same time, a big challenge for science and sustainability, and healthy, clean environment. Groundwater is the largest reservoir of fresh water in the world. About 75 % of the European population uses groundwater as their water supply. Groundwater is traditional water supply resource in Serbia, as well. It is exclusive source of water supply of central and northern Serbia - region of Vojvdina, with only exception in city of Belgrade, where part of the water supply originates from Sava river. More than half of the abstracted water is groundwater from alluvial aquifers, and therefore 80-90 % originates from infiltrated water from rivers. These aquifers belong to the so called rapidly rechargable aquifer since they depend on hydrologic regime of water. In some groundwater sources, during previous research, emerging substance of concern have been detected, such as carbamezapine and benzotriazole. Ten years after NATO's intervention in Serbia and the destruction of city of Novi Sad's oil refinery, bridges and water supply, providing safe drinking water and protecting the environment is still a major challenge. Integrated early warning system (EWS) for the detection of organic and inorganic contaminants in the source water will be developed and implemented, to gain more insight into the quality of the water used for drinking water production at Novi Sad and evaluate the risks for human health and the environment. The monitoring data of the EWS's individual components are stored in a database, fully compatible with standard European data collection systems, to share obtained knowledge and improve the protection of the Danube River and Novi Sad's inhabitants. This data is evaluated with the help of a novel QSAR-based procedure for the selection of hazardous substances. The results of the monitoring efforts and the QSAR-evaluation is then used as a basis for the improvement, integration and harmonisation of risk assessment and risk management standards for the protection of drinking water resources.

WE 297

Uncertainty assessment by a Monte Carlo simulation for methylmercury from hair of Colombian population

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Mercury (Hg) is a ubiquitous contaminant of aquatic ecosystems causing a global environmental concern. Mercury levels are biomagnified in fish tissues from prey to depredator in the food chain, and them humans as top consumer. Risk of exposure to mercury can be reached by eating freshwater fish, seafood, and shellfish. Mercury has been widely distributed in aquatic ecosystems by natural process and human activities in which anthropogenic sources are a consequence of the huge pollution that has been caused since the past 20th century. Mercury contamination in Colombia has been documented for different types of matrices related principally with informal gold mining activities, where the metal is released into the atmosphere after burning amalgam in the gold refining process and also poured directly into body waters. These studies reported concentration of mercury in fish from different trophic levels in the San Jorge and Magdalena basins, and the Cartagena bay. The aim of this report is model the exposure and risk assessment to methyl mercury associated with fish consumption from fishermen Colombian population. Type and amount of fish consumed by the individuals as well as the concentration of fish were obtained from previous published data from our research group. The probabilistic dose intake was accomplished by Monte Carlo simulation by 100.000 random samplings. Internal dose of methyl mercury in blood and hair were also simulated using one-compartment model based on a review of literature to the specific model input parameters. Overall, mean of methyl mercury dose intake estimated for Colombian population was 0.68 µg/day-Kg bw and mean values of methyl mercury in blood and hair were 42.5 µg/L and 10.5 µg/g, respectively. This report intended to related methylmercury concentrations of hair from a cross-sectional study on Colombian population that frequently consumes fish. The results of the model estimated the methylmercury concentration in hair in good concordance. However, the exposure assessment model is a good starting point to attempt predicts the risk-benefit analysis of fish consumption.

WE 298

Uptake of pesticides in potatoes grown in Colombia: monitoring, modelling, and human health risk assessment

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A dynamic model for uptake of pesticides in potatoes is presented and evaluated with measurements performed within a field trial in the region of Boyacá, Colombia. The model takes into account the time between pesticide applications and harvest, the time between harvest and consumption, the amount of spray deposition on soil surface, mobility and degradation of pesticide in soil, diffusive uptake and persistence due to crop growth and metabolism in plant material, and loss due to food processing. Food processing steps included were cleaning, washing, storing, and cooking. Pesticide concentrations were measured periodically in soil and potato samples from the beginning of tuber formation until harvest. The model was able to predict the magnitude and temporal profile of the experimentally derived pesticide concentrations well, with all measurements falling within the 90% confidence interval. The fraction of chlorpyrifos applied on the field during plant cultivation that eventually is ingested by the consumer is on average 10^{-4}-10^{-7}, depending on the time between pesticide application and ingestion and the processing step considered.

WE 299

Assessment of the effects of Anopheles breeding environment' chemistry on the development of malaria vectors larvae in Mount Cameroon Region: an ecofriendly control solution

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Mount Cameroon region is a malaria endemic area with high transmission level, particularly among women and young children in Cameroon Tea Estate (CTE) settlements. Understanding Anopheles ecology is therefore crucial to achieve effective control of malaria.

A study was therefore designed and carried out in 6 CTE settlements to assess the influence of physicochemical quality of water and environmental characteristics of Anopheles breeding sites on Anopheles distribution and densities. Breeding sites were surveyed and larvae collected and identified morphologically to species. 25 water samples from representative breeding sites were analyzed. The influence of environmental (vegetation, surface debris, predators, turbidity, exposure to sunlight, and distance to the nearest inhabited house- DNIH) and physicochemical (temperature, pH, calcium, potassium, sodium, chloride, carbonate, bicarbonate, ammonium, nitrate, phosphate, magnesium, sulphate ions, and conductivity) parameters on larval densities and distribution was assessed.

2096 Anopheles larvae were collected from the various breeding sites. The morphological identification yielded 8 species: An. gambiae, An. marshallii, An. funestus, An. moucheti, An. sergentii, An. hargreavesi, An. hancocki, and An. concolor. Potassium, DNIH, type of breeding sites and predators ($p < 0.05$) were key factors determining the presence of Anopheles larvae in breeding sites. .An. gambiae showed a clear preference for temporary breeding sites close to inhabited houses (DNIH > 20m) with high larval densities.

The potassium comes from the fertilisers profusely used by CTE farmers. Education of the farmers on appropriate hygiene measures and application of chemicals to be observed in CTE plantations, are essential to suppress the increasing vector densities and malaria transmission intensity in the area.

RA10 - Risk assessment of chemical mixtures: how can we crack the nut?

WE 302

Statistical approaches for distinguishing individual chemical toxicity thresholds in potentially complex mixtures

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It is not known how closely the protection afforded by an Environmental Quality Standard (EQS) corresponds to the definitions of 'Good' ecological status under the Water Framework Directive (WFD) i.e. are the requirements for 'Good' status between chemistry and biology consistent? This could have important implications for deriving EQS and it is important to understand how EQSs compare to field data. An analysis of "matched"biological quality element and chemical monitoring data from England and Wales was undertaken. It is important to consider that ecological communities are subject to numerous stressors (both physical and chemical), in-

cluding exposure to mixtures of both anthropogenic and naturally occurring chemicals. Quantile regression (QR) was used to investigate the relationship between chemical concentration and biological quality and to distinguish between the impact of a single chemical and the effects of complex mixtures. QR can be used to identify a concentration of a stressor which is likely to result in a limitation of ecological quality, and is particularly useful in cases where it is not possible to exclude all of the potentially confounding factors (e.g. co-exposure to other chemical stressors in a mixture). Forty determinands (including metals, pesticides and sanitary determinands) were included in the analysis. "Thresholds"of chemical exposure consistent with definitions of "high", "good"and "moderate"ecological quality under the WFD could be derived for some, but not all, chemicals; primarily because of limited dataset size for many of the chemicals and the high prevalence of censored chemical exposure data (i.e. below LOD). An analysis of co-variance was also undertaken. These thresholds are useful for calibrating the relative stringency of EQS compared with ecological protection goals. In most cases, existing EQS appear to offer adequate protection for ecological communities.

WE 303

Evaluation of tank mixing in British agriculture: environmental implications

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Tank mixtures of pesticides have the potential to introduce different levels of toxic impact to the environment than is accounted for by single product risk assessment and authorisation. We have reviewed the evidence for the action (concentration or response additive) and interaction (synergistic or antagonistic) effects of pesticide mixtures. In common with the conclusions of several other reviews over the past decade, we present clear evidence of concentration or response addition effects when chemicals, including pesticides, are found in mixtures. Toxic Units (TUs) were calculated for the individual components of commonly used UK binary and ternary tank mixtures, using waterflea, algal and fish EC50 data and conservative exposure estimates. These TUs were then summed under the assumption of concentration addition and compared with individual component TUs. This showed that reliance on single substance risk assessment could under-estimate the toxicity of the mixtures that were analysed by up to 70% for this data set, although the mean discrepancy was much lower, at around 12%. Risk assessment of mixtures should therefore include consideration of additive effects, under the assumption of concentration addition, which is marginally more conservative than the response addition model. Although it was difficult to demonstrate interaction effects such as synergy, the possibility of synergy should not be discounted. Rather it should be carefully evaluated on the basis of existing knowledge of synergy between pesticide active ingredients. A simple, reasonably conservative rule of thumb for synergy that could be used as an initial ranking tool for potentially risky mixtures for further, refined investigation would be to assume that all tank mixtures are 2.5 times more toxic than the concentration addition of their component pesticides.

WE 304

The fish embryo toxicity (FET) test as a screening method to support a component-based risk assessment of biocidal products

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The aim of the present project was to investigate the suitability of the fish embryo toxicity (FET) test as a screening method to check for concentration-additive behaviour of biocides. Five commercial wood protection products, the three biocidal active substances contained in these products as well as six different generic binary mixtures of these active substances were investigated in fish embryo toxicity tests. Median lethal concentrations (LC50) estimated from the concentration-response curves and corrected for measured concentrations of the substances were compared to the toxicity predicted by the concept of concentration addition. Less than 2fold deviation between measured and predicted toxicity was observed for all binary mixtures of the active substances and for three of the five biocidal products. The detected underestimation of the toxicity of two products by the mixture toxicity prediction could be explained by the influence of certain formulation additives. Overall, the FET was found to be a suitable tool for verifying whether the toxicity of formulated biocidal products can be reliably predicted by the concept of concentration addition. Applied as a quick and simple non-vertebrate screening test, the FET may support approachs of applying component-based mixture toxicity predictions within the environmental risk assessment of biocidal products in justified cases.

WE 305

Should the receiving environment be considered a mixture in chemical risk assessment. If so, how?

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There are three different types of mixtures that could be of concern in terms of impacts on humans and the environment: products consisting of multiple components, wastes and effluents which contain a variety of materials, and finally the receiving environment itself which will contain a vast number of substances of anthropogenic and natural origin often at very low concentrations. Establishing the effects of the first two mixture types is straightforward since the mixture itself is both bounded and physically available. As such the developing techniques for mixtures assessment can be readily applied. However in the case of the receiving environment, the problem is not how to measure the toxic effects of the mixture but how to identify which components, out of all those available, should be included in the mixture to evaluate. This poster presentation will outline this critical issue in more detail, review the current methods that might be used and suggest further avenues for research.

WE 306

Risk assessment of mixture of herbicides: the case study of lake Geneva

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In surface waters, aquatic organisms are not only exposed to single substances but typically to mixtures. Therefore joint action of toxicants should be taken into account in ecological risk assessments. However, few approaches have been proposed to reach this goal until now. In ecotoxicology, mixtures effects prediction is largely influenced by toxicological theory. In concrete terms, two concepts/models are particularly used and play a pivotal role: concentration addition (or dose addition) and response addition. Synergetic and antagonistic effects are generally neglected because of their complexity to predict. To assess the ecological risk of substances, these two models could be combined with either the Assessment-Factor method or the species sensitivity distribution (SSD) curves. Nevertheless these approaches aren't common applied for two major reasons: the gap of data (ecotoxicological data, information on mechanism of action, etc.), and the lack of predictions validation. In this study, we propose to assess the risk of mixture of similar and dissimilarly acting pesticides. This approach combines the use of SSD curves and joint action models and is illustrated based on long-term monitoring data of lake Geneva, Switzerland. The assumptions underlying the approach will also be presented and discussed. The results show a decrease of toxicity of herbicides mixture since 2004 and lead to the question whether this improvement is observable in term of algal communities response. However, due to the low toxicity and its correlation with phosphorous change in the lake, the main nutrient driving, it is difficult to link phytoplankton species abundance with toxicity change. A traits-based approach and a classification in functional groups may provide a better answer along this environmental gradient.

WE 307

Effects of s-triazines and metolachlor on chlorpyrifos toxicity in Zebrafish (Danio rerio) early life-stages

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Previous work showed the existence of ecotoxicity of water samples from the Alqueva reservoir (south of Portugal) due to the presence of significant concentrations of herbicides (e.g. atrazine, simazine, terbuthylazine and metolachlor) and the insecticide chlorpyrifos. Here we examine the effects of these pesticides singly and as binary mixtures on Zebrafish (Danio rerio) early life-stages through a Fish Embryo Toxicity (FET) Test, in the lab.

Our results indicate patterns where deviations from the conceptual models CA (concentration addiction) and IA (independent action) like synergism, antagonism, and dose ratio or level dependencies were observed. As examples, there was an increase in the toxicity of chlorpyrifos on the triazines-treated embryos, possibly due to the biotransformation of chlorpyrifos into more toxic o-analog metabolites.

This study represents an important step to understand the interactions among various pesticides detected in the Alqueva´s reservoir.

WE 308

Learning lessons from exposure assessment of complex metal mixtures

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REACH requires registration in 2010 of all substances that are produced above 1000 tonnes/year in Europe. As such, a chemical safety assessment (CSA) needs to be developed. Complex substances like intermediates from metal production processes, ores and concentrates, slags and alloys contain different metals in several species or mineral forms. The uptake of these species by living organisms is related to the solubility and bio-availability of the metal bearing species (determined through transformation/dissolution and in-vitro bio-solubility tests). However, guidance on exposure and risk assessment of such complex materials is scarce. Consequently, there was a need to develop a scientific but pragmatic approach to assess the complex metal mixture exposure and risks.

The copper slags risk assessment will be presented as an example. Cu slags are co-produced out of the process of recovering copper and used in various applications like abrasive blasting, embankments and road construction. The exposure and risk assessment of this substance is successfully developed. These assessments are focused on release/exposure of critical trace metals of potential concern and compared to the DNELs and PNECs for these selected metals. The (site-specific) exposure assessment for the production process is based on measured values of releases to environment and inhalation exposures of the selected critical metals. Relative contributions towards total dust or copper were derived and used if no measured inhalation data were available. The exposure scenarios of the downstream uses are also based on individual constituents and focused on the main driver(s) based on leaching and hazard information. The human health risk management measures include mainly automated and closed systems, local exhaust ventilation and/or respiratory protection. Fume/dust cleaning and removal of metal trace constituents in direct cooling water and effluent aim to protect the local environment.

Some challenges were identified during the assessment. The existing metal CSRs are based on different approaches, which in the slag assessment had to be combined: some CSRs took an added versus total approach, DNELs were based on respirable versus total inhalable dust and some risk assessments were only based on biomonitoring data versus others took into account biomonitoring and inhalation data. The question how to deal with "mixture toxicity"in such complex risk characterizations was another overarching issue.

WE 309

Environmental risk assessment of complex mixtures of some PPCPs in Tunisian WWTPs (Parabens/estrogens)

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Parabens are a group of alkyl esters of p-hydroxybenzoic acid. Parabens (or their salts) are widely used as preservatives and bactericides in cosmetics, toiletries, and pharmaceuticals (deodorants, bath gels, shampoos, cream etc.). Various in vitro assays shown paraben can bind to the estrogen receptor (Darbre et al. 2002). From the environmental point of view, information concerning levels and potential long term effects of parabens are missing. Some laboratory studies have confirmed that the presence of weak estrogenic species (genistein, bisphenol A, etc.), are able to act together to produce significant effect at concentration below its individual NOEC (Silva et al 2002). Alone or in combinations with other compounds, esters of 4-hydroxybenzoic acid may be present in the environment. The main goal of our works is to assess the occurrence of joint estrogenic action of various parabens present in water sample. We evaluate whether mixtures, between parabens and other estrogenic compounds (17-_-Estradiol (E2), Estriol) have synergistic or additive effect using a recombinant yeast (YES) assay, (Garcia-Reyero et al. 2001). To this end, concentration-response analyses with single agent were firstly carried out. Then, based on the activity of 17-_-Estradiol in the YES assay, we calculated predictions of concentration-response curves for different mixtures assuming additive combination effect. Evaluation of estrogenic activity of different treated sewage waters (influent and effluent of Tunisian WWTPs located near an industrial or tourist area) will be compared with a conventional HLPC DAD analysis. Thus the impact of estrogenic compounds on estrogenic activity of a water sample can be estimated

WE 310

Direct Toxicity Assessment of mixtures in effluents: current UK experiences

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Direct Toxicity Assessment (DTA) is the process of measuring the environmental hazard (toxicity) posed by a complex chemical mixture. The DTA approach has advantages over chemical-specific methods, in particular in its ability to provide an integrated assessment of the effects of all constituents, including unknown substances and those for which standards or analysis methods are not available. DTA was implemented into the UK regulatory framework under the Integrated Pollution Prevention & Control (IPPC) regime to assess the risk posed by complex industrial discharges to local receiving environments. DTA is used to provide a robust prediction of the acute hazard (toxicity) posed by a complex effluent which can then be used within a tiered risk assessment approach. Under the IPPC regime, industrial sites with liquid discharges must undertake DTA on their effluent(s) if they directly discharge more than 100 m^3 of effluent per day to controlled waters and cannot demonstrate that their effluent is "simple". A "simple"effluent is one where all the components have been identified and the toxicity of the effluent can be explained by the chemical properties of the components. Of the 150 UK sites estimated to have complex effluent discharges, approximately half have undertaken DTA as required by the regulations (2006 to date), and the majority have successfully demonstrated that the discharges posed no significant risk to the local receiving environment. In a small sub-set of sites, DTA demonstrated that the local environment may be at risk and has enabled measures to be taken to reduce that risk to an acceptable level. This poster reviews the current state of regulatory DTA in the UK, evaluates its success to date in addressing the environmental effects of complex effluents, and provides recommendations for the improvement of the existing UK regulatory DTA regime.

WE 311
Chemical mixture risk assessment in the context of multiple environmental stressors
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The importance of a multiple-stressor perspective when considering chemical mixtures in ecological risk assessment and management is demonstrated in a case study of the Hocking River watershed (Ohio, USA). A variety of assessment approaches ranging from single-chemical, chemical mixture, and multiple stressor assessments were applied to identify sites within the watershed at risk for biological impacts based on the environmental conditions present. The results of each approach were compared with observed biological impacts. The traditional single-chemical approach identified sites exceeding U.S. EPA water quality criteria based on single-chemical species sensitivity distributions, however this approach missed the majority of observed biological impacts. Chemical mixture approaches, such as toxic pressure quantified as multi-substance Potentially Affected Fraction ('msPAF') using a mixed-model approach, significantly improved the identification of impacted sites. The most effective identification of impacted sites was achieved when chemical mixture toxicity was integrated into a multi-stressor approach along with other stressors such as physical habitat quality and water chemistry. Within this context, the influence of chemical mixtures on biological impact in the watershed was determined to be outweighed by other stressors. The results suggest that a sole focus on chemical mixtures in retrospective assessments creates a scenario for potential over-management of chemicals, with less-than-expected ecosystem recovery after chemical exposure is reduced. Additionally, evaluation of chemical mixtures based on a criterion-risk assessment with a safety factor can further overestimate mixture effects, identifying more sites as impacted by mixtures when in fact other stressors are responsible for the observed impacts. This case study illustrates that while management of chemicals is enhanced by a chemical mixture perspective, the placement of this stressor in the context of other environmental stressors is critical for effective ecosystem protection and management.

WE 312
Assessing the impact of mixtures in the environment, an ECETOC taskforce
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The toxicity of mixtures can be predicted when the properties and chemical components are known. However, it is more problematic to assess the potential interaction of chemicals in the environment when not all components of the mixture are known. This leaves industry vulnerable to criticism for not determining whether chemicals present in the environment, including those at concentrations below their predicted no effect concentrations (PNECs) may act additively to cause an overall effect - so-called low dose effects.
As it is usually not possible to know all the potential combinations of chemicals in the environment, prospective risk assessments of mixtures may be limited. However, retrospective approaches that compare predicted risks from chemicals as well as other factors such as instream habitat, and altered hydrology and other factors to measured biological quality (e.g. structure and function) provide an integrated/holistic approach. This enables the relative importance of chemical mixtures in causing adverse biological responses to be determined. In essence, these retrospective approaches provide an ecological reality check by identifying priority concerns pertinent for appropriate management of water quality, including Environmental Quality Standards set within the WFD.
However, this is not a simple activity and requires the development of methods to discriminate the impacts of chemicals and other stressors from natural environmental variation. This poster summarises existing methods for retrospectively assessing the impact of mixtures in the environment, including methods to assess the degree to which a site is impaired and the eco-epidemiological methods used to attribute the cause of such impairments. Guidance will be presented on suitable methods and future research needs.

WE 313
Displacement of test chemicals from serum constituents in mixtures and its effect on free concentrations and in vitro assay results
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In vitro assays may be used to estimate the toxicity of chemical mixtures. The concentrations of chemicals in these assays are normally expressed as nominal concentrations. However, the freely available concentrations may be much lower than the nominal concentrations because the chemicals may bind to serum constituents in the culture medium. When chemicals are exposed to an *in vitro* assay in a mixture, one chemical that is normally bound to serum proteins (and thus has a low free concentration) may be displaced from serum proteins by another chemical that binds more strongly to these proteins, thus increasing the free concentration and response of the first chemical in the assay. When nominal concentrations are used, one could falsely attribute this increase in response as being a direct effect of the second chemical. Therefore, the aim of this study was to measure the free concentration of polychlorinated biphenyls (PCBs), individually and in a mixture, in a CAFLUX assay, using solid phase microextraction (SPME). Results indicate that mixture effects attributed to non-AhR agonists may in part be explained by the displacement of AhR-agonists (PCB 118, 126, 156) from serum constituents by non-AhR agonists (PCB 28, 138, 153).

WE 314
Mixture toxicity in practice: what are the needs?
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Addressing the topic of mixture toxicity and its integration into risk assessment is an important task, which requires close collaboration between science and regulatory authorities. Furthermore it is essential to sensitize different stakeholders, such as industry, authorities, and research organizations, as well as the general public to this problem. With this aim, a workshop on mixture toxicity organized by the Swiss Centre for Applied Ecotoxicology, in collaboration with the Swiss Centre for Applied Human Toxicology, took place in Switzerland in November 2010.
During/at this workshop, participants from various stakeholder groups and international experts on mixture toxicity discussed the available models and assessment methods (including risk assessment of mixtures), whereby not only component-based mixtures, but also interactions of substances with abiotic stressors were considered. Insight into regulatory challenges resulting from mixture toxicity was presented, and the workshop was concluded by group discussions and an overall discussion on important issues in mixture toxicity. Challenges for assessing mixture toxicity were identified by representatives from practice, regulation and science, and several examples were presented on how those difficulties can be overcome.
The participants addressed the need for good communication between science and regulation, and supported the effort to jointly - that is with participants from eco- and human toxicology - tackle the challenges in mixture toxicity and effect assessment. Effect based biotest systems were highlighted as useful tools to assess the toxicity of mixtures, however, the costs of those assays as well as the applicability for regulation were important points and concerns. Here the desire to have a 'tool box' for measuring and assessing mixture toxicity was expressed. The poster gives an overview of the problems identified, suggestions how to solve them and lessons learned.

WE 315
Environmental and health impact of unknown / unregulated chemical mixtures - case study of the State of Punjab, India
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Sunrakshan Foundation shares its experience in the mind set of the regulators, both at the Centre and State level. The problems of keeping a check on the complex chemical mixtures finding its way into agriculture and its impact on human health. The various laws available and enforced still have a loophole in controlling such mixtures finding its way into the environment, primarily as a result of unorganized industrialization. A typical problem in Punjab, the bread basket state and pioneer of the famous Green Revolution of INDIA.

S07P - Special Poster Session: 30 years of Environmental Systems Research - A tribute to Michael Matthies

In the last 30 years, considerable progress was achieved in the field of environmental research by means of systems analysis and application of mathematical simulation models with active and envisioning contributions from Michael Matthies. Some landmarks of his scientific career are highlighted in this poster session.

Predicting the fate of the numerous environmental contaminants is a major challenge in chemical risk assessment and management. The development of multimedia fate and transport models was motivated by the objective to simulate the environmental fate of organic chemicals more realistically. Explicit modelling of partitioning into and transport between different environmental compartments beyond simple equilibrium approaches allow considering the effects of the emission compartment, kinetics of inter-media transfer and loss processes. To reduce the effort in the determination of substance specific input parameters, quantitative structure-activity relationships (QSAR) were developed. These methods predict substance properties by correlating them with chemical structure.

Introduction of specific measures such as overall persistence (Pov) and characteristic travel distance (CTD) within the model ELPOS constitutes a well acknowledged contribution in the field of assessment of chemicals with respect to their persistence and long-range transport potential (LRTP). Bioaccumulation potential as of specific interest for PBT assessment was recently integrated into ELPOS by combining a food chain model with the fate model. In this way, multimedia models have become a powerful tool for the assessment of organic chemicals according to prevailing legislations like the Stockholm Convention and the European chemical legislation REACH.

Michael Matthies was among the first researchers to investigate the uptake of organic pollutants into plants and subsequent accumulation therein. With a simple one compartment model, he was able to predict internal concentrations of plants based on a few physico-chemical parameters. Advanced model approaches split up the plant into separate compartments such as root, stem, leaves or fruits and consider temporal variability. These models proved to be useful tools e.g. to predict concentrations of chemicals in plants in the scope of risk assessment procedures.

Basic research elucidating the possible need to refine environmental risk assessment for veterinary antibiotics took advantage of model-based analysis and integration of experimental results. Simulation models for the fate of the sulfonamide sulfadiazine in soil were coupled via cellular uptake with the effect on the cellular level and furthermore used to predict effects concerning soil nitrification and the selection of antibiotic resistance genes.

Early Environmental Information Systems basically followed database driven approaches and provided access to homogenous datasets. A major focus was on chemical data needed for environmental exposure assessment and hazard management. Later on, Matthies and colleagues used information systems to structure the growing amount of heterogeneous environmental data for use in appropriate simulation models. With the development of Geographic Information Systems (GIS) consideration of the spatial dimension of environmental problems became possible, for instance in impact assessment of atmospheric heavy metal deposition or regional groundwater contaminations.

Environmental exposure assessment of aquatic micropollutants needs to consider spatial and temporal variability of concentrations in river basins. The software tool GREAT-ER (Georeferenced Regional Exposure Assessment Tool for European Rivers) has been developed to simulate surface water concentrations as an effect of overlying multipoint and diffuse sources into receiving waters under the aegis of Michael Matthies. The model considers emissions from local sources and calculates concentrations in whole river basins on a high spatial resolution (≤ 2000 m) taking into account removal and transport processes. It has been successfully applied to simulate surface water concentrations of typical household chemicals, pharmaceuticals and metals in various catchments. Temporal variability can be included within a Monte-Carlo type probabilistic simulation routine. By coupling with a GIS, resulting concentration patterns can be visualized in form of digital colour-coded maps or concentration profiles of selected rivers. This enables investigation of spatial concentration variability and analysis of exceedance of environmental quality standards. The model can be embedded in integrated river basin management systems which constitutes a useful tool in water quality management of aquatic micro pollutants. It was already successfully linked to an environmental information system and coupled with analysis tools to build up a Decision Support System (DSS) for integrated management tasks in the Elbe catchment.

WE 320
From integrated modelling to decision support
J Berlekamp
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WE 321
unPOPular: defining long-range transport and persistence of POPs
A Beyer
Biotechnology Center, TU Dresden, DRESDEN, Germany

WE 322
Modelling and data analysis of chemicals
R Brüggemann
Leibniz-Institute of Freshwater Ecology and Inland Fisheries, BERLIN, Germany

WE 323
A GIS integrated dynamic model to predict runoff water pesticide concentrations in agricultural basins
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WE 324
Veterinary medicines in soils: Basic research for risk analysis
A Focks
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WE 325
Surface water exposure to chemicals - Recent developments of the GREAT-ER model tool
J Klasmeier
University Osnabrueck, OSNABRUECK, Germany

WE 326
Characteristic Travel Distance (CTD): The use of multimedia models to track the geographic range of pollutants
TE Mckone
University of California, BERKELEY CA, United States of America

WE 327
Intercomparison of multimedia environmental fate models and the OECD Screening Tool for Overall Persistence and Long-Range Transport Potential
M Scheringer
Swiss Federal Institute of Technology, ZURICH, Switzerland

WE 328
Simultaneous simulations of uptake into plants and leaching to groundwater
S Trapp
Technical University of Denmark, KGS. LYNGBY, Denmark

WE 329
Definition, calculation and measurement of POV and LRTP
M van de Meent
National Institute for public health and the environment (RIVM), BILTHOVEN, The Netherlands

WE 330
Poster for Special Session Michael Matthies
K Voigt
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WE 331
Evaluation of chemicals for PBT or POP properties using multimedia fate modeling
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SS06P - SETAC/ISES poster session - Integrating the sciences & development of methods, approaches & tools to meet emerging exp. needs in chem. reg.

WT 338
Black Carbon Measurements is Effective in Detecting the Benefits of Traffic Restriction Policy on Outdoor Air Quality - the Field Study of Ecopass Area in Milan, Italy
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OBJECTIVE: To verify if the Black Carbon (BC) measurements could detect and demonstrate more suitably than PM differences in local urban air quality among three traffic scheme zones: unrestricted traffic, Ecopass zone with traffic restrictions, and pedestrian zone in the Milan city center.
METHOD: Instruments: measurements of BC with Aethalometer AE51 by Magee Scientific, USA and PM_{10}, $PM_{2.5}$, PM_1 with a mass precalibrated Aerocet 531 by Metone Instruments Inc. USA.
Procedure: concurrent measurements in three fixed stations within the three different zones repeated in three different days and from 8.00 am till 7.00 pm and with different weather conditions.
RESULTS: campaign day 1: 6.3 (2.9); 3.1 (1.7); 1.6 (0.3), Campaign day 2: 5.2 (2.3); 2.8 (1.4);,2.0 (0.4) Campaign day 3: 3.3 (1.9); 2.6 (1.8); 1.5 (0.5) mean BC (SD) levels µg/m^3 for unrestricted, ecopass and pedestrian zones respectively. The differences in mean BC levels in the same day in the different traffic scheme locations were highly significant for each comparison ($p < 0.0001$). Mean PM_{10}, $PM_{2.5}$, PM_1 concentrations did not show significant differences among the different traffic zones on the different campaign days. BC/PM_{10} ratios decreased significantly from the unrestricted zone to the pedestrian zone: on average, the BC content in PM_{10} decreased by about 52% and 65% in the Ecopass zone and the pedestrian zone, respectively, as compared to the no-restriction zone.
CONCLUSION: Different city areas with different traffic intensity and quality showed different black carbon levels. Traffic reduction within the Ecopass zone results in a significant reduction in black carbon concentrations compared to the no-restriction zone, while the pedestrian zone rated even better. These data suggest that black carbon is a highly relevant metric of traffic pollution and should be taken into consideration in demonstrating the effectiveness of air quality mitigation measures.

WT 339
Influence of Outdoor Smoking on Urban Pollution. PM and Black Carbon (BC) Concentration Measurement over a Typical Summer Weekend in the Pedestrian Brera Historical District of Milan, Italy
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INTRODUCTION. Historical Milan City Center is characterized by "street canyons", some pedestrian islands and some open car traffic. On the pedestrian areas many people are smoking worsening the outdoor air quality.
SCOPE. To compare PM_1, $PM_{2.5}$ and PM_{10} and BC concentrations in pedestrian Via Brera with parallel high trafficked Via Pontaccio, on summer weekend.
METHODS. Two analysers (Aerocet 531, MetOne) were used to record PM_1, $PM_{2.5}$ and PM_{10} and BC with two micro Aethalometers AE51 (Magee). They were placed concurrently from Friday until Monday on the balcony at first floor flats, one in Via Brera, the other in Via Pontaccio. Outdoor nicotine vapour phase was sampled using passive samplers at the same position and time of the other instruments and analysed using gas chromatography. Cigarette consumption and traffic density were estimated.
RESULTS. In the rush hours :458 smokers/h in Brera and 922 cars and motorbikes/h in Pontaccio. Means (SD) of data recorded are reported in Table 1. BC in Via Brera reached but not overcome Pontaccio concentrations during crowded hours, but all PM increased more in Brera than in Pontaccio by a increase factor of 2.38, 2.30 and 2.08 in Brera and 1.19, 1.21 and 1.22 only in Pontaccio for PM_1, $PM_{2.5}$ and PM_{10} respectively. Nicotine was below detection limit on Pontaccio (>0.02 µg/m^3) and 0.26 µg/m^3 confirming presence of ETS.
CONCLUSIONS. Outdoor ETS increase PM concentrations during the crowded hours in the pedestrian street more than in the trafficked nearby street. BC also showed an increase in the pedestrian street during the rush hours, but the difference was not as much relevant.

WT 340
Measurements of Particulate Matter (PM) pollution in the Subway System of the City of Milan, Italy
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Background. Restrictions to vehicular traffic in cities are increasingly implemented in order to reduce pollutant emission with an increase in the utilization of the subways system in the metropolitan cities. A strict monitoring of the index of air quality (IAQ) in the underground transportation system should be therefore carried out.
SCOPE. To assess the concentration of particulate matter (PM) in the subway system of Line 1, 2 and 3 of the Milan subway
METHODS. Analyzer: portable laser mass analyzer model Aerocet 531, MetOne, USA precalibrated to record PM_1, $PM_{2.5}$ and PM_{10}. Procedure: complete journeys on the three subway lines measuring the stations outdoor, the platforms and the carriages concentrations.
RESULTS. Mean (SD) outdoor: PM_1 14.6(13.3); $PM_{2.5}$ 29.5(24.3); PM_{10} 49.5(27.2) ug/m^3. Mean(SD) platforms: PM_1 19.7(9.5); $PM_{2.5}$ 62.4(29.5); PM_{10} 141.8(64.6). Mean(SD) carriages:

PM_1 21.9(11.1); $PM_{2.5}$ 100.8(65.7); PM_{10} 253.0(134.1) ug/m^3. The PM concentrations on the platforms resulted 1.2, 2.1 and 2.9 times greater than the outdoor for the PM_1, $PM_{2.5}$ and PM_{10} respectively and in the carriages of 1.3, 3.4 and 5.1.
CONCLUSIONS. PM levels were much higher in the whole underground system as compared to outdoor pollution. In the whole network PM_{10} concentrations always exceeded EU outdoor limits of 50 ug/m^3 and the suggested 25 ug/m^3 for the $PM_{2.5}$ Extremely high concentrations (over 250 ug/m^3) were found inside subway carriages. Such an exposure is considered hazardous for sensitive groups of people (respiratory and cardiovascular patients, children, pregnant women). We suggest that the air quality should be constantly controlled with fixed monitoring sites at platforms and with portable analyzers in the carriages, as in other subways in the world. Improvement in filtration efficiency on the carriages and installation on all platforms of screen door equipments is envisioned to reduce avoidable exposure to peak PM concentrations of thousands of commuters.

WT 341

The impact of no-traffic Sundays on atmospheric pollution in the City of Milan, January 2011: black carbon concentration measurement as a reliable traffic pollution metric

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INTRODUCTION. On February 2011 the PM_{10} concentrations in Milan exceeded the limit of 50 µg/m^3 for 35 days and the Municipality decided to stop the traffic on two consecutive Sundays.
SCOPE. To evaluate the efficacy on air quality of Sundays traffic limitation measuring Black Carbon (BC) content in Particulate Matter (PM).
METHODS. Analyzers: A precalibrated analysers (Aerocet 531, MetOne) was used to record mass of PM_{10} and a micro Aethalometer AE51 (Magee) for BC. Procedure: PM and BC concentrations were measured at fixed minitoring site at the same hours at the curbside of a high-traffic street of Milan city center and from Friday 4th until Sunday 6th February, 2011. During the Sunday they also measured the concentration before and after the cessation of the traffic restrictions. Traffic density was also measured.
RESULTS. On Friday, Saturday and Sunday PM_{10} mean (SD) concentrations were 89.8 (10.6), 78.3 (15.9) and 120.6 (42.23) µg/m^3, while BC concentrations were 9.5 (3.0), 11.4 (3.7) and 8.1(1.8), respectively. The percent of BC in PM_{10} was 10.6, 14.6 and 6.6 percent respectively. Traffic density was 1,600/2,000 and 350/400 vehicles/h on no-restriction and restriction time, respectively. The BC in PM_{10} percent increased from 6.6 to 12.4 one hour after the cessation of traffic restrictions on Sunday. Mean (SD) BC was 8.1(1.8) and 17.6(3.9) µg/m^3 in the last hour of restriction and in the first hour after resumption of full traffic, respectively ($p < 0.0001$).
DISCUSSION. Despite the considerable increase in PM_{10} on Sunday as compared with Saturday, BC concentrations were lower both in absolute values and in percent in PM_{10} indicating a reduction in carbonaceous pollutants.
CONCLUSIONS. No-traffic Sundays seem to bring immediate benefits in air quality regardless the absolute values of PM concentrations. This effect is limited to the interested zones; however the benefits disappear after less than one hour after normal traffic resumption.

WT 342

Seasonal source apportionment of volatile organic compounds in Windsor, Ontario

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Air quality in Windsor, Ontario Canada is heavily impacted by the automotive industry within the city, the industrial sources in Ohio and Michigan (US), and the large volume of car and trucks crossing the border on Huron Church road leading to the Ambassador Bridge. Both emissions and ambient concentrations are expected to change with season. A source apportionment study was conducted using the Chemical Mass Balance (CMB) model. Once every 6-day, 6 Liter Summa canisters were used to collect 24-hr air samples at Windsor West Station operated by Environment Canada. There were 25 samples in 2005 during winter (Jan-March) and fall (Oct-Dec). The samples were analyzed, yielding concentrations of 188 volatile organic compounds. Source profiles were selected based on a previous study in Windsor. The CMB results showed large inter-season and intra-season variability. Vehicle emissions (Diesel Exhaust, Gasoline Exhaust, Liquid Gasoline, Gasoline Vapour) were consistently the greatest sources, 54% in winter and 60% in fall. Contribution from Industrial Refinery was higher in winter (21%) than in fall (18%). Significant contributions from Commercial Natural Gas (17%) and Liquefied Petroleum (7%) were observed in both seasons. The contributions from Biogenic and Coating sources were small and varied little between the two seasons.

WT 343

Simulation and instrumental data conjugation for ambient air monitoring

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The proposed method includes the conjugation of simulated air pollutant dispersion (in Russia, atmospheric diffusion simulation based on the comprehensive solution of atmospheric ground layer thermo-hydrodynamics equations and turbulent diffusion equations is used) and data obtained by measurements.
Air quality measurements are known to be characterized by high accuracy but low spatial reliability. Simulation of pollutant distribution provides a reliable spatial exposure assessment but it is not sufficiently accurate. The conjugation of spatial distribution characteristics obtained by measurements and simulation data allows us to reduce uncertainties in both methods.
The technique includes the conjugation of field data interpolated to the system of points within the studied area using Delaunay triangulation, and simulation data in the same points. It also calculates compliance coefficients in the points of measurements (fixed monitoring stations) and then approximates the coefficients over the studied area.
The method was applied in the city of Perm with the total area of 799.68 km2 and the population estimated at 985 000 people. In Perm, 125.0 thousand tons of more than 340 pollutants are annually emitted into the atmosphere. Pollutant distribution simulation and the conjugation of the simulation data and instrumental data from seven monitoring stations were carried out for 7.5 thousand points within residential districts. The verification of the obtained data by check measurements showed an improvement of simulation and instrumental data convergence up to 70-95% compared to the previously recorded convergence of 8-50%. We also determined zones with high air pollution levels which could not be identified using linear interpolation of instrumental data and/or were not estimated as polluted zones by the analysis of pollutant distribution simulation.
The method can be used for sanitation and epidemiology studies, investigations, expert examinations and assessments

WT 344

Application of Adsorption Sampling and Thermal Desorption with GC Analysis for the Measurement of Low-Molecular Weight Polycyclic Aromatic Hydrocarbons in Ambient Air

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Polycyclic aromatic hydrocarbons (PAHs) have been of particular concern since they are present both in the vapor and particulate phases in ambient air. Historically, polyurethane foam(PUF) or XAD-2 have been widely used for the vapor phase PAHs. However, they have shortcomings such as breakthrough of samples and inevitable pressure drop during sampling. Furthermore, collected samples may be significantly lost during solvent extraction and concentration procedures. In this study, a simple method was applied to determine gas phase PAH, and the performance of the new method was evaluated with conventional method. The method was based on adsorption sampling and thermal desorption with GC/MS analysis, which is generally applied to VOC measurement. Target compounds included two rings PAH such as naphthalene, acenaphthylene, and acenaphthene. As a reference method for comparison purposes, adsorption sampling using XAD-2 and solvent extraction with GC analysis was adapted. The performance of the adsorption sampling and thermal desorption with GC analysis for the measurement of low-molecular weight PAHs in ambient air were evaluated to be satisfactory because repeatabilities of standard sample and target sample are all within 20%. Also, lower detection limits value was estimated to be less than 0.1 ppb. The results from comparison studies between two methods for real air samples revealed the correlation coefficients were more than 0.8, and the difference between two groups of data from the two different methods appeared to be all statistically not significant. Although the adsorption sampling and thermal desorption method tested in this study has shortcomings that the method can not determine higher-molecular weight PAHs in the particulate phase, the method is very simple, rapid, and reliable for lower-molecular weight PAHs. In addition, the method can be used for the simultaneous measurement of toxic VOCs, including benzene, toluene, xylenes, and naphthalene.

WT 345

Exposure and toxicity assessment for size-related airborne particulate matter from nearby traffic in Seoul

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Automobiles are considered as the primary source of air pollution and many studies are being conducted especially on the risk of particulate matter (PM). We investigated the particle size distribution and chemical properties in the urban air and the capacity of PM to induce cytotoxicity in human bronchial epithelial (BEAS-2B) cells. We collected PM, sized from 0.056 to 10um on the road side, using MOUDI in Seoul, Korea from Dec. 2007 to Dec. 2009.
Concentrations of PM showed bi-modal distribution (peaking at 0.18-0.32 and 1.8-3.2um) and average concentrations of PM10 and PM1.0 were 38.3ug/m^3 and 21.9ug/m^3, respectively. Our study shows that the particle size was affected by the seasonality and traffic condition, such that the concentration increased in 0.1-1.0um size as the number of vehicles increased.
We observed PM concentration-dependent cytotoxic effects in BEAS-2B cells. We found that exposure to PM2.5 and PM10 from nearby traffic area induced significant increase in gene expression of inflammatory cytokines (IL-6 and IL-8). The cell death rate and release of cytokines in response to the PM2.5 treatment were higher than those of the PM10. The occurrence rates of reactive oxygen species (ROS) were also higher in the order of PM1.0, PM2.5 and PM10 at same treated PM dose. We constructed a dose-response profile by fitting BMD models on oxidative stress considering ROS generation as the endpoint.
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WT 346

Research and Analysis on the Current Situation of the Noise Pollution in Xi'an City's Acoustic Environmental Domains

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Objective: To find out the noise pollution situation in the acoustic environmental domains in Xi'an city. Method: Measure the noise level in Xi'an city's domains day and night during a year around, and report the monitor result in the seasonal average and year average of equivalent sound level. Result: The monitor result of the noise pollution in Xi'an city's five kinds of acoustic environmental domains is listed as follows: Special residential area's daytime noise all the year is within the national standard, yet the night noise is all beyond it; Residential culture and education area only has one kind of noise level within the national limit, which is the daytime of the second season; The mixed area's daytime noise is without the limit in the second season; The industrial area's daytime noise is all within the limit, however the night is beyond the limit; The area around the main transportation routes has qualified daytime noise for all four season, yet the night noise is beyond the limit. Conclusion: The situation of noise exceeding the national limit exists in all domains in the city, and the night noise situation is more serious. Besides, the noise levels in the night time of the third season in the industrial area and the daytime of the second season of the mixed area have an obvious increase compared with other seasons, which might affect the Xi'an city residents' normal quality of life, and should be controlled.

WT 347

Transfer of chlorinated volatile organic compounds from soil and groundwater into indoor air buildings.

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Soil vapor migration into house, with subsequent inhalation, is often the main exposure pathway to humans at sites contaminated with Volatile Organic Compounds (VOCs). Two approaches are commonly used for quantification of indoor concentrations: indoor gas measurement or transfer modeling from the source.
Model development is relatively well advanced [1-3] but measurements for model calibration and 'validation' hardly exist in the literature. Furthermore, predictions of indoor gas concentrations

from different models may vary by several orders of magnitude, depending on the application [4]. The PhD work presented here consists in comparing modelled results and experimental measurements on a test site. The site is a factory, contaminated with chlorinated solvents in unsaturated soils and groundwater. Measurements concerned contaminant concentrations and fluxes in different media and at different transfer stages, but also key model parameters. The equations of Johnson & Ettinger and VOLASOIL models were used for this comparison.

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WT 348

Characterizing the spatial heterogeneity of on-road motor vehicle intake fraction

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On-road motor vehicles (MVs) in the United States (US) emit approximately 10^9 kg/y of conserved (non-reactive and non-depositing) hazardous air pollutants (HAPs) to the atmosphere. There is a need to spatially resolve the population impacts associated with these HAP emissions. To address this need, we estimate population impacts using the intake fraction (iF)--the ratio of the chemical mass taken in by a population to the mass released. The population iF [ppm] is estimated from the on-road MV source-receptor relationships derived from the US Environmental Protection Agnecy's AERMOD model, which was employed in the latest (2005) National Air Toxics Assessment (NATA). AERMOD is an advanced local-scale air quality model that provides S-R relationships (χ/Q), in mg/m^3 per ton/y emitted, for the roughly 65,000 census tracts in the continental US.

With AERMOD, we estimate iF for within county receptors, and separately for extra-county receptors. In our California case study, the resulting population iF ranges between 0.5 to 328 ppm--four orders of magnitude. We also compare the within county AERMOD iFs with a county-level iF derived from a box model, based on breathing rate, population, mixing height, wind speed and county area. The box-model iF tracks the lower range of the within-county AERMOD iF. The AERMOD extra-county iFs are generally 1-2 orders of magnitude lower than the range of the within-county iFs. Exceptions are counties with smaller land area and high population density. We are expanding our approach to characterize the within-county and extra-county iF for every county in the continental US. Additionally, we are introducing persistence (reaction and degradation) of each on-road-MV-emitted HAP to the box-model derived iF. Results can be easily incorporated in health impact assessments of on-road MVs at various spatial scales, such as county, regional, and national scales.

WT 349

A population-based modeling framework to assess health impacts from indoor pollutants emitted from gas cooking burners

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Natural gas cooking burners - including cooktop, oven and broiler burners - emit substantial quantities of air pollutants, such as CO, NOx, and HCHO, that can affect residential indoor air quality, pollutant exposures, and related health risks. To characterize health impacts from gas cooking burners we use a physics-based, data-driven simulation model to build virtual cohorts of homes that are representative of residences in the state of California. Time dependent indoor pollutant concentrations are calculated for each residence, taking into account emissions, dilution, deposition to surfaces, removal by air exchange, as well as pollutants entering with outdoor air. Household and building characteristics influencing these processes are drawn from several data sources, including a representative statewide residential appliance use survey of over 10,000 anonymous households, the US Environmental Protection Agency's (EPA's) National Human Activity Patterns Survey, and a web-based cooking activity survey we conducted to gather additional data on household cooking activity patterns. By linking household specific cooking activity data and residential characteristics we account for the co-variances between the inputs. Based on a literature review, source-proximity effects to the "cooker"and to young children are also incorporated in the model.

Health impacts can be assessed at various spatial scales (county to regional). Impacts are quantified based on modeled peak and time-averaged concentrations in residences and for individual occupants, and the frequency at which health-protective exposure limits are exceeded. Results from a recent model application suggest that nearly 40% of all households in Southern California that use gas cooking burners exceed the US EPA's 1-hr ambient NO2 standard (190 μg/m^3) at least once during a typical week in winter. The model is ultimately intended to inform and guide public health policy related to changing gas supplies in California.

WT 350

Validation of the deterministic indoor air model SprayExpo

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The deterministic indoor air model "SprayExpo"calculating inhalation exposure to aerosols released during professional liquid spraying processes and containing non-evaporable active components has been validated. The model calculates the concentrations of spray related non-volatile aerosols in various health-relevant particle size fractions independent from the room volume. It takes into account turbulent diffusion, droplet evaporation, and gravitational settling.

Sensitivity analysis has been performed to reveal the most influencing parameters. For validation, modelled values have been compared with measured values carried out at real workplaces in the fields of antifouling treatment and stored-product protection. The exposure concentrations of the active substances used were determined by time-resolved and particle size-segregated personal sampling and subsequent chemical analysis. In addition, the modelling results of SprayExpo have been compared with similar modelling using ConsExpo and BG-Spray.

SprayExpo was found to be an appropriate model for assessing exposures during indoor spraying processes and especially suited for large room volumes. The sensitivity analysis confirmed the assumption that besides the substance release rate, the droplet spectrum of the spraying device is the process parameter which decisively influences the exposure.

Validation of SprayExpo is of relevance for acceptance of this tool to assess the exposure to hazardous substances in indoor air. For regulatory purposes such as the Biocidal Products Directive 98/8/EC, an improved validated model is now available to estimate inhalation exposure to non-evaporable active substances during spray applications.

WT 351

A mechanistically-based model for estimating exposure to volatile insecticides from a solid resin matrix ('pest strip')

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A variety of pesticides and other chemicals are formulated in solid resin matrices (e.g., flea and tick collars, pest strips, air fresheners and cleaners, etc.), which permit a controlled release of the active substance from the resin. Controlled-release formulations are very important drug delivery vehicles in pharmacology, but also in other areas, such as chemical synthesis, agricultural fertilizers, pheromone dispersal, and pest control. Controlled-release of a solute from a solid matrix can be governed by diffusion of the solute through the matrix, 'degradation' of the matrix, or both. This poster describes a mathematical model that can accurately predict the release of chemicals contained in such resin formulations. The model incorporates the diffusion-based slow release mechanism, with parameters estimated using experimental chamber data, and it is independently validated against a large database of measured room concentrations for one of the most common applications of chemical-laden resins, i.e., dichlorvos (DDVP) contained in commercially available "Pest Strips". This poster also outlines an exposure assessment that combines reliable monitoring data for long-term ambient dichlorvos concentrations in relevant indoor situations with mathematical simulation modeling of other situations for which monitoring data are lacking. The exposure assessment demonstrates the accuracy of the model predictions compared to the existing monitoring data.

WT 352

Perfluorinated Compounds in Human Blood, Freshwater Fish and Seafood from China: Regional and Global Implications for Human Exposure

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Despite the growing public interest in perfluorinated compounds (PFCs), very few studies have reported the sources and pathways of human exposure to these compounds. In this study, concentrations of PFCs were measured in human blood (n=93) and freshwater fish and seafood (n=73) samples collected from China to determine residue levels, dietary intakes, regional differences in human exposures, and risk associated with ingestion of PFCs from a primary food source for the Chinese population. The highest mean perfluorooctane sulfonate (PFOS) concentration in human blood was 12.5 ng/mL (from Tianjin), and 25.4 ng/g wet wt (or 0.92 ng/g wet wt after excluding an outlier value) in freshwater fish and seafood. An anomalously high concentration of PFOS was found in a crucian carp collected from Wuhan at 1610 ng/g wet wt. The daily intake of PFOS, perfluorooctanoic acid (PFOA), and perfluoroundecanoic acid (PFUnDA) via fish and seafood consumption (EDIfish&seafood) ranged from 0.10 to 2.51, 0.13 to 0.38, and 0.16 to 0.32 ng/kg bw/d, respectively, for different age groups (i.e., toddlers, adolescents and children, and adults) from selected locations (i.e., Tianjin, Nanchang, Wuhan, Shenyang). The daily dietary PFC intake values increased ($p < 0.05$) with age. Comparison of EDIfish&seafood values with the modeled total dietary intake (TDI) of PFCs by adults from Tianjin, Nanchang, Wuhan and Shenyang, showed that contributions of fish and seafood to TDI of PFOS varied depending on the location. Fish and seafood accounted for 6%, 25%, 80%, and 85% of PFOS intake in Nanchang, Shenyang, Wuhan and Tianjin, respectively, suggesting regional difference in human exposure to PFOS. Based on the export of fish and fishery products from China in 2009, the outflow of PFCs was estimated to range from 0.22 (perfluorohexane sulfonate, PFHxS) to 3.42 kg (PFOS); the outflow of PFOA, PFUnDA and sum of PFCs was 0.87, 0.84, and 6.59 kg, respectively.

WT 354

Human biomonitoring to assess exposure of Norwegian infants to perfluorinated compounds

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Perfluoroalkyl compounds (PFCs) comprise a large group of man-made fluorinated organic compounds used in numerous consumer products and industrial applications. Several PFCs have been shown to be persistent and bioaccumulative and have been found widespread in the environment and in humans. Animal studies have demonstrated hepatotoxicity, developmental toxicity, immunotoxicity as well as hormonal effects. In order to explore pre- and postnatal exposure to PFCs, we have initiated several studies.

Up to 7 PFCs were detected in the 123 paired samples of maternal and umbilical cord blood plasma. The maternal and fetal levels were significantly correlated for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA). The relative proportion of PFHxS was higher than that of PFOS in cord blood compared to maternal blood, and it was higher for PFOA than for PFNA and PFUnDA. This indicates that the chain length of the fluorinated compound is an important determinant for placental passage. Mean PFC concentrations in cord blood were 34 to 84% of the maternal concentrations.

Concentrations of PFOS and PFOA were determined in paired samples of serum and breast milk from 19 Norwegian mothers. Breast milk concentrations were only 1.4 and 3.8% of the serum concentrations, respectively, and the relationships were linear. Assuming a consumption of 700 ml breast milk/day the intakes from breast milk are 61 for PFOS and 29 ng/day for PFOA. In comparison, a dietary intake of 113 and 44 ng/day has been estimated for the adult Norwegian population.

Finally, to study rates of elimination through breastfeeding, 9 primiparous mothers collected

breast milk samples monthly from about two weeks after birth and up to 12 months. Using linear mixed effect models, the depuration rates for PFOS and PFOA were calculated to be 3.1 and 7.7% per month, respectively.

WT 355

PBDEs, HBCDs and TBBPA internal level in blood serum from Neonates-Mother paired samples in Korean population and estimating various parameters on internal accumulation of 3 BFRs in human serum

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This study investigated three brominated flame retardants compromising 23 tri- to deca- BDEs, TBBPA, alpha-, beta- and gamma- HBCDs in 38 pair blood serum samples of mother and neonates pair samples from Korea population. Also, the effect of environmental factors or impact to health status like thyroid function on internal accumulation of BFRs with 76 blood serum samples were analyzed statistically. Total PBDE concentration in blood serum was ranged from 1.559 to 50.85 ng/g lipid (mean: 17.72 ng/g-lipid) in normal group mothers and from 0.829 to 252.9 ng/g lipid (mean: 46.29 ng/g-lipid) in normal group neonates. In case of patient group, Σ3-7 BDEs concentration was detected from 3.692 to 1563 ng/g lipid (mean: 83.58 ng/g-lipid) for mothers and from 2.216 to 860.2 ng/g lipid (58.25 ng/g-lipid) for neonates. Total HBCDs concentration in normal mothers was ranged from 0.450 to 99.79 ng/g lipid (mean: 12.72 ng/g-lipid) and in normal neonates was ranged from <LOQ to 165.6 ng/g lipid (mean: 17.47 ng/g-lipid). In patient group, total HBCDs concentration was detected from <LOQ to 14.15 ng/g lipid (mean: 3.164 ng/g-lipid) for mothers and from <LOQ to 69.96 ng/g lipid (mean: 7.884 ng/g-lipid) for neonates. Total HBCDs were possessed less than 20% among 3 BFRs in four groups of in this study but relatively highly observed in normal mothers as 31%. TBBPA was analyzed for 76 blood samples and ranged from below LOQ to 73.96 ng/g-lipid (mean: 10.93 ng/g-lipid) in normal group mothers and from below LOQ to 457.4 ng/g lipid (mean: 77.65 ng/g-lipid) in normal group neonates. In case of patient group, TBBPA concentration was detected from <LOQ to 48.25 ng/g lipid (mean: 8.888 ng/g-lipid) for mothers and from <LOQ to 713.6 ng/g lipid (mean: 83.42 ng/g-lipid) for neonates. Among various environmental factors-BDEs with computer use hours and chemical substances or industrial chemicals, HBCDs with production year of car, TBBPA with coffee intake frequency was shown the highest correlation.

WT 356

A biomonitoring study designed to estimate human exposures to pyrethroid pesticides in residential settings using an exposure reconstruction framework

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Biomonitoring is an important tool for understanding the linkages between external chemical exposures, internal doses and potential health outcomes in humans. In recent years, there has been an explosion of available urinary biomonitoring data. However, urinary biomarkers by themselves cannot be used to quantitatively reconstruct human exposures to non-persistent chemicals (e.g., pyrethroids). Exposure reconstruction is a process of estimating an external exposure that is consistent with biomonitoring data and requires modeling tools and other key data such as environmental and biomarker measurements, activity pattern, and chemical toxicokinetics. To aid in identifying critical research components and parameters to improve biomarker-based exposure assessments for non-persistent chemicals, we have designed an exposure reconstruction framework. Using the framework, we identified the major data gaps and modeling inputs that were likely needed to improve exposure estimates for the pyrethroid insecticides. 'The Pilot Study to Estimate Human Exposures to Pyrethroids Using An Exposure Reconstruction Approach' was designed to collect these specific types of data including the temporal variability of pyrethroids and their metabolites in environmental media, cumulative oral intake estimates of pyrethroids/metabolites, temporal variability of urinary pyrethroid metabolites, and temporal food, pesticide-use, and activity pattern data. Adults collect their own environmental (food, water, dust, and surface wipes) and biological (urine) samples and diaries at their homes in North Carolina with repeated sample collection for a total of three weeks over a six-week monitoring period. Current-use pyrethroids and their metabolites (environmentally-occurring and urinary) are being measured in the environmental and/or urine samples. This study will assess the feasibility of using an exposure reconstruction approach to estimate 50 adult exposures to pyrethroids using urinary biomarkers.

WT 357

Community Duplicate Diet Methodology: A New Tool for Estimating Dietary Intakes of Pesticides

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True dietary exposure to pesticides is determined by collection and analysis of food samples. Participant responses to questionnaires about acceptability during a recent field study showed the Community Duplicate Diet approach to be feasible as a dietary collection strategy while maintaining low participant burden. The calculation of dietary intake of selected pesticides was accomplished using food samples collected from individual representatives of a defined demographic community. Successfully determining the intake of pesticides through the dietary route will allow for an accurate assessment of the potential exposures to individuals within the community; thereby significantly enhancing the research conducted under large scale epidemiological studies.

For nine participants selected from among a community in Apopka, Fl, intakes of organophosphate (OP) and pyrethroid pesticides were 140 - 1335 ng and 1017 - 24,282 ng, respectively, based on analysis of individual samples collected over one week. Individual samples were collected for breakfast, lunch, dinner, and snack; although not all individuals collected samples at each event. Utilizing individual samples, a community intake was calculated to be 1650 ng for OPs and 37,746 ng for pyrethroid pesticides.

The results showed higher measured levels of pyrethroid pesticides than for OPs, which is consistent with their decreased usage. The diversity of the pyrethroid pesticides detected in food samples was greater than expected. Bifenthrin was detected in several samples, indicating an increase in its use compared to permethrin. Continually changing pesticide usage patterns need to be considered when determining the analytes of interest for large scale epidemiology studies. The Community Duplicate Diet Methodology is a tool for researchers to meet emerging exposure measurement needs that will lead to a more accurate assessment of intake which may in turn enhance decisions for chemical regulation.

WT 358

Estimating the number of Canadian agricultural workers exposed to pesticides

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Introduction

Pesticide exposure surveillance for Canadian agricultural workers is challenging due to a lack of detailed occupational data. Large amounts of pesticides that are classified as "possible"human carcinogens by the International Agency for Research on Cancer (IARC) are used for agriculture. Using a single province (Alberta) as a prototype, the objective is to estimate the number of agricultural workers at risk of carcinogenic pesticide exposure and to geographically characterize exposure by region.

Methods

Canadian Labour Force Survey (LFS, 2006) data on number of Alberta (AB) workers by job title (NOCS - 2006) is combined with regional (ecodistrict) estimates of annual pesticide use (APU). APU's are derived using data from the Interpolated Census of Agriculture (2006) and pesticide use information from the Ministry of Agriculture and Pesticide Management Regulatory Agency. Ecodistricts are categorized into four exposure groups based on APU quartiles, and mapped using a Geographic Information System (GIS).

Results

LFS reports 18,030 agricultural workers in AB broken down by NOCS titles: 'farmers and farm managers' (n=11,510), 'agricultural and related service contractors and managers' (n=20), 'farm supervisors and specialized livestock workers' (n=635), general farm workers (n=5,715), and 'harvesting labourers' (n=150). Agriculture occurs in 110 of 150 (73%) AB ecodistricts. APU estimates (tonnes) were derived for 2,4-D (mean 111; range 0-61) and Chlorothalonil (mean 100; range 0-1029). Using GIS, ecodistricts were mapped by exposure group to visually identify high risk exposure regions for agricultural workers.

Conclusions

Information on the number of agricultural workers at risk of exposure to IARC "possibly"carcinogenic pesticides is necessary to understand the impact of these pesticides. This work is limited by only having access to occupational data at the provincial level, when estimates of pesticide use are most useful at the sub-provincial level.

WT 359

Estimated Dietary intake of Bifenthrin and Indoxacarb in Leaf Mustard

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This study was carried out to estimate the dietary intakes of pesticide residues of commonly used insecticides, bifenthrin and indoxacarb in leaf mustard. The pesticides were sprayed onto the leaf mustard at recommended and double doses at the 14 days before the harvest and then sampling was done at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 14 days after spraying under greenhouse conditions. The amounts of pesticides residues in the crop were analyzed with GC-ECD. Limites of detection(LODs) of both bifenthrin and indoxacarb were 0.008 mg kg^{-1}. Mean of the recoveries were from 110.1 to 112.9% in case of bifethrin and from 102.8 to 111.0% in case of indoxacarb, respectively. Biological half-lives of bifenthrin and indoxacarb were 6.390 and 6.211 days at the recommended dose and 5.158 and 4.716 days at the double dose, respectively. The estimated daily intakes (EDIs) of the pesticides in the crop harvested at 14days after spraying were less than 0.05% of their acceptable daily intakes (ADIs), representing that risk of the pesticides were generally low.

WT 360

Human dietary exposure to Uranium in Catalonia, Spain

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Uranium (U) is a health concern as a potential carcinogen and as a causal agent of kidney dysfunction. Human exposure to U can be through dermal contact, inhalation, or ingestion through water or food. In natural environments, uranium isotopes include 238U (99.3%), 235U (0.71%), and 234U (0.006%).

At the moment, we had no information on the concentrations of U in foodstuffs. The main purpose of this study was to establish the temporal trend in the daily dietary intake of U by the population of Catalonia, Spain. Fish and seafood, vegetables, fruits, and rice, were randomly acquired in 12 localities of the zone under evaluation between March and June. U levels were determined by high-resolution gas chromatography-high-resolution mass spectrometry. The dietary intake of U was subsequently estimated for the population of Catalonia, and the results will be the basis for a future survey. The highest U concentrations were found in fish and seafood, dairy products, meat and meat products and bakery products, with mean levels of 0.0199, 0.0105, 0.0061 and 0.0053 ng/g of fresh weight. U intake was estimated for four population groups: children, adolescents, adults, and seniors (aged >65 years). The highest and lowest U intake corresponded to children and senior, respectively. All the intakes are considerably lower than the World Health Organization tolerable daily intake, which is 0.5μg and 5μg per kg of body weight per day in humans of soluble and insoluble DU respectively. In summary, the results of this study indicate that, currently, the dietary intakes of U should not mean additional health risks for the consumers.

WT 361

Consumer exposure to food chemicals: using probabilistic modelling to determine aggregate exposure and assess susceptible human subpopulations.

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The outlined approach is to use a combination of Monte Carlo simulations, large data sets, and

probabilistic modelling to create a software tool that can determine the exposure in consumers to chemicals in food. Sources of exposure that are considered include (but are not limited to) pesticides, Food Contact Materials (FCMs), additives, flavourings, contaminants, and food ingredients. In order to accurately determine exposure to a food chemical for a population, an extensive diary of food consumption events needs to be combined with the appropriate chemical concentration data for each food. This concentration data may be may uncertain, have known variability, be the output of a model, or be at a greatly aggregated level. In order to adequately determine the risk to consumers, thousands of simulations are required. These simulations can then be collated to determine susceptible subpopulations by determining e.g. the high consumers, the high percentiles, and the main drivers of exposure. This is also the approach currently being taken in FACET (Flavourings, Additives and Food Contact Materials Exposure Task, a four year EU FP7 project).

WT 362

Evaluating different approaches to uncertainty in fugacity-based multimedia modeling: Probabilistic and non-probabilistic method

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Multimedia environmental fate models are used for evaluating the fate and distributions of organic chemicals in the environment. Uncertainty is associated with the input parameters used in these models and will thus influence the assessment of exposure and risk. Monte Carlo-simulations using assumed parametric distributions have been applied previously, but suffer from the problem of capturing true knowledge uncertainty, for example regarding the choice of distribution or uncertainty in its parameters. Uncertainty can also be described by fuzzy numbers or in combination with distributions as probability boxes. Here we report preliminary results from applying these three different approaches (fuzzy arithmetic, probability bounds analysis and parametric distributions) to characterize uncertainty in a fugacity-based equilibrium criterion level-I mass balance model in an evaluative environment. The uncertainty analysis was performed for three test compounds: benzene, pyrene, and DDT. Experimental data to characterize uncertainty in the Henry's law constant, the organic-carbon water partition coefficient, and bioconcentration factors for fish were collected from literature. Both the measurement uncertainty and the availability of data were substantially different between investigated properties and test compounds, directly influencing the description and modeling of uncertainty. The connection between possibility theory and probability theory was used to implement similar constraints for the fuzzy arithmetic and probability bounds analysis. These calculations were subsequently compared with a description of uncertainty assuming log-normal distribution. The results demonstrate a considerable uncertainty in the estimated uptake in biota, while the general distribution in the environment seems to agree well with a traditional deterministic calculation. The evaluation also highlights some of the possibilities and constraints in the assessment and description of uncertainty.

WT 363

European Solvents Industry Group Consumer Generic Exposure Scenario Tool

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The European Solvents Industry Group (ESIG) has developed a novel tool to undertake an evaluation of the safety of consumer uses of solvents, as required by the Registration, Evaluation and Authorization of Chemicals (REACH) Regulation. This tool builds upon the consumer portion of the European Center for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment tool (ECETOC - TRA, a recommended lower tier tool under REACH) by implementing refinements described in Appendix F of ECETOC technical report 107, while accounting for the application hierarchy described in Chapter R15 of the REACH Technical Guidance. The technical enhancements include utilization of additional data to refine scenario defaults and the ability to include the impact of indoor ventilation rates. When appropriate, additional exposure scenarios were developed to cover those not currently contained within the TRA. Via the inclusion of substance-specific Derived No Effect Levels, vapor pressure, and molecular weight information, the TRA tool structure was also modified to automatically determine conditions necessary for safe use (Risk Characterization Ratio <1). Using specific standard phrases, this information is then automatically reported for relevant exposure scenarios, in order that the outputs can be readily assimilated within Material Safety Data Sheet and other similar information technology systems. Transparency within the tool is maintained by including all original defaults and associated exposure predictions, as well as the refined defaults and exposure predictions.

WT 364

REACT - REACH Exposure Assessment for Consumers Tool

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REACH, the EU chemical legislation, implies the principle 'no safe use - no market'. This means that the safe use of chemicals must be demonstrated and requires that exposure assessments be performed as part of the risk assessment process. For consumer products such as cleaning and maintenance products, human exposure resulting from the domestic use of such products has to be assessed. Chapter R15 of the REACH guidance on Data requirements and Chemical Safety Assessment outlines the basis for tier-1 exposure assessments for consumers. A.I.S.E., the international association of the manufacturers of cleaning and maintenance products, concluded that the current tier-1 exposure assessment methodology results in significant over prediction of consumer exposure. Consequently, to refine exposure assessments for chemicals used in cleaning and maintenance products, A.I.S.E developed its significant habits and practices database for these products and built REACT, a dedicated consumer exposure assessment tool. REACT covers the full range of consumer products marketed by A.I.S.E. member companies. It is based on simple, transparent algorithms and has a similar structure to that of the consumer ECETOC TRA tool. REACT has been used in the 2010 registrations for raw materials by A.I.S.E. member companies.

WT 365

Evaluation of intake fraction for near and far field exposure using Computational Fluid Dynamics models

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Inhalation exposure can be represented through Intake Fraction (IF) which is defined as the mass of pollutant inhaled by an individual over a given time per total mass of pollution emitted in the environment in which the individual resides. This term has been proposed by a number of researchers as a robust tool in order to establish the link between emission sources and health effects without applying full chain modeling including mechanistic models. However, the estimation based on the Intake Fraction may contain significant errors, especially when based on the assumption of homogenous concentration in indoor environments. This is mostly the case in environments where emission sources are strongly localized and thus indoor concentrations may vary significantly even within a small distance from the source.
Various analytical models have been developed in order to assess the near and far field exposure for indoor environments, where multiple zones are considered. Among the disadvantages of such an approach is that the division of the internal environment into various zones has to take place before the analysis, thus influencing the final assessment. In the present work the use of CFD techniques is proposed in order to evaluate more accurately the near and far field exposure and thus create a more detailed map of the indoor pollutants concentration close to the occupant. The comparison of the CFD model with the available analytical multi-zone models can help to create a guide for the applicability of simplified analytical models for near and far field exposure assessments. A typical indoor office environment has been chosen for performing the case study and comparing the analytical multi-zone models with the CFD approach.

WT 366

Refined REACH consumer exposure assessment for adhesives

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REACH, the EU chemical legislation implements the principle 'no safe use - no market'. This implies that the safe use of dangerous chemicals be demonstrated and requires that exposure assessments be performed. For adhesives and sealants intended for consumer use the exposure resulting from the domestic use of such products has to be assessed. Chapter R15 of the REACH guidance on Data requirements and Chemical Safety Assessment outlines the basis for tier-1 exposure assessments for consumers. FEICA, the international association of the manufacturers of adhesives and sealants concluded that the tier-1 exposure assessment methodology results in significant overprediction of consumer exposure. Consequently, to refine exposure assessments, FEICA developed its knowledge base on the consumer use of adhesives and sealants into a dedicated exposure assessment tool. As a dedicated tool for adhesives, it covers the major categories of adhesives and sealants in consumer use. It is structurally similar to the consumer ECETOC TRA tool and based on simple, transparent algorithms. The tool has been widely used in the 2010 registrations for raw materials of FEICA member companies.

WT 367

Connectivity: causal web for exposure and risk assessment of combined exposure to chemicals

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This article presents an exposure biology approach to mechanistically-based exposure and risk assessment of environmental chemical mixtures comprising the following steps:
a) Characterization of exposure factors quantifying the parameters that affect human exposure to environmental chemicals, such as time-activity relationships, seasonal and climatic variation, and consumer choice. These exposure factors can be used to derive aggregate and cumulative exposure models, leading in probabilistic exposure assessments.
b) Current toxicological state of the art combines estimations of biologically effective dose with early biological events to derive dose-effect models, which can be used in combination with the probabilistic exposure estimates to derive biomarkers of exposure and/or effect. Combined use of epidemiological, clinical and genetic analysis data may shed light on the effect of risk modifying factors such as lifestyle choices and DNA polymorphisms. Observation of real clinical data and / or results of biomonitoring, if coupled with the exposure/effect biomarker discovery systems, can produce biomarkers of individual susceptibility and thus allow estimations of individual response to toxic insults. Toxicogenomics (transcriptomics, proteomics, metabolomics) is key to this kind of analytical and data interpretation process.
c) The integrated analysis of the biomarker data (including results on biomarkers of exposure, effects and individual susceptibility) results in the integrated assessment of risk factors. Use of information on risk factors with molecular dosimetry data (i.e. estimation of the actual internal and biologically effective dose of xenobiotic substance found in the target organ and, indeed, perturbing cellular response) enables population risk studies to be done, by converting generic exposure profiles into population risk metrics having taken into account inter-individual variability of response and exposure uncertainty.

WT 368

Tiered aggregate exposure assessment methodology and toolbox: the TAGS approach

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The objective of the TAGS project is the development of a tiered approach to aggregate exposure assessment and the compilation of a computational platform, able to perform quantitative aggregate exposure assessments for environmental and consumer products following a full chain approach (including emission-migration, media concentrations, exposure and internal dosimetry). The use of biomarkers to verify model predictions, to reconstruct population exposure and allocate to apportion exposure to sources (reverse modeling) constitutes a part of the tiered approach and the accompanying guidance. The tiered approach guides the user through the preparation of the exposure assessment. It is foressen to make use of qualitative and quantitative information to evaluate the need for an aggregate assessment and will allow delineation of the assessment to its relevant aspects. Clear criteria for the advancement from Tier 0 (qualitative assessment) to Tier 2 (fully quantitative assessment, coupling probabilistic and deterministic information) have been defined for use in the frame of EU chemical and consumer product safety legislation.
The methodology for quantitative aggregate exposure assessment has been implemented into a proof-of-concept computational platform, the core of which is a synthetic dynamic modeling environment able to track and describe in mathematical terms all the steps of the full chain approach, implementing both mechanistic (e.g. dispersion models, Physiology Based ToxicoKinetic Models) and probabilistic methodologies (Markov Chain Monte Carlo or maximum likelihood

estimates) based on outcome optimization and the current status of knowledge and data availability. The tool provide not only "forward"mode of analysis, but it also describe in a more interactive way the procedure from the source to the target tissue in a way that all intermediate stages can be estimated when needed, with a well defined level of uncertainty.

WT 369

The GExFRAME: IT reflections of the Global Risk Assessment Dialogue

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Since 2008 the European Commission through DG SANCO supports the Global Risk Assessment Dialogue, a Forum to discuss and collaborate on harmonising risk assessment of chemical substances among the OECD member countries.
One of the main common issues dealt with in the Forum is Exposure Assessment. Key issues of Exposure Assessment identified include reviewing and comparing existing approaches worldwide, facilitating the dissemination and sharing of exposure data and assessment methodologies and exposure training. Model credibility and uncertainty, systemic exposure scenarios development (via the establishment of collaborative case studies) and applicability of models in various tiers of assessment are specific issues to focus on.
The GExFRAME is a web-based platform developed by the Joint Research Centre (JRC) of the European Commission and designed to host exposure assessment models for inter-comparison and harmonisation purposes that includes also scenario and new model building capabilities.
It allows exposure assessors to apply different, new or existing, low or high tier assessment approaches, under the same model and data management system and compare input assumptions and output results. As such, it provides the technology to tangibly address the key issues identified in the Global Dialogue on Exposure assessment using a central, reference platform.

WT 370

Potential Screening Approaches for Identifying Indicator Compounds at Land affected by Contamination in the UK

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The assessment of land affected by contamination in the UK follows a risk-based framework, relying on the early identification of source-pathway-receptor (SPR) linkages, which could present unacceptable risk. Both qualitative and quantitative methods are used routinely, allowing refinement of the conceptual site model as site-specific data are gathered and eliminating SPR linkages from further assessment. However, there are multiple examples of global semi-quantitative methods that can be used to support discounting SPR linkages without having to undertake a full quantitative assessment. These methods are less commonly used in the UK, resulting in either qualitative assessments that are not robust, or quantitative assessments that are more complicated than is needed. Examples of these semi-quantitative methods which can be used include (1) source-receptor off-set criteria, used in the United States, (2) volatility and toxicity screening criteria used in the United States and promoted in good practice guidance in the UK, and (3) an in-house system to rank contaminants based on their toxicological characteristics and behavior in the environment. Each of the three forms of semi-quantitative is described in detail, highlighting the background to how each approach was first developed, and examples scenarios where they have been applied successfully in the UK.
If applied early in the process, these semi-quantitative assessment approaches would allow UK risk assessment practitioners to more successfully identify indicator compounds, negating the requirement to undertake a detailed quantitative assessment for multiple contaminants.

WT 372

The European Exposure Factors (ExpoFacts) Sourcebook

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The ExpoFacts database is a unique European tool for exposure assessors and risk managers involved in health and environmental issues, particularly in the areas of indoor air quality, dietary exposures and safety of consumer articles. It implies risk scientists as well. The database contains information on exposure factors and population data covering 30 European countries with extensive links and references.
European exposure factor data have been scattered around national and international institutes, in scientific articles and reports. The ExpoFacts team reviewed available sources, collected reliable exposure factors and evaluated the quality of the data. They classified them into seven main categories: countries, housing, dietary ingestion, non-dietary ingestion, physiology, population and time activity. Two main and complementary search interfaces are made available: either by guided procedure or by free text search. ExpoFacts is accessible through the website http://expofacts.jrc.ec.europa.eu and does not require any registration.
ExpoFacts Sourcebook, financed by CEFIC was developed by THL and JRC, with a steering group composed of key EU academic and regulatory experts as well as opinion formers and supporting peer review process. The database was placed on the JRC web server on 15th January 2007.
The ExpoFacts follow up project started in 2009 and aims to collect, maintain, and provide the most updated reference data on exposure factors specific to European populations. Following its second meeting, the expert group of the ExpoFacts follow up project (including BfR, THL, InVS, LIGA.NRW, ANSES and VWA in addition to HETUS and RefXP ambassadors and ExxonMobil) evaluated new datasets and recommended the dissemination of its use as well as its mutual link with the U.S. Exposure Factors Handbook. The new datasets will be available starting the second trimester 2011.

WT 373

Children exposure to mixtures: Parameters, Scenarios and exposure estimates

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Exposure to a variety of chemicals occurs since conception and then at all stages of age. It varies in intensity as well as in the multiple combinations of many determinants of exposure. Adverse health effects may result from, some effects showing up during the same life stage at which the exposure has occurred while others being delayed until later. There are determinants and parameters that, per se or in combination with other factors, contribute to the occurrence of windows of vulnerability to chemicals. This paper deals with a body of information covering several temporal intervals and with a series of situations involving the inhalation route and dust ingestion. Respiratory volume has been measured in children from 5 to 12 years old, depending on physical activity. Time budget have also been recorded at various ages. An assessment of ingested dust is obtained in another part of the study. These data are used in exposure scenarios involving exposure to mixtures. Children exposure estimates to dust, to ambient and indoor air pollutants are provided for the case of cumulative exposure to lead and organic compounds.

WT 374

PAHs, Nitro-PAHs and Diesel Exhaust Toxins in the North American Great Lakes

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This study investigates the composition and selected sources of polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and hopanes and steranes used as diesel exhaust markers in several compartments in the North American Great Lakes region. While concentrations of nitro-PAH compounds are generally well below those of PAHs, their greater toxicity may result in greater ecological and human health risks. Several types of samples were collected and analyzed: diesel exhaust emissions sampled as particulate matter (PM) from engines combusting both conventional diesel and biodiesel fuels; atmospheric deposition samples obtained along transects of major roadways and at urban and remote locations; and biological samples including predator fish (whole sample) and birds (livers) from the Great Lakes. Diesel exhaust emissions showed many PAHs and nitro-PAHs, with levels and compositions that depended on emission control technology, fuel type, and load, e.g., the use of biodiesel fuels significantly lowered both PAH and nitro-PAH emissions as compared to conventional diesel. Graidents in the deposition samples helped to confirm the importance of highway emissions. Concentrations of individual PAH and nitro-PAH compounds in fish and bird samples were detected in the 1 to 100 ng/g range, indicating a need to enhance monitoring, examine trends, assess risks, and apportion emission sources. The development and application of source profiles that can help to identify sources and quantify emissions of these toxic compounds is discussed.

WT 375

Time Trends of Brominated Flame Retardents in Fish from German Water Bodies

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Brominated Flame retardents (BFR) are mainly added to plastics to prevent them from catching fire and hence, contribute to consumer product safety. However, they may be released into the environment during use and after disposal of those products and have been found as ubiquitous pollutants in all environmental compartments.
We analyzed muscle tissue homogenates of bream (*Abramis brama*) sampled from six German river systems (Rhine, Elbe, Danube, Mulde, Saale and Saar) for 23 PBDE congeners, five brominated benzene derivates, DBDPE, BTBPE, and HBCD. Samples were taken in biennial intervals in the period 1995-2009 within regular sampling campaigns of the German Environmental Specimen Bank.
Tissue samples were extracted with toluene by PLE, followed by a cleanup using GPC and multilayer silica gel column chromatography. All analytes were quantified by GC-ECNI-MS except HBCD which was analyzed by LC-MS/MS.
Being banned in the EU, many PBDEs are currently no longer in use. Nevertheless, they can still be detected in environmental samples. For the period 1995 to 2009, we found declining PBDE concentrations for most of the congeners while coincidentally concentrations of other BFR, in particular of HBCD were increasing. BFR levels at different sampling sites showed significant differences with lowest concentrations seen in breams from the reference site, Lake Belau, and highest concentrations in those from the River Saar, an area characterized by a high degree of industrialization. Our study clearly indicates that since regulatory actions to limit the use and marketing of PBDEs were taken, their levels in fish from German Rivers declined. However, they were still detected in all fish samples.
Other BFR like HBCD and DBDPE, used as substitutes for banned PBDEs, are of growing concern as their concentrations in biota are on the rise.

WT 376

Environmental toxicology and carcinogenesis

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In today's scenario of increasing industrialization, different factors including indiscriminate use of various agrochemicals for the protection of crops; release of toxic chemicals and other industrial and automobile exhausts into air, soil and water; have been responsible for imbalance in ecosystem. Accumulation of residues of these environmental pollutants produce adverse effect on various body functions, immune system in particular, making man and animals more vulnerable and susceptible to diseases. The incidence of cancer is on the rise, with multiple risk factors that involve interplay between genetic and environmental components. In India, the annual estimate of cancer was 0.98 million (2001).
Recently PAHs in the environment have become serious concern worldwide since the exposure to high concentrations has been linked to carcinogenic risk. The International Agency for Research on Cancer (IARC) has classified 48 PAH compounds likely to be carcinogenic to humans. Presently in India, light duty diesel cars are selling in large numbers due to the fuel economy. Levels of PAHs such as fluoranthene, pyrene, chrysene, benzo[a]pyrene etc. are higher in emissions of diesel cars than petrol versions. Widespread dieselization of light duty cars and commercial vehicles might further exacerbate the health risks of the urban people of India. According to CPCB, ambient air PAH in Delhi ranged between 9.4-60.9 ng/m3 during 1999-2000 with higher values recorded in winter.
Environmental toxicants have been responsible for the rise in breast cancer cases in females in India. The general population is exposed to organochlorines through food chain but water, ambient and indoor air, dust, and soil are also thought to be involved. As a result, several organochlorines are commonly detected in serum, adipose and breast milk of human origin (Aronson K.J. et.al. 2000). Pollution due to heavy metals is also a big threat to the plant and animal communities, including the human race. Saha (1983) made an extensive survey from 61 villages of 7 districts of West Bengal. He detected 1214 cases of chronic arsenical dermatoses, having skin cancer in 6 cases.
Thus the paper highlights the role of environmental toxicants in rapidly increasing cancer cases, cardiovascular mortality and several other health abnormalities in the current Indian scenario.
Key Words: Cancer; Environmental toxicants; Carcinogenic; Industrialization

WT 377

Evaluation of specific Environmental Release Categories (spERCs) developed by industry associations

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At an early stage of RIP 3.2 members of industry associations claimed that the emission factors listed in the guidance documents often are excessive when compared with real emission data. Therefore different industry sectors developed specific environmental release categories (spERCs) with corresponding fact sheets documenting the derivation of the spERCs.
Together with Oekopol GmbH, the Federal Environment Agency Germany analyzed several spERCs at a general level to examine how they were derived and documentated. Additional questions were how existing guidance for the development of spERCs was used, and how different actors in the REACH process understand the role and scope of spERCs. Industry understands spERCs as specification of ERCs. However, according to industry associations who developed the spERCs it is not intended that spERCs are used by registrants directly and without adaptation to his (a single registrants) specific situation. This intention differs from the general expectations of other REACH actors (registrants, downstream user, authorities etc.) not involved in the development of spERCs.
It became clear that conditions of use assumed for a spERC are described at a very general level in most of the fact sheets. The covered uses are frequently rather broadly defined, respective descriptions are spread over several sections in the fact sheets and are partly inconsistent. The default values of the spERCs are derived using different methods and different information sources. In many cases the documentation of the justification of values is regarded as not sufficient.
An essential aspect of the further development of spERCs is the clear and precise information whether or not release factors apply before or after risk management measures. This is necessary for downstream users and evaluators because this information gives indication on reliability and accuracy of the exposure assessment and avoids an underestimation of risks in the Chemical Safety Assessment.

WT 378
SPERC - Refining REACH Environmental Emission Assessments
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REACH, the EU chemical legislation implements the principle 'no safe use - no market'. This implies that the safe use of dangerous chemicals be demonstrated and requires that exposure assessments be performed. The emission estimation constitutes the starting point for environmental exposure assessment. Chapter R16 of the REACH guidance on Data requirements and Chemical Safety Assessment provides the environmental release categories (ERC). They provide very broadly applicable first-tier emission estimates which often lead to significant overprediction of environmental exposure. Consequently, a considerable number of sector groups of chemical industry and their downstream customer industries refined the emission estimates by introducing so-called specific ERCs (SPERCs). This poster presents the outcome of a workshop involving representatives of national competent authorities, European Chemicals Agency, OECD and industry. The workshop is held on April 14. It addresses the current state of emission assessment under REACH, the future development needs for emission assessment under REACH (and elsewhere) with a focus on SPERCs, options for communcation on SPERCs and other emission assessment approaches in order to improving the general level of knowledge and adapting generic emission assessments to specific local conditions.

WT 379
Development of release classes for chemicals used during their various life-cycle stages
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Under REACH, the safety of chemicals needs to be demonstrated by industry by means of the preparation of chemical safety assessments. A crucial step is to estimate the environmental releases of chemicals during the chemicals' life-cycle stages. To facilitate these estimations, the concept of Environmental Release Categories (ERCs) has been developed. ERCs do not assume any Risk Management Measures (RMMs) and are not based on the chemical's properties. To facilitate more detailed release estimates, they can be considered as blueprints to start building emission scenarios applicable to numerous applications covering the entire life-cycle of a chemical. In total, 24 ERCs have been developed each covering different parts of industrial processes, applications in products and in articles with corresponding assumed emission fractions to air, water and soil. ERCs were intentionally designed to provide very conservative (high) release estimates of substances.
In cases when there is a need for more refined emission information under REACH, the design of specific ERCs (SPERCs) defined and used by industry is optional. SPERCs contain release factors that are based on more process-specific knowledge, including information on implemented RMMs and operational conditions. Hence, SPERCs will be less conservative and, therefore, more useful in attaining realistic release estimates of substances. A source of information for making a SPERC is release information from existing Emission Scenario Documents (ESDs).
Based on two existing OECD ESDs, the potential of ESDs to transform ERCs into SPERCs was investigated. The feasibility of this approach as well as the observed shortcomings in the information requirements will be presented in this poster.

WT 380
A decision tree for Exposure Based Waiving of toxicological studies under REACH
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The EU 6th Framework Project OSIRIS (Optimized StrategIes for Risk assessment of Industrial chemicalS through integration of non-test and test information) aims to improve integrated testing strategies for REACH to increase the use of non-testing information for regulatory decision making, and thus to minimize animal testing. A decision tree on the possibilities of Exposure Based Waiving (EBW) for human health endpoints has been developed and will be presented in this poster. The regulatory requirements of REACH determine the legal possibilities for waiving toxicological studies. Waiving is legally possible for repeated dose, reproductive toxicity and carcinogenicity studies, but it has to be adequately justified. In general this requires sound argumentation that exposure levels are already so low that more knowledge on the hazards will not trigger further reduction. Therefore the decision tree focuses on parameters of substance, product, process, conditions and risk management measures that will ensure these very low exposure levels. The following factors are taken into account:
- Substance concentration in products
- Encapsulation
- High integrity closed systems
- Personal Protective Equipment
- Process and product characteristics
- Absorption, distribution, metabolism and excretion
- Frequency and duration of exposure

For some of the factors, e.g. frequency of exposure and process characteristics an expert elicitation process delivered input to specify criteria or examples.
A further option is to compare quantified exposure estimates to so-called 'Thresholds of Toxicological Concern"(TTC), which are general thresholds for groups of substances below which the chance of any of these substances exerting an effect is very low. Proposals for TTC's for inhalation exposure and for dermal exposure have been made within OSIRIS.
The final decision tree is included in the OSIRIS Integrated Testing Strategy Tool at http://osiris.simpple.com.

WT 382
The protection of groundwater and drinking water within the REACH-system
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Chemicals and uses that fall within the scope of the REACH regulation (1907/2006 EG) have to be registered at ECHA in Helsinki. By doing so, industry guarantees the safe use of chemicals throughout the whole life cycle. Registrants are requested to ensure a high level of protection of human health and the environment. It is common sense for the public and beyond controversy between industry and authorities that groundwater and drinking water need the highest level of protection. The conservation of clean drinking water is one the most prominent examples where the precautionary principle in our society should be applied.
In Europe drinking water is obtained mainly from groundwater, reservoirs or rivers by bank filtration. If these environmental compartments are contaminated by chemicals a contamination of the drinking water itself is possible. As a result society as a whole - and not the polluter - would have to pay for the costs of water treatment. These are often considerably higher than the costs of precautionary action. Thus the protection of groundwater and drinking water gives reasons for a sound assessment of the physico-/chemical substance properties and the exposure of the environment in the registration under REACH.
Here we present an inventory of exposure pathways to be considered and a review of guidance available. We indicate and discuss gaps and offer a definition of the exposure path "men via the environment"in relation to drinking water. The Federal Environmental Agency (UBA) has the aim to support industry to fulfil their responsibility by providing support and guidance. In addition our aim is to identify chemicals for which regulatory action may be necessary. For the protection of drinking water we focus on those combinations of physico-chemical properties and uses that cause a potential contamination. Consequently we also discuss here the key physico-chemical properties, the key uses as well as the regulatory action that should be envisaged.

WT 383
Past, Present and Future - Human Health and Ecological Exposure Assessment under Canada's Chemical Management Plan
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Assessing risk as part of Canada's global commitment to address legacy chemicals by 2020 is implemented through the Chemicals Management Plan. In 2006, Canada categorized approximately 23,000 existing commercial substances through a priority-setting exercise using available data, QSAR modelling, and simple tools that ranked substances based on a number of parameters including their potential for human exposure. Since 2007, Health Canada and Environment Canada have addressed the highest priorities from this exercise by jointly conducting screening assessments on approximately 200 substances as part of its 'Challenge' initiative. Exposure was characterized using empirical data and exposure models to estimate environmental concentrations in air, water, soil, sediments, food and dust, as well as exposure resulting from use of consumer products. Rapid screening approaches were also developed for lower priorities. An overview is presented of both human and ecological exposure tools and methods used to estimate environmental releases and assess ambient environmental exposure, and approaches to assess exposures in indoor environments (personal care products, toys, building materials, etc.). Moving forward, it is recognized that novel approaches to complete the assessment of approximately 3000 remaining prioritised substances and that enhancements to exposure tools available to regulators are needed. Understanding where and how chemicals are used throughout their life cycle is a crucial step in exposure assessment and continued efforts, be it through international initiatives (OECD, WHO, Transatlantic Risk Assessment Dialogue) or stakeholder engagement, are key to improving the quality of exposure assessments. This poster will highlight strengths and limitations from past exposure-based prioritization exercises, exposure-based lessons learned from the 'Challenge' initiative, and current data and exposure tool needs moving forward within the context of chemical regulation.

WT 384
Metabolomic study of rat exposed to pentabromodiphenyl ether
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Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants, which have been manufactured in large quantities and widely used in a variety of consumer goods. They spread ubiquitously as environmental contaminants and have been detected in a wide range of environmental samples and humans.
Although tetra- to hexa-brominated congeners tend to dominate the patterns observed in wildlife, the penta-BDE product, which contains mainly tetra- and pentabrominated congeners, is a minor portion of the commercial PBDE flame retardant market.
PBDEs may leach or volatilize from products and have been shown to bioaccumulate in the environment. Bioaccumulation of PBDEs has been extensively studied several decades ago. But little information is available about the metabolic characterization of PBDEs in animal models. In this work, we investigate the metabolic changes in rat force-fed with pentabromodiphenyl ether(PeBDE) by NMR-based metabolomics. 1H-NMR spectra obtained from rat urine and

liver tissue samples.
In order to examine the bioavailability and bioaccumulation of PBDEs at low exposure levels, we have performed a mass balance study in rats fed a low dose of a penta-BDE for 7 days. Suitable extraction and analytical methods were developed to achieve the chromatographic separation of pentabromodiphenyl ether metabolites formed in vivo and to study their structure.
The identification of the OH-PBDE metabolites was also supported by full scan electron ionization mass spectra and MS/MS spectra of LC-orbitrap mass spectrometer.
Six biomarkers were candidate from NMR analysis of urine metabolites isolated from rat dosed with BDE119.
This study provided the valuable information of metabolic difference between treatment and control group by using multivariate analysis and could be applied for understanding the toxicity of PBDEs.

CS01 - Climate changes, biological invasions and pollution

TH 002
Life under climate and pesticide conundrum: can the plethora of life below-ground endure concomitant stresses part 1 - functional response in terrestrial model ecosystems
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Soil microorganisms are key drivers of many important ecosystem services, including those that support agricultural production. However, climate change may pose a threat, directly through its impact on soil properties (especially temperature and moisture content) and indirectly through the shifts in soil communities. We hypothesise that pesticide risks to microorganisms may change as a result of climate change. The key question is how the plethora of soil inhabitants will endure simultaneous stresses. Aiming to study the interaction of climatic and pesticide stressors on soil microbial activity and function, a Terrestrial Model Ecosystem experiment was conducted under two climatic conditions - Mediterranean (Portugal) and Continental (Germany). The fungicide pyrimethanil (PYR) was used as chemical stressor and extreme rain events (drought and flooding events) were used as climatic stressors. We measured enzyme activities (dehydrogenase, β-glucosidase, phosphatase, potential nitrification) and carbon metabolism profile (BIOLOG). In Portugal, enzyme activities related to C and P cyclings shifted significantly at the maximum application rate (MAR) of PYR (1kg a.i./ha) and so was overall enzyme activity (dehydrogenase) while those related to N and S cyclings were unaffected by PYR. Extremes in precipitation elicited few effects in the measured parameters. The interaction between the two factors did not alter pesticide risks to soil microbial function in long term (8 weeks after treatment) although a short-term (2 weeks after treatment) shift in metabolic profile was observed as a result of the interaction between the extremes in precipitation and pesticide. Biotic and abiotic factors beyond soil moisture seemed to interweave to produce the observed patterns. For German data, where a wider gradient of PYR concentration were used (from 0 to 1400X above MAR), we were able to determine stronger PYR effects and stronger interactions between factors, even for a short period of time, causing irreversible shifts. Such a shift was observed for N cycling.
These findings suggested that microorganisms are sensitive to the interaction between climate change and pesticides even in short-term for low concentration and at high concentration, the effect can be persistent. These changes indicate potential shifts in nutrient cycling in the future. Whether these changes represent a risk or opportunity to ecosystem health will depend on the biotic legacy and immediate pressures.

TH 003
Life under climate and pesticide conundrum: can the plethora of life below-ground endure concomitant stresses part 2 - structural response in terrestrial model ecosystems
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As the primary chemical pollution from agriculture, pesticide use imposes major stress to soil. Climate on the other hand, has an overriding influence on landuse, particularly agriculture. In Portugal, the Intergovernmental Panel on Climate Change foresee greater risks of flash floods and droughts that will start earlier and last longer, while Germany will see hotter summers with lower rainfall together with wetter and warmer winters. In face of the confounding effects of pesticide and climate change, we hypothesised that microbial community may respond with a shift in its community structure. We used terrestrial model ecosystem to examine the interaction between these factors under two climatic conditions - Mediterranean (Portugal) and Continental (Germany). The fungicide pyrimethanil (PYR) was used as chemical stressor and extreme rain events (drought and flooding events) were used as climatic stressors. We examined the response of bacteria community structure via PCR-DGGE.
PYR was found to be the dominant factor driving bacterial diversity in Portugal, with increasing bacterial diversity up from 0X PYR to 5X PYR (whereby maximum application rate (MAR) of PYR = 1kg a.i./ha). Climatic extremes were not significant at all times and a significant interaction was observed only for a short time (2 weeks after pesticide application), with no lasting impact. Where climate is relevant in the interaction, a mixture of biotic and abiotic factors beyond soil moisture appeared to direct the trend of diversity. Similar short-term effects of PYR were found on bacterial diversity in Germany even though the highest PYR application was 1400X above MAR.
The findings of this study suggest that bacteria communities are sensitive to the interaction between climate change and pesticide in short-term even at low concentration although no long-term shifts in the bacterial diversity were observed even at concentration above 1400X MAR PYR. These changes indicate that microbial communities are resilient to simultaneous stresses.

TH 004
Influence of drought and flood stress on carbaryl toxicity to the reproduction and survival of Folsomia candida (Collembola).
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Edaphic organism can experience a large range of environmental fluctuations in the field and drought and flood stress can influence the life-traits of organisms, influencing their growth, reproduction and survival. Another source of stress is the presence of chemicals in the environment. The aim of this work is to study the effects of soil moisture on the chemical toxicity of carbaryl to a soil-dwelling collembolan Folsomia candida. The effects on survival and reproduction were investigated in single and combined exposures. The main conceptual model for mixture evaluation data, Independent Action, and deviations to synergism/antagonism, "dose ratio"and "dose level"dependency were used to predict the joint toxicity. The reproduction of F.candida was strongly affected by the water content in soil, decreasing the number of juveniles produced at extremely conditions of drought and flood stress. Carbaryl also induced changes on adult survival and number of juveniles produced. Regarding combined exposures, the observed effects were compared to the predicted effects considering stressors single effects and patterns for synergism or/and antagonism were observed.This study highlights the importance of including natural stressors as an extra source of stress that can occur in the environment and that conventional ecotoxicolgical studies with controlled and optimum conditions do not regarded these exposure scenarios.

TH 005
Dose level dependent interactions of binary mixtures of Cd and Zn under different temperatures in the earthworm Eisenia andrei
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To investigate whether binary mixtures of heavy metals in soil would affect organisms differently from exposure to the metals sperately, earthworms (Eisenia andrei) was exposed to a fixed ratio (1:1) of mixture concentrations of Cd and Zn in artificial OECD soil for four weeks. As climatic conditions such as rising temperatures have recently become an increasing concern and to determine if different temperatures might affect these metal interactions, the exposures were conducted at three different temperatures (15oC, 20 oC and 25oC)
Selected adult specimens of E.fetida from a stock culture maintained at 20oC, were exposed to 0+0, 50+50,100+100; 250+250, 500+500, and 750+750 mg/kg Cd+Zn (these concentrations were later transformed to toxic units -TU). The experiments were conducted in triplicate and excecuted in incubators at the three temperatures mentioned. Soil moisture, pH and light were kept constant and food, in the form of dried cattle manure was added weekly to all replicates. Biomass change was monitored and the results were analised following a stepwise approach as suggested by Jonker et al (2005), using MixToxModules. The interactions between Cd and Zn at all three temperatures were antagonistic at the lower mixture concentrations but became synergistic at higher concentrations. Analysis parameters indicated that the type of interactions between Cd and Zn were dose level dependent rather than dose ratio dependent. It was also found that a change from antagonism to synergism occurred at higher dose levels than the EC50s measured at all three temperatures. These results showed that these temperatures had no direct effect on the type of interactions present.
Results obtained for interactions between these heavy metals in mixtures indicate that conclusions should always be made with caution, taking into account the concentrations used. Further, that the effects of mixtures on test organisms should preferably be assessed using a wide range of exposure concentrations. In binary mixtures of Cd and Zn the interactions between the metals vary from antagonism (at dose levels lower than the mixture EC50) to synergism (at dose levels higher than the mixture EC50). Thus, metal interactions in mixtures should always be assessed by using a wide range of doses.

TH 006
Effect of freezing and thawing on the availability and elution of PAH in soils
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The fate of organic contaminants in soil depends on soil related factors like carbon content, microbial activity as well as on intrinsic contaminant properties. However the impact of environmental conditions such as water content, freezing and thawing, occurrence of cations e.g. is of significance as well. Research on these effects is of high interest in the context of interactions of contaminated to climate conditions and climate change In different approaches - in parts with ^{14}C labeled PAH - the effect of freezing and thawing on the fate of PAH in soil samples was investigated. We conducted column experiments to study the elution, column experiments to study the dislocation during slow freezing processes and batch experiments to study the stability of non-extractable residues. In waste material the application of previous freezing and thawing cycles had an impact. The elution rate and availability of PAH was higher than in the untreated control experiments. However in soil material this effect was not detectable. The stability of not-extractable ^{14}C-PAH bound residues was not significantly influenced by freezing and thawing cycles as well. The extractable fraction and the mineralization to $^{14}CO_2$ in treated samples were comparable to controls.
In soil columns slow freezing processes (4°C per day) which occur under field conditions were simulated and a possible dislocation of organic contaminants was investigated with respect to a differentiation in a frozen and an unfrozen part of the soil column. First experiments on this topic could not confirm expected effects of phase separation and squeezing process due to ice formation. The results of the different investigations will be discussed in comparison and with regard to conditions of permafrost soils.

TH 007
The bait-lamina test as a tool to assess differences in soil activity caused by season, fire and invasion by alien grasses in Cerrado, Brazil
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The Cerrado is a Brazilian savanna recognized as a conservation hotspot. In this environment, the vegetation has to cope with several types of stress, either natural or originated by human activities as a) water-stress, caused by a marked seasonality of the rains, b) fire, which is a natural ecological feature but its frequency has been intensified by human activities, and c) invasion by exotic grass species, which can markedly change the natural environment by displacing natural grasses and increasing the total fuel biomass. It is known that changes in plant community can also affect soil health, since plant-species composition is a key factor in ecosystem nutrient cycling. In this context, a promising method for biological assessment in tropical soils is the Bait-Lamina test which provides a general overview on soil functioning, and can be performed under different conditions. Although it is mostly used to assess effects of chemicals on soil fauna, some studies also report a correlation between feeding activity in the soil and the existing vegetation. The aim of this study was to assess soil-feeding activity using the bait-lamina test in different environmental conditions. Four experiments were conducted in the National Park of Brasília (S 15º 47' e W 47º 56'). Firstly, the sites, within a study area of 5.400 m2, where sites were classified according to vegetation cover: 1- Melinis minutiflora, an invasive species, 2 - Native grasses/dicots, 3- Andropogon gayanus, an invasive species. For each of the three groups, five replicates of 16 probes were placed in the soil, totalizing 256 probes, for each of the four experiments. The bait used in the

experiment was composed by cellulose powder, oat flakes and agar (7:2:1), and the probes were maintained for 10 days in the soil. This procedure was conducted 1- before fire and during the dry season, 2- after a prescribed fire and during the dry season 3- in the same burned area in the beginning of the rainy season, and 4- in an equivalent adjacent unburned area in the beginning of the rainy season. Data was analyzed by Mann-Whitney ranking test, using the software R. Results suggest that both climate, fire and species composition may affect soil feeding activity.

TH 008

Does the altered thermal regime measured in the Cerrado soils after fires have a significant effect on germination of native and invasive grasses?

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Fire is an important ecological factor in many ecosystems worldwide. In the Brazilian savanna, known as Cerrado, it has been occurring naturally for more than 30,000 years, being ignited by lightening during the rainy season from September to May. However, human activities have been changing fire regimes, and this ecological feature became more frequent during the dry season. This shift may generate losses of annual seed production and depletion of soil seed bank, especially of grass species, that correspond to the major part of the diversity (richness and biomass) of the herbaceous layer. Still, soil seed bank is not completely extinguished by fire, once soil works as a thermal isolator, protecting the seeds from lethal temperatures. It is also known that fires that occur in the dry season cause a significant change in soil thermal regime at 1cm of depth, where more than 90% of soil seed bank can be found. The normal amplitude of temperature is of 13°C (22°C-35°C), and it can reach almost 40°C (10°-50°C) in a burned area. The direct effect of peaks of high temperatures has been reported to cause little effect on germination of Poeaceae seeds. Nevertheless, the effect of different storage temperatures in soil is considered to affect germination of many seeds. Regarding the lack of information on this field and the importance of the Cerrado region as a tropical hotpost for conservation, the aim of this study was to assess the effect of the increased soil temperature, and the time of exposure to these conditions, to ability and mean time of germination of 10 grass species (nine native and one invasive). The post-fire soil conditions were simulated in a germination chamber, where seeds were kept dry in the dark in a oscillating temperature of 10ºC/45ºC, 14h/10h for 7, 15 of 30 days, simulating time between fire and the beginning of the rains. Each time of exposure was followed by a germination period of 30 days, in a photoperiod of 10h of light in 22ºC/37ºC 14h/10h, where seeds were kept watered and seed germination verified daily. Results suggest a species-specific response to these conditions. Germination ability was species and time of exposure dependent. Changes in time for germination were also verified, which can have a significant effect on site recolonization dynamics in post-fire environments and in competition between native and invasive species.

TH 009

What's up inside the reactor - biotests for risk assessment of biofuel fermentation

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As a consequence of the increasing use of biofuels and bioenergy as alternative energy sources to replace fossil fuels, there is an urgent need for ecotoxicological evaluation, in particular within processes that are used to gain these alternative energies. Although research in the field of energy sciences was steadily growing during the last years, ecotoxicological data on the intermediates in the fermentation processes have not yet been sufficiently investigated.

Thus, a project was initiated to assess the pollutant development by microbial conversion from substrate to end product in cooperation between Cornell University and RWTH Aachen University. Cornell University works on the conversion of lignocellulosic wastes into n-butyrate, which can be further converted to the liquid biofuel n-butanol in a subsequent fermentation step. To ensure sufficient biological breakdown, lignocellulosic materials are pretreated with several different techniques, such as hot water acid and hot water base treatment. This process, however, has a known disadvantage of generating toxic compounds, which can inhibit microbial cultures that are grown in down-stream bioprocesses. Undefined mixed cultures may destroy the toxic compounds, which would prevent inhibition. However, if intermediates are not degraded they are assumed to be of ecotoxicological relevance due to possible release into the environment.

In this approach, we want to meet this hypothesis applying a battery of acute and mechanism specific in vitro biotests. Biological analysis will focus on different pretreated and untreated substrate samples, as well as on complementary effluent samples. In detail, cytotoxicity will be assessed using the neutral red retention assay, and Ah receptor agonist activity will be detected with the EROD assay, both using RTL-W1 cells. In addition endocrine activity will be determined in the yeast endocrine screen (YES) assay. Further biotests are considered. Finally, toxic impacts of effluents resulting from the production process will be assessed.

TH 010

Climate change damage functions in LCA - (2) data availability and selection of indicators

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Emissions of greenhouse gases among other things lead to increasing atmospheric CO_2 concentrations, increasing temperatures, changed precipitation patterns and thus multi-factorial changes in the growth environment (1). Primary producers in both terrestrial and aquatic ecosystems and consumers in the food web will experience ecophysiological changes as a consequence of this. To date, only very few truly multi-factorial ecophysiological experiments at the field scale exist. Results from these suggest that the sensitivities of species and ecosystems towards a changing growth environment will be variable (2). Modeling exercises suggest large-scale range shifts of the major biomes of the world (1). The unknown magnitude of future GHG emissions and the complexity of the climate-carbon system induce large uncertainties in the projected changes. A changed climate may result in new interactions and new directions of ecosystem change due to differing adaptive capacities and new species assemblages.

Within the framework 'ecosystem services' both marketed and non-marketed utilities of the natural environment are formulated (3). Provisioning, cultural, supporting, and regulating ecosystem services have been described. How will these services be affected by the increasing atmospheric GHG concentrations ? How can the changes be expressed in a damage model for LCIA? For the area of protection 'Natural environment' both sensitive and robust responses to climate change may be foreseen for different species within ecosystems and between ecosystems. A common metric may thus show high variability. Plural metrics may be needed to adequately describe the variety of different ecosystem services in different regional settings.

By evaluation of available data from e.g. global monitoring initiatives of ecosystem services such as UN's Food and Agriculture Organisation (FAO), UN-REDD (reducing emissions from deforestation and forest degradation in developing countries), and other available sources (e.g. the Global Biodiversity Information Facility), we discuss the selection of indicators for different environmental services from the natural environment, how these can be related to life cycle inventory results for GHG emissions and what would be appropriate metrics for the resulting damage to the area of protection 'Natural environment'.

References

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TH 011

Water temperature, ultraviolet radiation transparency, and motorized watercraft activity interact to control invasive warm-water fish in a Montane Lake

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Montane lakes have been shown to be good sentinels of and are sensitive to the impacts of climate change. In addition, because they are typically oligotrophic and located in aesthetically desirable surroundings easily reached by humans, montane lakes are under increasing pressures due to the impacts of climate change and human recreational activities. The purpose of this study was to determine how multiple stressors affect both non-native and native fish species in Lake Tahoe, a montane lake located at an elevation of 1,900m in the Sierra Nevada mountain range of western United States. Lake Tahoe is a large, ultra-oligotrophic lake that has a history of impacts due to recreational activity and introduced species, including recent introductions of warmwater fish such as bluegill sunfish. Warm-water fish must spawn in shallow littoral waters because of adult requirements for warmer spawning temperatures. In transparent lakes this potentially exposes eggs and larvae to high levels of ultraviolet radiation (UVR). We examined how water temperature, transparency to UVR, and polycyclic aromatic hydrocarbons (PAH) from motorized watercraft activity influence the suitability of nearshore habitats for invasive warm-water fish in Lake Tahoe. Due to climate change and human development, nearshore temperature, UVR attenuation, and PAH levels are increasing, affecting natural processes of the lake. Responses to these changes in the native Lahontan Redside minnow (*Richardsonius egregius*) and the non-native warm-water Bluegill sunfish (*Lepomis macrochirus*) were compared in *in situ* exposures and in controlled outdoor exposures. These results were combined with UVR transparency data from monthly profiles to predict fish survival in each nearshore site as a function of UVR exposure. Our results suggest that current UVR transparency and water temperature limit establishment of non-native fish in most, though not all, nearshore sites in Lake Tahoe. PAH increased the level of UVR toxicity in both species and added an additional stressor to nearshore areas. Determination of the effects of these multiple stressors clarifies an understanding of the invasive success of bluegill and similar species in Lake Tahoe and other oligotrophic, montane lakes that are susceptible to climate change, habitat alteration, nutrient inputs, and recreational activity.

TH 012

Combined effects of UV radiation and triclosan in zebrafish early life stages

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Climate change is an imperative problem compromising human and nature well-being. At ecosystems level, environmental parameters such as temperature, pH, dissolved oxygen concentration and intensity of UV radiation have an important role on the efficiency of organisms' physiological and behavioural performances and consequently on the capacity of response to contaminants. Insignificant alterations of these parameters may compromise this response; thus, toxicity of pollutants is not only conditioned by its concentration and bioavailability but also by possible interactions with abiotic factors. Understanding the combinatory effects of chemicals and environmental parameters is absolutely necessary for an adequate prediction of risk in aquatic environments.

This work aims at studying the combined toxicity of UV radiation and triclosan, a biocide commonly used in personal care, acrylic, plastic, and textiles products. Zebrafish (Danio rerio) newly fertilized eggs were used and the draft OECD guideline on Fish Embryo Toxicity Test was followed. Embryos were exposed to nominal concentrations of 0.1, 0.3, 0.5 and 0.7 mg/l of triclosan, plus a control, under 5 different UV intensities for 8 hours. After this period, embryos were exposed to visible light until the end of the test (96h). Embryos/larvae were daily inspected for mortality, developmental anomalies and development delay. A general Morphology Score (GMS) (based on tail detachment, somite formation, pigmentation, eye development, heartbeat, movement, blood circulation, pectoral fin, mouth and hatching) was used to quantify development delay. Observed results were compared to the ones predicted by the Independent Action (IA) model, usually used in chemical mixture toxicity assessment, considering effects induced by single exposures. It was observed that for the parameters chosen patterns for synergism and/or antagonism were observed as deviations from the IA model. Results will contribute to the understanding of possible interactions of UV radiation and triclosan at lethal and sublethal levels to zebrafish embryos.

TH 013

Ultraviolet radiation increases sensitivity to pesticides - Synergistic effects on population growth rate of Daphnia magna at low concentrations

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Ozone depletion and climate change have resulted in increased UV exposure within both terrestrial and aquatic habitats. These processes highlight the need for improved consideration of UV and other environmental stressors in ecological risk assessment of toxicants. However, the combined effects of UV and contaminants on organisms remain poorly understood, and such effects have not been studied for pesticides. In the study reported herein, we aimed to investigate whether UV"ðB radiation and three selected pesticides at environmentally realistic sublethal doses/concentrations had additive or synergistic effects on the survival, reproduction, and population growth rate of the standard test species *Daphnia magna*. We observed synergistic effects of UV and pesticides on both cumulative reproduction and population growth rate for fenoxycarb and pirimicarb, but a less"ðthan-additive effect for tebufenpyrad. The results indicate explicitly that concentrations of toxicants that are nontoxic in standard tests can cause harmful population"ðlevel effects when combined with UV.

TH 014

Effects of parasitism and pesticide exposure on life-history and biochemical traits in two Daphnia magna clones

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In nature multiple chemical stressors occur and interact, however, also other environmental stressors (biotic as well as abiotic) can have an influence on the environmental effects of a pollutant. In this study, the influence of the gram-positive bacterial parasite *Pasteuria ramosa* on the toxicity of the insecticide carbaryl to the waterflea *Daphnia magna* was studied. In a three-way full factorial design two different *D. magna* clones were exposed to carbaryl, the parasite and the combination of both. Several key life-history (growth, survival, reproduction, filtration- and castration rate) and biochemical traits (energy reserves, electron transport system, acethylcholinesterase- and phenoloxidase activity) were measured. The two selected *D. magna* clones differed in the presence (clone P) or absence (clone A) of a previously detected synergistic effect of the combination of both stressors on the castration rate.
The insecticide carbaryl had a direct effect on reproduction in both clones. No effect on any of the other measured traits was detected for clone A. In contrast, a significant decrease in filtration rate, growth and survival were observed for clone P. Exposure to *P. ramosa* initiated a shift in energy reserves in both clones: protein reserves decreased while lipid- and carbohydrate reserves increased. This suggests a shift from lipid- and carbohydrate metabolism to protein metabolism. Although the previously detected synergistic effect on the castration rate was not confirmed, the above results clearly demonstrate clonal sensitivity differences towards carbaryl toxicity and enhance knowledge on the biochemical mode of action of the parasite *P. ramosa*.

TH 015

The use of biomarkers to assess effects of binary combinations of chemical and natural stressors to Daphnia magna

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In aquatic ecosystems several stressors may act together and affect the life traits of organisms. Pesticide runoffs are usually associated with high inputs of organic matter and depletion of oxygen in aquatic systems. This study aimed to use biomarkers (eg. AChE, LDH, CAT and GST) to assess the single and combine effects of imidacloprid and thiacloprid and imidacloprid and low food levels to the life traits of Daphnia magna. Biomarkers were measured after a 96h exposure period showing that chemical stress impaired the activity of several biomarkers used (e.g. related with nervous system). Whenever possible, the two conceptual models, Independent action and Concentration Addition, usually used for assessing response patterns in binary combinations of chemical and natural stressors, were used to fit our data.
Our results showed that biomarkers can be used as useful tools to understand the physiological processes that undergo detoxification processes or to understand stressors mode of action.

TH 016

Multiple stressor effects of predation and chemical pollution in green algae

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To investigate the toxicity of chemicals for environmental risk assessment, standard toxicity tests were established which are conducted with single species under controlled and reproducible exposure conditions. In nature, however, species do not live isolated or under constant conditions, but due to changing environmental factors they are permanently challenged with abiotic and biotic stressors such as extreme temperature, nutrient limitation or predation stress. Still, little is known about the effect of the combined stress condition caused by changing environmental factors and chemical pollutants, and how the tolerance to such multiple stressor conditions correlates with the tolerance to the individual stressors. In order to investigate the effect of predation stress in combination with the exposure to toxic chemicals in algae, we tested the sensitivity of eight different natural isolates of the green alga *Chlamydomonas reinhardtii* to grazing stress by the rotifer *Brachionus calyciflorus* and to various herbicides with different modes of action. First, dose-response relationships of individual single stressor were measured to determine 50% effective concentrations for each strain. Based on these experiments, several strains with different susceptibilities to grazing stress and the individual herbicides were selected for combined exposure experiments. The results of these combined exposure conditions will be presented and interpreted in order to determine the role of individual stress factor on the susceptibility of the selected strains to the combined stressors.

TH 017

Effects of temperature variation on the acute toxicity of florfenicol to Daphnia magna and Chlorella vulgaris

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In the last decade veterinary pharmaceuticals have been increasingly found in aquatic ecosystems raising concern on their possible adverse effects on non target species. Florfenicol is an antibiotic widely used in animal production, including in aquaculture, which has been detected in freshwater ecosystems. The aim of the present study was to investigate the effects of temperature variation on the toxicity of florfenicol to the freshwater producer Chlorella vulgaris and its grazer Daphnia magna. Standard acute bioassays with C. vulgaris and D. magna were carried out in accordance to OCDE guidelines at three different temperatures: 15, 20 and 25 ºC. Test concentrations ranged from 31 to 750 mg/L for C. vulgaris bioassays and from 300 to 1000 mg/L for D. magna bioassays. Six different treatments were prepared for each bioassay and two control groups were used: (i) test medium and (ii) solvent - control. The results indicate that temperature changes the acute toxicity of florfenicol both to the algae and to the water flea, with increased toxicity at 25 ºC relatively to lower temperatures, raising concern on effects at the ecosystem level due to possible disruption of phytoplankton-first consumers balance in freshwater systems contaminated with florfenicol and experiencing water temperature increase as a result of global warming. This may be a factor acting in favour of non-native invasive species (NIS) in their competition with native species, especially in the case of NIS with sub-tropical or tropical origins.
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TH 018

Does temperature acclimation alters cadmium sensitivity in the green alga Pseudokirchneriella subcapitata ?

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Growing attention in environmental toxicology is directed towards the effects of combined - abiotic and biotic - stressors. In aquatic (ectotherm) animals it is frequently observed that metal sensitivity is temperature dependent. Similar research for phytoplankton is limited. In the present study we have cultured the green alga *Pseudokirchneriella subcapitata* for 8 weeks at 12, 20 and 25°C. At the beginning (week 1) and the end (week 8) of this period the algae were exposed to a Cd range (0-320 µg/L Cd) at the respective temperatures. Cd tolerance ($72hE_rC50$), Cd accumulation (internal and adsorbed) and pigment ratios (chlorophyll a and b, carotenoids) were used as endpoints to investigate if temperature alters Cd sensitivity and if changes in Cd sensitivity occurred due to temperature acclimation. At week 1, it was demonstrated that the Cd toxicity at 20 and 25°C was significantly higher than at 12°C with $72hE_rC50$ values of 159 (95% CI=140-181), 157 (139-178) and 197 (172-227) µg/L Cd, respectively. Similarly, Cd accumulation was significantly higher at 20 and 25°C compared to 12°C. At week 8, only at 25°C the $72hE_rC50$ was significantly different from 12°C. Cd accumulation was significantly higher than at week 1 but in general no significant differences between temperature treatments were observed. For the pigment ratios it was shown that temperature did not influence the effect of Cd on pigment ratios, although differences were observed between week 1 and week 8. As a conclusion for this species of green algae we can state that temperature in the range of 12 to 25°C does affect Cd sensitivity and that the effect of temperature can be modified by temperature acclimation.

TH 019

Genetic adaptation potential of Chironomus populations to temperature stress

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The evolutionary potential of natural populations to environmental stress, is virtually unknown for most species. Under rapid anthropogenic modifications, such as water pollution and Global Climate Change the potential of populations to adapt to environmental changes is of increasing importance. In order to test, if populations of the freshwater model organism *Chironomus riparius* are adapted to local water temperatures we performed common garden life history experiments and used measures of genetic diversity and differentiation. Six populations of *C. riparius* from a climatic gradient in Europe were subjected to three different temperatures (20, 24, 28 °C). While we found significant life history differences among populations and a significant reduction in fecundity in all populations under temperature stress, there was no clear overall sign of genetic adaptation for most parameters investigated. In contrast to southern populations, for instance, midges from northern sampling sites produced egg masses of significantly reduced size under temperature stress. We correlate life history traits with several other factors, such as geographic origin, local climate differences, genetic variation, and differentiation among populations. Our results clearly show that genetic factors have to be considered in assessments of organismal stress sensitivity.

TH 020

Effects of short-term exposure to pyrene on mussels (Mytilus galloprovincialis)

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed environmental pollutants that pose a potential risk to aquatic organisms. Pyrene is one of the priority PAHs, widespread distributed in estuarine and marine ecosystems that has demonstrated its toxicity in several species. According to some climate changes scenarios, the pH of sea water is expected to decrease in the future and this may interfere with the toxicity of PAHs. Thus, the objective of this study was to investigate the effects of pyrene at different pH values on *Mytilus galloprovincialis*, a mussel that has been commonly used as a sentinel species in the marine environment. Mussels were exposed for 96 hours to a range of sub-lethal pyrene concentrations at three different pH values. At the end of the bioassay, several biomarkers involved in energy production, neurotransmission, biotransformation, antioxidant defences and indicative of oxidative damage were determined. Furthermore, the accumulation of pyrene metabolites was also investigated. Significant effects of pyrene on biomarkers were found and pH variation was able to change the response of some biomarkers to pyrene. This study highlights the importance of investigating combined effects of chemicals and abiotic factors variation on marine organisms.
This work was conducted in the scope of the project RAMOCS (ERA-AMPERA/0001/2007) supported by the Portuguese Foundation for the Science and Technology and FEDER funds in the framework of the EU AMPERA ERA-NET (ERAC-CT2005-016165) and by FCT (European Social Fund and Portuguese "Ministério para a Ciência e Ensino Superior") through a postdoctoral research grant to Miguel Oliveira (SFRH/BPD/65188/2009).

TH 021

May pollution act as limiting factor of the invasive behaviour of the Asian clam (Corbicula fluminea) by decreasing its capability to face chemical stress?

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The Asian clam Corbicula fluminea is one of the most important non-indigenous invasive species (NIS) in aquatic ecosystems that has been causing considerable negative ecological and economic impacts, especially in Europe and North America. In the present study, the effects of the reference compound aniline on C. fluminea populations from two adjacent estuaries of the Portuguese NW coast with similar hydromorphological characteristics but different types and levels of pollution were compared as a first step to investigate if chemical pollution may act as limiting factor of the invasive behaviour of this species. Advantage was taken from the comparison between a population inhabiting a low polluted estuary (Minho River estuary) that colonized all the freshwater tidal area and has at the present extremely high densities and a population living in a more polluted estuary (Lima estuary) where it is restricted to small area for years in the vicinity of a paper mill. The hypotheses to be tested is that pollution is decreasing the health status of the population inhabiting the most contaminated estuary, decreasing its capability to face chemical stress and thus the population is not able to spread. Animals were collected in both Lima and Minho estuaries and were separately exposed to the reference compound aniline for 96h, individually, in semi-static conditions with appropriate medium renewal to prevent toxicant degradation. At the end of the assay, several biomarkers involved in functions determinant for survival and performance of the animals were determined (e.g. neurotransmission, energy production,

biotransformation and anti-oxidative stress defenses). The results are discussed in relation to field data on the invasive behaviour of the species and health status.
This study was done in the scope of the project NISTRAKS (PTDC/AAC-AMB/102121/2008) funded by the Portuguese Foundation for the Science and the Technology (FCT) and COMPETE funds

TH 022
Forecasting the effects of global change on bioaccumulation patterns in Great Lakes species
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Climate change will have substantial impacts on biodiversity, particularly for aquatic species. Warming temperatures and changing weather patterns will also remobilize and modify chemical partitioning. Holding millions of cubic yards of sediments contaminated with persistent legacy chemicals such as polychlorinated biphenyls (PCBs) and dioxins, the Laurentian Great Lakes are a laboratory for observing interactions between biological and chemical responses to climate change. They provide a wide range of habitat to a variety of species, from littoral forage fish to deep-water predators. In this paper, we couple bioenergetic and bioaccumulation models to investigate the biological and chemical effects of climate change in the Great Lakes. We consider three species: round goby, a warm-water invasive forage fish; mottled sculpin, a cool-water native forage fish; and lake trout, a cold-water native predator. Using our coupled models, we calculate the accumulation of a representative persistent chemical, PCB-77, under four climate scenarios for Lake Erie and Lake Superior. Predator-prey (lake trout-round goby) interactions and food availability (high-low) are incorporated into our simulations. For cool- to cold-water species (sculpin, lake trout) we find that warm temperatures limit growth. For warm-water species (round goby) cold temperatures limit growth. The impact of climate warming on growth depends on the winter lows as well as the summer highs of the scenario, in combination with the species' critical upper and lower thermal limits. We find conditions for high growth and consumption rates generally lead to high bioaccumulation. However, this can be confounded by predator-prey dynamics, as mismatches in the temperature preferences of predator and prey can lead to mismatches in relative growth and uptake rates. As predator-prey dynamics are expected to undergo substantial shifts with changing climate, these relative thermal sensitivities will be key in determining the implications of climate change for bioaccumulation, particularly in top predator species.

TH 023
The impact of climate change related events on water quality
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Climate change may not only affect river water discharges in terms of water quantity, but also in terms of water quality. During flooding periods, for instance, higher water discharges may cause a dilution of normal pollution levels while increased transport of suspended particulate matter (SPM) may strongly reduce the bioavailability of contaminants. On the other hand, higher discharges may cause increased mobilization of polluted sediments and surface run-off from possibly polluted flood plains and inundated urban areas. To study the impact of climate change on the quality of urban and coastal waters, a battery of in vitro bioassays was applied to determine the toxicity profiles of environmental samples collected during climate change related events. SPM samples were collected in rivers from Norway, Sweden, Denmark, Germany, and The Netherlands during periods of dryness, flooding and extreme run-off. In addition, passive sampling was performed using silicone rubber sheets during 4 to 6 weeks periods with different river discharges. Extracts from SPM and passive samples were tested for respiratory toxicity, dioxin-like activities, (anti)androgenic, (anti)estrogenic, and thyroid hormone-like activities, and for mutagenicity. At the time of submission of this abstract, sample collection has been completed and toxicity profiling is subsequently executed. Results will be presented in the context of the sampling events. The work described is performed within the context of the DiPol (Diffuse Pollution) project, which is funded by the Interreg IVB North Sea Region Programme.

TH 024
Impact of climate change on the drinking water resource - focus on emerging substances on the Vilaine's basin
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The Vilaine's basin is located in Brittany, the first agricultural and food industry region of France. This watershed covers two thirds of the region (10,500 km^2). Its main river named the Vilaine is about 220 km long and crosses Rennes, which is the main city in this area (approximately 210,000 inhabitants).
All these activities (urban, industrial and agricultural ones) could have an impact on the water quality of this river and its tributaries. Heavy rains could otherwise play a role. This region is often facing significant rainfall events, it rained approximately 170 days and 730 millimetres in 2009 in Rennes.
The DWTP located in Ferel (close to the mouth of the Vilaine) is the biggest facility in Brittany and it is operated by Saur company. So this study aims at following the evolution of the water quality under different hydrological conditions from the source of the Vilaine to the entrance of the DWTP. According to the resource characteristics this study could help optimize the different treatment steps on the DWTP.
Thirty one strategic sampling points have been identified with a preliminary study of land use. To assess the global quality of the watershed, three sampling campaigns on the Vilaine and its tributaries are planned during dry periods. Another one, related to the impact of climate change on water quality, is planned after a rainfall event (preceded by a long dry period).
After analyzing the global physicochemical characteristics of these samples, pesticides and human and veterinary pharmaceutical products will be looked for by developing adapted and robust analytical methods.
Results consist on the description of amounts of all these emerging substances along the Vilaine's basin. Furthermore the main objectives are the comparison of data under dry weather and rainfall events to provide information about the consequences on the drinking water resource quality. Finally the better understanding of the river basin quality could assist the water treatment companies in managing their plants in responses to a sudden climate change, particularly by modulating the quantity of consumables to implement.

TH 025
Predicting the effect of climate change on agricultural insecticide exposure and risk for freshwater communities
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Climate change exerts direct effects on ecosystems but has additional indirect effects due to changes in agricultural practice. These include the increased use of pesticides, changes in the areas that are cultivated, and changes in the crops cultivated. It is well known that pesticides, and in particular insecticides, affect aquatic ecosystems adversely. To implement effective mitigation measures it is necessary to identify areas that are affected currently and those that will be affected in the future. As a consequence, we predicted potential exposure to insecticide (runoff potential, RP) under current conditions (1990) and under a model scenario of future climate and land use (2090) using a spatially explicit model on a continental scale, with a focus on Europe. Space-for-time substitution was used to predict future levels of insecticide application, intensity of agricultural land use, and cultivated crops. To assess the indirect effects of climate change, evaluation of the risk of insecticide exposure was based on a trait-based, climate-insensitive indicator system (SPEAR - SPEcies At Risk). To this end, RP and landscape characteristics that are relevant for the recovery of affected populations were combined to estimate the ecological risk (ER) of insecticides for freshwater communities. We predicted a strong increase in the application of, and aquatic exposure to, insecticides under the future scenario, especially in Central and Northern Europe. This will result in a severe increase in ER in these regions. Hence, the proportion of stream sites adjacent to arable land that do not meet the requirements for good ecological status as defined by the EU Water Framework Directive will increase (from 33% to 39% for the EU-25 countries), in particular in the Scandinavian and Baltic countries (from 6% to 19%). In the most affected regions adaptation and mitigation strategies including vegetated buffer strips and recolonization zones along streams should be fostered.

TH 026
May results of aquatic microcosm and mesocosm experiments with pesticides be extrapolated between climate zones in Europe?
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Higher tier studies to assess the environmental risks of pesticides have been performed mainly in Atlantic Central Europe and North America and results of such studies have been extrapolated to other climatic regions such as South Europe. Since climate (e.g., sun hours, rainfall, temperature), agroecosystems (e.g., crop rotation, field size), and edge-of-field surface waters (e.g., hydrology, ecology) in those regions are quite different, it may be expected that exposure profiles and effects of pesticides in surface waters are also different. To account for the lack of studies in the South zone, a research project was initiated that started with the set up of an outdoor microcosm facility in Lisbon (Portugal). One of the aims of this facility is to study the possibilities and limitations in the spatial extrapolation of regulatory acceptable concentrations (RACs) derived from outdoor micro-/mesocosm experiments between European climate zones. As a preliminary evaluation, differences in (1) ecology of edge-of field surface waters, (2) exposure conditions to pesticides, (3) direct and indirect effects, and (4) recovery potential in pesticide stressed (semi-) field freshwater ecosystems under South zone compared to Central zone conditions that a priori may be anticipated, will be discussed.

TH 027
Ecological assessment of situation in Shirvan region of Azerbaijan
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The total area of flat and mountain part of Shirvan makes 748 thousand in hectares. Anthropogenous factors influensis on changes of biogeocenosisare are drainage and flooding of territories, agricultural land development, set of agrotechnical actions for increase of soils fertility, cutting down of woods and bushes, building of roads and industrial targets. The region climate is non-uniform: climate is damp in high-mountainous and middle- mountainous parts, in foothill-hilly - droughty, warm, in low-flat - dry and warm. The mountain zone of area is rich by mineral waters of medical and resort value. 7 basic rivers proceeding in region: Kur, Goycaychay, Turyanchay, Girdimanchay, Agsuchay, Pirsakhatchay, Gozluchay, their chemical, bacteriological structure influences an ecological condition of a soil-vegetative cover of Shirvan. Pollution of the rivers occurs both superficial and sewage where get a waste and products of live ability of the person. The springs rich basically by sulphur and hydrocarbonates are used as a source of medical and potable water. The mountain part is located at height of 700-3000 m over level of s.l. Soil resources of region differ by variety. Deterioration of physical and chemical properties of soils are observed, has amplified water and wind erosion. It inseparably linked with a soil erosion, salification, chemical pollution and as a whole soil degradation. Development of degradation processes of the soils depends on an overexploitation, unstable agriculture and irrigation, destruction of woods, biodiversity pauperization. These processes are caused by factors of political, economic character, absents of special knowledge, internal both regional conflicts and natural factors. The basic industries of region are food-processing industry, processing local agricultural production (winemaking, fruit growing) and light industry (sewing, carpet weaving). Besides, wood-working enterprises functions in Ismayilli district, stone open-cast mines, brick-works, the enterprises for agricultural machinery repair in Shamakhi and Gobustan districts. Instability of Shirvan ecosystems negatively affects on it biogeocenose and there is actual a working out of measures on its preservation. Constant monitoring of a soil and vegetative cover gives the chance monitoring of an ecological condition of region. The full and all-round information is necessary for decision-making on protection of Shirvan environment.

EC02 - Atmospheric chemistry, transport and deposition

TH 035
Dioxin-like pollutants in sub-alpine Northern Italy: results from 1 year of monitoring at the EMEP Ispra Station
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Dioxin-like pollutants (i.e. polychlorinated dibenzo-p-dioxins and dibenzofurans, PCDD/Fs

and dioxin-like polychlorinated biphenyls, DL-PCBs) are considered potent toxicants capable of producing a wide spectrum of adverse health effects in biota and humans. There are still some areas in Europe for which none or little information is available on atmospheric ambient levels, occurrence and deposition of these contaminants. Such is the case with Southern-Western Europe for example. Moreover, the scarcity of consistent experimental data sets on atmospheric concentrations poses a problem for proper validation of models. The objective of this study was to initiate POPs atmospheric measurements (weekly resolution) in order to assess air concentrations, seasonal variations and atmospheric deposition of PCDD/Fs and DL-PCBS in a sub-alpine location where little information is available on POPs ambient levels. To achieve this goal, a monitoring and research station was set up at the Joint Research Centre EMEP site (Ispra, Italy). A one year data set (2005-2006) on PCDD/Fs and DL-PCB air concentrations (gas and particulate phases) is presented and discussed.
∑2,3,7,8-PCDD/Fs and ∑DL-PCB total (gas + particulate) atmospheric concentrations ranged from 70 to 3000 fg m-3 (4 to 215 WHO98-TEQ fg m-3) and 3 to 16 pg m-3 (2 to 6 WHO98-TEQ fg m-3), respectively. Both POPs families exhibited different seasonal patterns, PCDD/Fs presenting higher concentrations in the cold season and DL-PCB in hot season. Atmospheric deposition fluxes are under calculation and will help to complete the picture.

TH 037

Levels of PCDD/Fs and PCBs in ambient air from an highly industrialized city of North of Italy

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In the present study ambient air samples around a polychlorobiphenyls (PCBs) production plant placed in Brescia, an highly industrialized city in north of Italy, were collected. This plant was the sole Italian PCBs producer and it operated from 1930 to 1984. Previous investigations demonstrate that in the surrounding areas soil levels of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and PCBs were higher than limits of the law. The most contaminated area was of particular interest because it may affect several farms producing milk and meat. The first aim of this work is to complete the information rising from the previous studies with an air monitoring in the city of Brescia. PCDD/Fs were measured and expressed as TEQ concentrations according to the international (I-TEF), and World Health Organization toxicity equivalent factors (WHO-TEQ) schemes were calculated. However ambient air may be affected by several emission sources, thus the second aim of this study to understand if there are other potential PCDD/F and PCB emission sources affecting the atmospheric concentrations. Principal component analysis (PCA) was used to compare the "fingerprint"of the air data obtained on the city of Brescia with some source-related data selected from experimental and published data. Finally all the data collected were included in risk assessment analysis applying the Italian guidelines in order to estimate the possible adverse effects induced by the pollutants levels on human and ecological targets.

TH 038

Bulk atmospheric deposition of legacy and current-used persistent bioaccumulative toxic pollutants to remote regions of Europe

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Persistent bioaccumulative toxic substances (PBTs) cover a wide range of chemical with different physico-chemical properties which pose serious concern for human and environmental health, not only for their toxicity but also because they remain in the environment for long periods of time. In addition, some of these chemicals have high environmental mobility being detected in Polar Regions on they were never produced nor used. Terms like global distillation or selective trapping have been used for describing the processes responsible of the global distribution of these compounds base on their physico-chemical properties and ambient temperature.
Remote mountain regions have been considered as the more pristine areas at temperate and tropical latitudes; however several studies have shown that, as Polar Regions, mountains can act as a cold trap for persistent organic pollutants or even for those chemicals with a limited atmospheric transport, like current-use pesticides.
Moreover, mountain regions represent pronounced gradients of environmental conditions at small scale, which allows studying transport and distribution processes of contaminants that can be representative of the mechanisms operating at global scale.
In order to gain insight into these mechanisms, PBT substances have been determined in bulk atmospheric deposition samples collected in four mountain areas throughout Europe from 2004 to 2006. Sampling sites cover from the south (Pyrenees), to central (Alps), east (Tatra Mts), and west Europe (the Grampians Mts).
Compounds determined encompass both legacy (PCBs, HCHs and HCB) and current-use (endosulfans and PBDEs) pollutants.
Significant spatial differences have been found for all studied compounds, being Tatra Mts the site with the highest deposition fluxes and Pyrenees with the lowest. Interestingly, similar deposition fluxes were found for light BDE congeners between sites, while decaBDE deposition shows the above-mentioned spatial distribution. In general, higher fluxes are found during warmer seasons except for PBDEs. Comparison of the total deposition levels found in this study with fluxes determined in the Pyrenees and the Alps in the late 90s reveals a marked decrease of HCHs and endosulfans deposition to these remote sites while levels of PCBs have increased.

TH 039

Linking polycyclic aromatic hydrocarbons in alpine lake sediments from the High Tatras with atmospheric deposition

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European alpine lake systems are used as indicators of air quality over the continent. Preliminary data showed high polycyclic aromatic hydrocarbons (PAH) loads in the High Tatras (Eastern Europe) in comparison to other mountain regions (1, 2). Here, insight on the spatial distribution of PAH is provided from analysis of top-core sediments of 27 alpine lakes distributed along the High Tatras mountain range.
Top-core sediment concentrations were higher than those in deep-cores, and they were higher than those observed in other European high mountain regions. The PAH profiles were uniform and comparable to those observed in aerosols and snow, indicating that atmospheric deposition was the predominant PAH input pathway to the lakes. Good agreement between estimated atmospheric deposition and sedimentation fluxes was observed. However, in several lakes in the western mountain range higher sediment fluxes may respond to induced PAH deposition. These higher concentrations may also reflect inputs from potential emission source areas west of the mountain range.
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TH 040

Influence of residential wood combustion on the air quality (PM10 and its chemical composition) in Bremen, Germany

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The recent price development of fossil fuels increased the attractiveness of wood firing for residential heating. Therefore, many people decide using wood as sustainable and renewable energy source. However, wood firing, particularly using old furnaces, results in enhanced particle emissions leading to an impairment of the air quality. In this study, the impact of residential wood firing on air quality in an urban area of Bremen, Germany was investigated in winter time 2009/2010. PM10 samples were taken in 1 day intervals from October 2009 until March 2010 and analysed for arsenic (As), lead (Pb), cadmium (Cd), nickel (Ni) as well as polycyclic aromatic hydrocarbons (PAH), including the 3-ring PAH retene. PM10 concentrations ranged from <4 $\mu g/m^3$ to 89 $\mu g/m^3$ in periods of low temperatures and inversion and were higher than those of comparable measurements at other locations in Bremen. Concentrations of As, Cd, Ni, and Pb were between <0.2 and 4.1, 0.05 and 6.3, <1 and 3.6, and 2.4 and 38 ng/m^3, respectively and were always below regulatory limits. ∂'__PAH+retene concentrations were between 17 and 335 ng/m^3. PAH profiles clearly revealed the presence of wood firing at the investigated site which was possibly caused by the individual wood combustion used for residential heating.

TH 041

Air quality assessment by tree barks biomonitoring in urban, industrial and rural environments of the Rhine Valley: PCDD/Fs, PCBs and trace metal evidence

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Tree barks have been used as passive samplers to evaluate past atmospheric pollutions because they accumulate organic and trace metal compounds in their structure. The studied sites were the urban areas of Strasbourg (France) and Kehl (Germany) near the Rhine river and a remote forested environment in the Vosges mountains. The urban zone is situated close to an important industrial harbour with steel plant, waste incinerators, thermal power plant and others. Tree barks have been collected in spring 2008, 2009 and 2010 in this industrial area, but also in the urban and remote areas. One tree bark sample was collected in the forested part of the Vosges Mountains. Dioxins (PCDDs), furans (PCDFs) and polychlorinated biphenyls (PCBs) were analyzed in barks, as they own to Persistent Organic Pollutant (POP) group. These pollutants are emitted by industries and human activities. Trace metals were also analyzed to quantify the toxic metal concentrations in bark, and identify the emitters.
PCDD/Fs concentrations in the industrial area (509-1420ng/kg dw) were in the range of variation usually found in this type of zone. 22 PCB congeners were analyzed in bark by GC-2ECD on a range of concentration between 15±4 and 270±40 ng/g. Median level of PCB were found to be close to industries and highways (40-60 ng/g) while low level were observed in rural and forested zone. An important concentration was measured in the urban environment (170-270 ng/g) close to a former landfill. At this place, not only organic but also inorganic pollutants were enriched in tree bark.
High trace element (Cd, Sb, As, Sn, Co, Cr and Ni) concentrations were found close to the steel plant. Waste incinerators also appear to be important emitters of trace metall enriched particulate matter. Barks still have high Pb concentrations close to traffic but also Sn, Sb, Mo, and Ni (280 ppm, 7 ppm, 4 ppm, 3 ppm and 10 ppm respectively). In this study organic and inorganic pollution were not necessarily coupled. However a strong relationship exists between barium and PCB concentrations (r^2=0.85) in tree bark. Barium could be associated with sulphate in the atmosphere and has halogenated affinities.

TH 042

Dry deposition measurements of persistent organic pollutants, including PAHs, PBDEs and selected currently used pesticides in the Lake Victoria watershed.

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As part of the global monitoring plan under the Stockholm Convention on persistent organic pollutants (POPS), member states are called upon to regularly take record and report atmospheric POPs data. There remains severe shortage of general data on POPs, especially from atmospheric measurements, from the region of Africa. The results presented here are part of the first and, so far, the longest study to measure atmospheric prevalence of POPS and currently used pesticides (CUPs) in the East African region. We present data from measurements of PCBs, Organochlorine Pesticides (OCPs), PBDEs, PAHs and CUPs. High volume active samples (taken weekly at Entebbe, Uganda) and passive samples (taken quarterly at Entebbe and Bukasa Island in Lake Victoria, Uganda) were taken between November 2008 and March 2010 and analysed for the above chemicals at the Environment Canada laboratories (PCBs, OCPs, PBDEs and CUPs) and AirZOne Inc. (PAHs) in Burlington and Mississauga, ON respectively. Samples were extracted by accelerated solvent exraction (Dionex ASE 200) using hexane: acetone 70:30 v/v and analysed by GC/MSD. PBDEs and endosulfan were determined in negative chemical ioniza-

tion mode (GCMS-NCI). PCBs and OCs were determined by GC-µECD. Preliminary PAH results show a mean of 27.97 ng/m3 (51 samples and a range of 4.05 - 58.97 ng/m3) with high PAHs from combustion of biomass. We will present further analysis of the PAHs data by source. Preliminary PBDE (18 congeners) partial results show a mean of 25.74 pg/m3 (20 samples and a range of 7.40 - 53.94 pg/m3) with a relatively high abundance of BDE 47, 99, 183 and 153 congeners. Partial and preliminary ð'-PCBs data show a range 29-159 pg/m3 and average ð'-DDT concentration of 40pg/m3. Preliminary partial data for CUPs shows a predominance of Chlorpyrifos with a mean of 33.42 ng/m3 (20 samples and a range of 0.08 - 161.87 ng/m3) and ∑- endosulfan with a mean of 126.85 pg/m3 (20 samples and a range of 0.40 - 340.70 pg/m3) with the α-endosulfan isomer being the major isomer at a mean concentration of 109.85 pg/m3 (20 samples and a range of 0.4 - 295.99 pg/m3). More data from all the samples will be presented. Analysis of all the data against rainfall and air mass flow will be presented.

TH 043

Assessing legacy and current-use pesticide levels in air from an agricultural region of Valle del Cauca, Colombia

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Passive air sampler (PAS) consisting of polyurethane foam (PUF) disk, were deployed over three month period from August to October 2010 in three different areas of the agricultural center (CORPOICA), in the city of Palmira, Valle del Cauca Region - Colombia. The purpose of the study was to assess the gas-phase concentrations of current use pesticides (CUPs) and legacy (OCPs) pesticides. PUF disk samples were extracted with 300 ml of petroleum ether for 20 hours in soxhlet system and analyzed by gas chromatography and mass spectrometry (GC-MS). Target compounds that were regularly detected include Chlorpyrifos ethyl and Trifluralin for CUPs and Endosulfan and chlordanes for OCPs. Air concentrations (ng/m3) of CUPs were very high ranging from 50 to 95 (74 ± 13) for Chlorpyrifos ethyl and from 17 to 26 (22 ± 49) for trifluralin. However, OCPs air concentrations (pg/m3) were one order of magnitude lower than CUPs with Endosulfan showing air concentrations (pg/m3) that ranged from 190 to 1990 (770 ± 70) and for CC ranged from 42 to 136 (88 ± 63). In the case of CUPs these results are much higher than those reported in other agricultural areas of the world suggesting an intensive use of this pesticides in the agricultural activities in the Valle del Cauca region of Colombia.

TH 044

Sources of and temporal trends in occurrence of Legacy Pesticides in atmosphere of eastern United States

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Legacy Pesticides, mostly Organochlorines, are pesticides whose use in most countries has been banned or severely restricted. To elucidate the atmospheric sources and temporal trends in concentrations of these chemicals in the mid-Atlantic region of USA, weekly air (n= 265) were collected over 2000 to 2003 from three locations in the Delmarva Peninsula, part of the Chesapeake Bay watershed, USA. Legacy Pesticides (n = 15; from four pesticide groups: chlordanes, HCHs, DDTs and Diels-Alders related) were consistently present in the gas phase with infrequent detection in the particle phase. Patterns in gas phase concentrations at all three locations indicated that cis- and trans-chlordane and both HCH isomers are present continuously (95-100% detection) in the region at low levels averaging 20 to 50 pg/m3. Other pesticides including oxychlordane, heptachlor, dieldrin, and 4, 4'-DDE were also detected frequently. The highest mean air concentrations were for dieldrin (60-84 pg/m3), g-HCH (37-83 pg/m3), and 4, 4'-DDE (16-80 pg/m3).

Multiple regression analyses of air concentrations with temperature and wind conditions using modified Clausius-Clapeyron equations explained only 30 to 60% of the variability in concentration for most chemicals. Combining the data for mid-Atlantic (this study) with Central America, the southern United States (U.S.), and the Great Lakes region, reveals a general decrease in concentration with increasing latitude for cis- and trans-chlordane, indicating the Southern U.S. and areas of Central America remain source areas. However, examination of chlordane isomer ratios indicates some local and regional contributions. Spatial comparison of concentrations at the three Delmarva sites and the enthalpy of phase transfer indicates that the sources of chlordanes, HCHs, and heptachlor are primarily long range transport and that 4, 4'-DDE and dieldrin have substantial local sources at some sites. Results of this work indicate that soils on the Delmarva Peninsula are contaminated with dieldrin, g-HCH, and persistent pesticide degradation products (4, 4'-DDE, oxychlordane, and heptachlor epoxide).

Dissipation half-lives in air were well below 10 years for all chemicals and below published values for the Great Lakes except dieldrin, which did not decline during the sample period. The concentrations of most legacy pesticides are likely to decrease to below 10% of today's values over the next 30 to 40 years.

TH 045

High sea to air fluxes of aliphatic hydrocarbons and implications for formation of marine secondary organic aerosol

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Aliphatic hydrocarbons such as n-alkanes, branched alkanes, other hydrocarbons and the unresolved complex mixture (UCM) are among the most abundant semivolatile organic compounds in seawater and the atmosphere. Here we report the concentrations of aliphatic hydrocarbons in seawater and gas phase samples from the Mediterranean sea. The samples were taken in two East-West sampling cruises covering all the major Mediterranean regions. The concentrations of individual alkanes in seawater are in the order of tens of ng/L, but the UCM concentrations are two orders of magnitude higher. The estimation of air-water diffusive fluxes show an ubiquitous sea to air volatilization flux in all the Mediterranean Basin. Most of this flux is due to the contribution of the unresolved complex mixture. In fact, the UCM fluxes are higher than dimethylsulfur and isoprene fluxes, two well known precursors of sulfphate aerosol and marine secondary organic aerosol. Implications of these fluxes are discussed.

TH 047

Pollution of atmospheric precipitation and predict of their acidification at agricultural ecosystem

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The pollution of an atmosphere by sulfur and nitrogen oxides can cause acid deposits, which can put significant harm to agricultural cultures dropping out in agricultural areas and rivers' basins. In this connection monitoring of atmospheric precipitation chemical compositions is necessary. Such 12 years monitoring (1994-2005) was carried out in agricultural area of Crimea (Ukraine). It was determined ions $SO4^{2-}$, NO_3^-, Cl^- and NH_4^+ and pH value. The annual mean atmospheric precipitation pH was 5.43 with range of 3.82-7.63. In the first 6 years period (1994-1999) deposits with pH less than 4 met only in the cold period of year, but in 2001 the precipitation with such pH values dropped out in June. The ion SO_4^{2-} prevailed in deposits for all years. It was marked a negative trend its concentration during time basically at the expense of decrease it in a cold season. The contents of anion NO_3^- in atmospheric precipitation was high enough. The concentration was increased in time and was higher in the cold period of year in comparison with warm. The relation two basic acidification ions (SO_4^{2-} : NO_3^-) was 2.8 with significant fluctuations on years. A tendency to decrease of this ratio in time was marked The annual mean concentration of Cl- was 60 µeq l-1. In the cold period of year chloride ions were more than in warm. The increasing of Cl^- concentration from year to year is marked. The content of an ion NH_4^+ in deposits was rather high, and accrued with current of time. Concentration of Cl- ion in summer deposits was more, than in winter that is characteristic for agricultural area of Salgir river basin. The pH value of deposits depends on their content and was defined in the basic concentrations of ions SO42-, NO3- and Cl-. In warm period of year the pH in the greater measure depends on concentration of anion NO_3^-, in cold - SO_4^{2-}. The ions SO_4^{2-} and NO_3^- participated in acidification of precipitation in the warm period of first 6 year period. In the second 6 years (2000-2005) in a cold season ions SO_4^{2-} and NO_3^- is authentic, Cl- insignificant influence to deposits acidity. Closest and significant (1 % level) connection of pH values and sum of ions SO_4^{2-}, NO_3^- and Cl- is found out in the cold period (r = - 0.74). Thus, if we know the content of acidifying ions in the certain periods of year, it is possible to predict acidification of atmospheric precipitation in concrete territory.

TH 048

Study of the reentry interval and worker exposure after applying granular type pesticide formulation on soil in greenhouse

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This study carried out to establish reentry interval after applying granular type pesticide formulation on soil in greenhouse for preventing farmer's pesticide intoxication.

The recovery of pesticide, cadusafos, ethoprophos and probenazole on absorbent in air were ranged 80.9~121.1% in charcoal and 90.6~99.0% in XAD-4, respectively. Emission rate of pesticides from top soil added water in lysimeter was higher 3~5 times than that of pesticides from top soil not added water at 35ð'ð plot after applying a mixture of granular formulation and soil. The ethoprophos concetration in air, 50cm high from soil surface at greenhouse, was reached the highest 186.4 µg/m^3 within 13 hours and were ranged 17.8~186.4 µg/m^3 during 46 hours after applying granular formulation at dose rate 150 g a.i./245m^2. The cadusafos concetration in air at greenhouse was reached the highest 37.3 µg/m^3 within 39 hours and were ranged 10.0~37.3 µg/m^3 during 46 hours after applying granular formulation at dose rate 180 g a.i./245m^2. The probenazole concetration in air at greenhouse was reached the highest 1.45 µg/m^3 within 37 hours and were ranged 0.23~1.45 µg/m^3 during 46 hours after applying granular formulation at dose rate 144 g a.i./245m^2.

The pesticide exposure in air was measured in actual site generally using pesticides. The result of the reentry interval study demonstrated that reentry intervals for ethoprophos and cadusafos are longer than 48 hours.

TH 049

Seasonal trends of airborne VOCs and microbiological pollution in the vicinity of mechanical-biological plant. Human health risks.

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Although composting may be, in principle, a good alternative for the treatment of organic waste, adverse health effects related to this practice are not entirely known. The important accumulation of chemical and microbial agents released during the process may pose a risk for populations living close to composting facilities. Therefore, it is crucial to update information on health risks in non-occupationally exposed people before enhancing the active regulation to develop composting and similar waste treatment methodologies as the basis to manage organic residues. The aim of this study was to analyze the environmental concentrations of volatile organic compounds (VOCs) and bioaerosols (bacteria, fungi, and specifically Aspergillus fumigatus) in the vicinity of an organic waste treatment facility (Ecoparc-2) located in Montcada i Reixac (Catalonia, Spain), as well as to determine seasonal trends in their levels. The human health risks associated to the exposure to those pollutants were also characterized. Air samples were collected at 12 sampling sites around the plant. Mean VOC concentrations showed values of 32.4 and 15.7 µg/m3 in winter and summer respectively, although seasonal differences were not statistically significant. In addition, non-significantly higher concentrations of most microbiological contaminants (total bacteria, Gram negative bacteria, and fungi at 37ºC) were found in summer, with the only exception of fungi at 25ºC and A. fumigatus at 37ºC. The concentrations of both groups of agents, VOCs and bioaerosols, were slightly higher than those previously found in reference sites where no composting plants are operating. Anyhow, the current levels detected around the Ecoparc-2 do not pose an important carcinogenic or non-carcinogenic additional risk for the population living nearby.

TH 050

VOC emissions in offset printing plants

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Volatile organic compounds (VOCs) are organic chemical compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere. Printing inks and coatings used in the commercial graphic printing industry contain VOCs in varying amounts, depending on the type of ink, the drying and printing processes, substrates and end-use application requirements. Mean VOC concentrations in four different printing plants that use distinct types of vegetable based and water based inks and solvents were monitored for the purpose of quantifying the various VOCs in these areas and to relate the results with conventional printing processes. In each site VOCs were monitored during one workweek. They were sampled and analysed by mobile GC Voyager Photovac. Concentration levels of BTEX were often below limit of detection. Concentration levels of isopropanol and acetone were in range 232 - 4630 ppb

and 144 - 1813 ppb, respectively. BTEX concentrations and concentrations of the other VOCs founded in printing work environments of "ecological"printing plants were below the limits considered by the Serbian law and recommendations prescribed by NIOSH (National Institute for Occupational Safety and Health).

TH 051

Ozone and volatile organic compounds as prominent pollutants of printing indoor

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In the present work we investigated the concentration levels of ozone and total volatile organic compounds (TVOCs) measured during working time in a screen printing facility of Novi Sad. Our objective is to report a detailed analysis of TVOCs and ozone data exceeding the thresholds established by the air quality guidelines in order to investigate the workers exposure level of TVOCs and ozone and to identify correlation between ozone and TVOCs in a printing environment.

The air sampling was conducted in press department, including two different types of screen printing machines: semi-automatic and automatic. Air samples were collected and analyzed in situ for 4 times, once per 2 hour, during working time of 8 hour by ozonometer, Aeroqual Series 200, Aeroqual Ltd. and mobile gas chromatograph Voyager, Photovac, Inc. Results were obtained promptly, registering current situation and status of the working environment, which enables swift and adequate reaction in case of accidents and abnormal activities. The ozone concentrations were from 0.18 to 0.33 ppm for semi-automatic printing machine and 0.69 to 0.81 ppm for automatic screen printing machine. The TVOC concentrations were from 27.21 to 36.74 ppm for semi-automatic and 8.05 to 12.17 ppm for automatic screen printing machine.

The experimental data showed that ambient ozone concentration slowly increases with increasing of TVOCs concentration and illumination intensity during screen printing process. The ozone concentration levels were above the permissible exposure limit (PEL) for 8-hour TWA (time-weighted average) recommended by the OSHA (Occupational Safety and Health Administration), while the TVOCs concentrations were much below the PEL prescribed by the OSHA. In the Republic of Serbia, the Regulation of permitted concentration levels of TVOCs and ozone until now has not adopted.

Keywords: Ozone, total volatile organic compounds (TVOCs), exposure level, printing
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TH 052

Contribution of surface/air exchange to deposition potential for cyclic methylsiloxanes: a modeling assessment

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Partition of airborne contaminants to surface media such as soil, plant and aquatic suspended particulates have been identified as significant contributors to deposition potential for known persistent organic pollutants (POPs) such as PCBs and pesticides. In this study, the effects of partitioning of airborne cyclic volatile methylsiloxanes (cVMS) to soil, plant biomass and suspended particles in the remote region on the deposition potential of cVMS were estimated by equilibrium modeling. For each process, partitioning equilibrium between surface medium and air was assumed and the partitioning coefficients were estimated based on available data and approaches reported in literature. It was found that cVMS have surface media/air partition coefficients 3 to 6 orders of magnitude lower than those of the most volatile POP reference materials such as hexachlorobenzene (HCB) and PCB 28 congener. This is especially true for octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). Consequently, gas absorption via soil/air, plant/air or suspended particulates/air partitioning contributes minimally (< 1% of total airborne D4 or D5) to the deposition potential of D4 and D5 in the remote region. For dodecamethylcyclohexasiloxane (D6), plant/air and suspended particulates/air partitioning should contribute minimally to the deposition of airborne D6 to surface media, while soil/air partitioning may account for a small fraction (e.g., 3%) of airborne D6 transferred from air to soil under the low temperatures (e.g., -20 °C) in the remote region.

TH 053

Sorption behaviors of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) on various atmospheric aerosols

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The study investigated sorption and desorption behaviors of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) with nine major atmospheric aerosols including both primary and secondary aerosols. Sorption and desorption took place via an initial rapid and a rather slow processes. The initial rapid processes were favored especially at low concentrations. Both distribution coefficients and irreversible sorption fractions of D4 and D5 on the aerosols were determined based on isotherms of sorption and desorption. Distribution coefficients of D4 and D5 varied in a range of 8.32[GREEKX]10^{-5} ~ 4.17[GREEKX]10^{-2} m^3/m^2 and 1.47[GREEKX]10^{-3} ~ 1.52[GREEKX]10^{-1} m^3/m^2, respectively, depending on aerosol types due to unique interactions between D4 & D5 and each type of the aerosols tested. Kaolinite and sulfate aerosols showed high irreversible sorption for D4 and D5 whereas carbon black and sea salt aerosols reversibly interacted with the adsorbates. It was discovered that the irreversible sorption was caused by surface catalytic hydrolysis on the reactive aerosols. After the ring of the cyclic siloxanes was opened in the first step, consecutive surface reactions took place resulting in further cleavage of siloxane bonds. The degree of partitioning and transformation vary so widely that it is impossible to deal with the mixture of atmospheric aerosols as a single entity. Thus, atmospheric aerosols can play two major roles on volatile siloxanes: to remove airborne cVMS by sorption and to transform sorbed molecules to less volatile compounds such as silanols.

TH 054

Filtration performance characteristics of depth filter media using KCl particles

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Initial filtration performance of the filter media is usually evaluated by measuring two of the most significant parameters: the initial pressure drop and fractional collection efficiency. In the literature [1,2,3] these parameters are described as indicators weather the filter media is suitable for their intended application. Initial pressure drop is tested and usually regarded as the pressure difference (DP) measured up- and downstream of the filter media specie. Since the initial condition tests are conducted in a very short testing time, the loading of the filter media and thus its effect on pressure loss is usually neglected. For the purpose of filter media particle collection efficiency, test particles injected in the upstream of the test filter media can be of poly- or monodisperse nature. The test with monodisperse particle has been conducted and results are reported In our previous work [4]. In case of polydisperse test particles it is possible to evaluate the most penetrating particle size (MPPS) by comparing the concentration of particles of that particular size in the up- and downstream. This parameter is considered as of great interest by some testing standards available today [5,6]. These test standards specify the use of the KCl particles to determine the particle collection efficiency. Two depth filter media samples are used for this study to evaluate the filtration performance of depth filter media. During this study, the interest is to evaluate the above mentioned parameters by varying the face velocity

Due to the inconsistency of the data from the measurements for particles of the range 20 to 46 nm were not taken in consideration. From the initial KCl-water solution we could achieve a high particle concentration which in return had some slight effect on the pressure difference between two runs. The two media tested during this study have shown different results, having the media TFM1 which exhibits a more or less constant particle collection efficiency for the whole particle range while TFM2 media exhibits an increasing efficiency with the increase of particle size. Further test are required to confirm the behaviour of the filter media media during the test with submicron particles and the effect of particle loading. .

TH 055

A novel process to remove SO2 in internal circulating fluidized bed reactor

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Currently, reducing SO2 emission from power plants is a main issue for the environmental protection. Accordingly, fine powder based semi-dry FGD was proposed for higher SO2 removal efficiency which was accomplished by interacting flue gas and powder sorbent to be adhered on to the surface of particle1. The aim of the present research is to study a semi-dry FGD in fluidized bed reactor to be appropriate in the one-body cleaning process, evaluating the effects of water injection flow rate, Ca/S and NaHCO3/SO2 molar ratio, superficial gas velocity and specific water volume on SO2 removal efficiency.

Study of SO2 removal was carried out using fine powder of Ca(OH)2 and NaHCO3 as sorbents to achieve high desulfurization (>98%) with continuously fed water. The effect of operating variables such as water injection flow rate, Ca/S and NaHCO3/SO2 molar ratio, superficial gas velocity, and specific water volume on SO2 removal was investigated.

The target efficiency (>98%) was achieved by specific water volume of 0.035 liter-water/Nm3-gas as optimum operating condition at Ca/S molar ratio of 1.3 and gas flow rate of 20 Nm3/min. It was observed that the required specific water volume increases with the increase in superficial gas velocity or decrease in Ca/S molar ratio. SO2 removal efficiency of about 50~60% was achieved using NaHCO3 as sorbent in dry-FGD test and SO2 removal efficiency used with Ca(OH)2 was higher it used with NaHCO3 in semi-dry flue gas desulfurization.

TH 056

FT-IR as promising method for rapid determination of adulteration of gasoline with kerosene

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ABSTRACT
Adulteration of automotive gasoline is widespread throughout the world and Kenya is not left out. Adulteration takes its toll both in terms of the air quality and loss in tax revenue. Increase in prices of fuels and fuel intermediates is often cited as a cause for adulteration. Adulterated fuels deprive the consumers of assured quality fuels and lead to increased tail pipe emissions. In this study a method, using FTIR, has been developed to provides an alternative rapid analysis of adulteration of gasoline with kerosene using a peak at 1380.9cm-1. This method has been found to be economical in terms of amount of gasoline used (2µl) and the time for a single scan which is less than 1 minute.

Key words: (1) Adulteration (2) Gasoline (3) FTIR (4) Kerosene

EH01 - Ecosystem services in natural, agricultural and urban areas

TH 064

A novel approach to assess exposure for pollinators

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One of the main drawbacks in ecotoxicological risk assessment for terrestrial ecosystems is the lack of suitable methods for assessing exposure to plant protection products (PPPs) for epigean communities (bird, insects). This has particular relevance in risk assessment for organisms with a large foraging area like pollinators. At this stage, pollinator risk assessment is based on a procedure specifically addressed to *Apis mellifera*, comprehending a preliminary risk assessment with the Hazard Quotient, calculated as the ratio between the application rate of the PPP and a toxicological end point, refined by cage, semi-field and field studies. Therefore, any exposure assessment is not included in this kind of approach. In this work a procedure to assess exposure for pollinators was fully developed and applied. Starting from exposure models developed for the small scale, specific equations to evaluate the distribution of pesticides outside the field, as a function of the distribution of the treated crop, were obtained. The small scale approach was than adapted to the large scale; a semi quantitative index to determine the concentration of pesticides on non target vegetation was therefore elaborated. The input data of this approach are the application rate and land use. The developed approached was applied and experimentally validated in different field sites of 4x4 km located on NE Italy. A good agreement between predicted and measured concentrations was observed. Generally, the ratio between predicted and measured data was within a factor of about 2. The main results of this work are here presented.

TH 065

Relevance of an Integrated Pest Management approach towards understanding the possible causes in decline of honeybee population in the State of Punjab, India

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India has been popularizing the concept of Integrated Pest Management through out the country since early 1980's, having a whole division dedicated to it under the Directorate of Plant pro-

tection, Quarantine and Storage. The Ministry of Agriculture, Government of India has been encouraging the promotion of bio-pesticides and bio-fertilizers in this regard. However, the ignorance of the farmer in understanding the hazard linked implications of the indiscriminate use of pesticides they practice, are not only resulting in having its impact on the environment and human health, but also are having an impact on the honey bees population existing naturally or commercial breed bee hives. The current practice of agriculture, indiscriminate use of pesticides and plant growth regulator's, have given rise to the reduction of the honey bee populations and other beneficial insects of pollination importance. We tend to highlight our field experience and survey information collected in assessing the causes of decline in insect pollinators in Indian Agriculture, in the State of Punjab.

TH 066

Honeybee brood development: automated evaluation with the help of image analysis

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Adverse effects of plant protection products on development of the honeybee (Apis mellifera) brood under conditions close to the open environment are assessed according to the OECD guidance document 75. This evaluation requires assessment and correlation of the developmental stages in a large number of cells in several treatments and replicates at several time points. Manual counting of such systems is time consuming, of limited reproducibility and difficult to verify. These deficits can be resolved by purpose-built computerised image analysis tools.
Optimized computer-assisted digital image acquisition was conducted in order to deliver high resolution images with maximal contrast and minimal reflections. For the analysis of the images, customized macros of the freeware program "ImageJ"were created, providing automatic identification of the combs and an optimal interface to assess the developmental stage of the larvae. The features include:
1) jumping from comb to comb at optimal magnification,
2) non-destructive user-defined labelling of the staging of individual eggs, larvae or pupae,
3) galleries of the same comb on consecutive images of the same frame at different time points,
4) results of individual combs to facilitate data evaluation.
Ongoing development is aimed to implement pattern recognition of the combs' content, allowing the completely automated assessment of entire bee brood studies in a batch mode and also enabling the measurement of additional morphometric parameter (e.g. size, shape, staging).
The digital evaluation significantly accelerates the assessment of honeybee brood studies and allows full documentation of these studies with possibility of control and revision at the individual comb level.

TH 067

The value of using ecological models in the ecosystem service approach within ERA

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From theoretical concepts to applied conservational and management studies, ecological models have been proven to be useful on many levels. Recently, they started gaining popularity in chemical, mainly pesticide, ecological risk assessment (ERA). Their first and foremost advantage is gaining deeper insight into and understanding of ecological processes and mechanisms. Of special value for the risk assessment sector is the possibility of quantifying and extrapolating effects across spatial, temporal and biological scales, e.g. relevance of impact on individuals for the whole population, extrapolate from the local to the landscape scale etc. Moreover, they offer ample opportunities for extensive scenario testing and comparison without accompanying high costs of experimental setups or field-surveys. Finally, if well tested and validated, they can also have predictive potential.
The ecosystem services (ES) concept offers a new currency for discussing protection goals, evaluating and mitigating environmental issues, of course not only restricted to chemicals. The drivers behind different types of services are ecosystem processes, structures and functions, which can be described by the use of ecological models. First to identify the exact driver, or a service providing unit, (can be a local population, functional group, community etc.), then for quantifying the service provided, for testing and comparing different usage scenarios, and, if necessary, for identifying possible trade-offs between different services. Putting an economic value to a specific service, or a part of it, makes cost-benefit analyses much more feasible. This makes ecological-economical models very useful in quantifying and negotiating trade-offs between services.
In this poster, we discuss advantages and caveats of using ecological models for ERA, using protection goals defined through the ecosystem service framework and especially focusing on how ecological models can link the ES with ERA. We provide examples of the usefulness of models developed for other purposes and models developed for the specific purpose of service trade-off analyses, and propose future steps for involving the knowledge obtained with applying ecological models into a more meaningful and protection goal relevant risk assessment process.

TH 068

Understanding ecosystem services provided by rice fields

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While the concept of ecosystem services is not new, there is revived interest in how these services should be measured, monitored, and valued. Focused research over the last four years has provided insight into the mitigation capabilities of rice fields in the lower Mississippi River Valley of the United States. In an effort to assign an ecosystem value to this mitigation capacity, two field studies were evaluated for their effectiveness at decreasing concentrations and loads of various pesticides. In the large scale study (0.19-0.27 ha), aqueous diazinon concentrations decreased 81±18% from inflow to outflow after passing through a rice field. Given assumptions of the mean rice production in the state of Mississippi and current rice commodity prices, this area would have produced $355 - $507 worth of rice crop for a farmer. However, using ecosystem services values in published literature, the waste treatment value alone of these fields ranged from $1176 - $1670. A second, smaller scale study (0.02 ha) found similar results, with rice fields able to decrease aqueous atrazine and diazinon concentrations 77±20% and 82±15%, respectively, from inflow to outflow. Given the same assumptions above, $47 worth of rice could have been produced from this research plot, with approximately $150 worth of waste treatment achieved by the same system. Although these ecosystem service values are estimates, these studies demonstrate the potential for a 3-fold benefit in environmental services versus traditional crop production revenue. There are many additional issues to consider, including maintenance and other costs, but environmental practitioners must begin placing values on such systems to promote global sustainability.

TH 069

Soil invertebrate communities and urban pollution

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Soil invertebrates play an important role in the functioning of soil ecosystems. So far, focus has been mainly on natural systems. Little is known about urban soil and the impact of urban activities on soil invertebrate communities. This study investigated the functioning of urban soils with different characteristics, through the evaluation of the diversity and abundance of invertebrate communities. Final aim was to identify key species and their functional role in the urban soil ecosystem. Five sites with different grades of human impact were sampled in the urban area of Naples (Southern Italy). Soils were analyzed for physico-chemical properties (pH, granular composition, organic matter content, etc.) and Cu, Fe, Pb and Zn concentrations. To assess the abundance and diversity of the enchytraeid and microarthropod communities, soil samples were extracted using wet or dry Tullgren methods. Species richness varied among the sampled soils, and community composition differed, with certain taxa being present only in some soils. Four taxa (Collembola, Diplopoda, Chilopoda and Acarina) were extremely widespread and abundant. The study revealed that the sharply differentiated soil invertebrate community structure seems closely linked to soil properties, with the abundance and species richness of the soil invertebrate community decreasing with decreasing pH and organic matter contents and increasing metal concentrations. Further investigations are done to evaluate the effect of metals on the structure of the soil invertebrate community. For this purpose, bioassays with Collembola (Folsomia candida), earthworms (Eisenia andrei) and enchytraeids (Enchytraeus albidus) will be performed to assess toxicity of the different soils. These bioassays will follow the standard test guidelines (ISO, OECD) and focus on effects on survival and reproduction. In addition, metal uptake by the test organisms will be evaluated.

TH 070

Decision analysis for a sustainable environment, economy & society

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Environmental decisions are often made without consideration of the roles that ecosystem services play. Most decision-makers do not currently have access to useful or usable methods and approaches when they are presented with choices that will have significant ecosystem impacts. The goal of the Ecosystem Services Research Program (ESRP) Decision Support Framework (DSF) Team is to help provide that access by identifying or developing effective and user-friendly decision methods and approaches that empower decision-makers to explicitly and routinely incorporate ecosystem services into their decision-making. To this end the ESRP DSF team is developing an open-source, web-based decision analysis framework called DASEES: Decision Analysis for a Sustainable Environment, Economy and Society. DASEES integrates guidance and decision support tools to implement a five step iterative Bayesian decision process:
Step 1 - Understand Content
Step 2 - Define Objectives
Step 3 - Develop Options
Step 4 - Evaluate Options
Step 5 - Take Action
DASEES will be developed with stakeholder and decision-maker input, through case studies, to ensure the guidance, tools, and templates meet user needs and facilitate the incorporation of ecosystem services in the decision-making process.

TH 071

Ecosystem service models for balancing nature protection and wind energy development

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Renewable energy development has been the focus of energy policy development in many countries worldwide, particularly among European Union Member States who have agreed to obtain national targets in support of multiple Directives. However, renewable energy development is often associated with fundamental conflicts between policies that mandate environmental protection and policies that mandate energy independence and sustainability. In October 2010, the European Union released guidance on wind energy development that is specifically designed to balance prior nature conservation legislation with renewable energy development and siting policies. The resulting guidance proposes a development framework to guide assessment of potential impacts and resulting measures to mitigation stressors while balancing the need to provide the renewable energy development and financing community with predictable processes and mechanisms to implement wind generation projects. Successful implementation of such an approach will ultimately involve identification of the suite of stressors that may be associated with wind energy development, properly describing their relationship to ecosystem or nature protection services, and quantifying the value of ecosystem or nature protection services affected to properly mitigate for environmental impacts. Examples from related technical fields and regulatory frameworks in the U.S. and other countries such as Natural Resource Damage Assessment, Ecological Risk Assessment, and Environmental Impact Assessment provide ideal opportunities to describe the stressors associated with wind energy development, capture their relationship to ecosystem services, and appropriately quantify mitigation to compensate for potential environmental impacts. This presentation will draw upon approaches currently used in the U.S. to quantify stressor impacts and develop mitigation measures to protect biodiversity such as federally threatened or endangered species and their habitats. The model will demonstrate the utility and inclusiveness of an ecosystem service approach for evaluating wind energy development projects that balances the need to protect nature with the need for sustainable energy sources consistent with current environmental directives in the European Union and comparable regulatory requirements in the U.S. and other countries.

TH 072

Quantifying ecosystem service considerations during restoration of contaminated lands and waterways

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While a significant part of regulatory decision making has focused on the protection of human health, regulatory agencies and international non-governmental organizations have re-emphasized their mandate to protect the environment by encouraging long-term sustainable practices and an increased focus on the ecological benefits of environmental policies and actions. This renewed effort reflects recognition by business, government, and other institutions of the need for more rigorous and comprehensive accounting of the ecosystem impacts of their activities. As such, an understanding of ecosystem services, how services are quantified, and how the values of different types of services are ascertained and evaluated has become of paramount importance. Several models and methods for quantifying ecosystem services and evaluating tradeoffs between different policy or management alternatives have emerged that enable decision makers to assess the implications of decision alternatives on preservation, enhancement, or reduction of different types of services. These tools make clear the ways in which regulatory policy and management choices affect the type, quality, and magnitude of services we receive from ecosystems - such as air, water, productive soils, food, timber, and other resources and processes that sustain humans and biota alike. This presentation will show example results of tool applications, including a framework for screening and cataloguing ecosystem attributes and the application of tools such as Habitat Equivalency Analysis (HEA) and Net Environmental Benefits Analysis (NEBA) to quantify and aggregate ecological service flows (both detrimental and beneficial) of various restoration actions on resources and ecosystem attributes. A weighting scheme for compiling net changes in habitat value and ecosystem services, and approaches to monetize the relative changes in habitat value and ecosystem services between restoration alternatives will be demonstrated. It is evident from the environmental directives in Europe and environmental policy changes occurring in the U.S. and elsewhere that business and government can both benefit - financially, as well as in terms of stewardship and reputation - by incorporating ecosystem service considerations into the management strategies guiding their environmental obligations.

TH 073

A comparative analysis to select the best remedial alternatives for the Ecosystem of the Pallanza Bay (Lake Maggiore)

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In the context of site remediation, a Comparative Analysis is an approach that provides a formal quantification of the change in ecosystem service values (ecological and human use) that would be associated with the implementation of a remedial action and compares those changes to costs and predicted changes in risk. The goal of a CA is to provide the necessary information to support the selection of remedial alternatives that maximize benefits to the public, while managing site risks and remedial costs.
A CA is typically conducted to evaluate if the potential exists for a remedial action to create more natural resource harm and/or risk than that predicted by the risk assessment that drove the remedial action in the first place (i.e., create or increase natural resource liability) or provide a marginal benefit compared to the effort expended.
In this study four main remedial alternatives for the Pallanza Bay in the Lake Maggiore were identified for evaluation: monitored natural recovery (MNR) of Pallanza Bay; MNR of Pallanza Bay combined with riparian enhancement along the Toce River(tributary of the Pallanza Bay); capping of Pallanza Bay, and dredging of Pallanza Bay.
The CA required the:
1. Quantification of the ecological service losses and gains with the Habitat Equivalent Analysis, associated with implementation of each remedial alternative;
2. Quantification of the human use service values relying on tools used in benefit-cost analysis (e.g., benefits transfer methodology) associated with implementation of each remedial alternative;
3. Evaluation of how the human health and ecological risk profiles would change given implementation of each remedial alternative;
4. Development of order-of-magnitude cost estimates for each remedial alternative.
Based on the results of the Comparative Analysis it is recommended that the engineered remedial alternatives (capping and dredging) not be considered as viable options for Pallanza Bay. Capping and dredging appear to provide no benefit associated with overall ecological and human health risk, result in substantial detrimental effect on both ecological and human use service values and are characterized by costs that appear disproportionate to incremental benefits derived.

TH 074

Implications of the European Commission's September 2010 decision on measuring 'Good Environmental Status' in marine waters

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On 1 September 2010, the European Commission released a "Decision"providing the clearest explanation, to date, of what precisely is required to achieve "Good Environmental Status"(GES) in the different environmental directives arising from European Union environmental legislation during the past 10- years. According to the "Decision", achieving good environmental status requires that all relevant human activities are carried out in coherence with the requirement of protecting and preserving the [marine] environment and the concept of sustainable use of [marine] goods and services by present and future generations. The "Decision"provides the first indications of what constitutes applicable and acceptable quantitative metrics for judging environmental quality in the marine environment, though several of the metrics are likely applicable to freshwater, soil, air, and biodiversity considerations as well. The "Decision"also points to areas where further scientific work is needed to improve the understanding of certain environmental metrics or to develop tools that are either absent or inadequate. Recognition of the limitations of the current state of knowledge and ability to collect meaningful measurements represents a significant acknowledgement that much work remains to be done to achieve the environmental quality goals envisioned in European Union environmental legislation. These same limitations are evident in North America and elsewhere; thus suggesting a possible path forward for future environmental and ecological research not only in Europe but also around the world. The "Decision"is particularly relevant to two concepts of environmental management evolving rapidly in both Europe and North America - preserving, restoring, and/or enhancing ecosystem services; and, adaptive environmental management. This presentation explores further the implications of the "Decision"on environmental management in the European Union and elsewhere. Additional insight on the limitations of the current state of monitoring practices and knowledge of ecosystem services and quality are discussed by reviewing the Part A annex, which addresses general conditions governing application of criteria and related environmental indicators, and the Part B annex, which specifies criteria for assessing the extent to which GES is achieved and lists related indicators and applicable methodological standards, where available, to make such criteria operational.

TH 075

The 'Green Liver System' - using single ecosystem function of aquatic macrophytes for sustainable water purification

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The aquatic ecosystem must face a large input of xenobiotics, which are affecting organisms, populations and whole ecosystems. But not only anthropogenic substances is one cause of stress in aquatic ecosystems, also compounds synthesized by organisms within the ecosystem is another cause. To have clean water and stable aquatic ecosystems is the main goal for the future, as water is a limited resource in this world. Water purification could be one of the most expensive techniques, so methods cheaper and more sustainable must be developed to secure clean water for stable ecosystems and also for human use.
The ecosystem has many ways for selfpurification and stabilization and the river Tiete in Sao Paulo (Brasil) is a famous example for that. Crossing the city of Sao Paulo, the river is one of the most polluted water bodies with nearly no life concerning fish, plants etc, inside. But a few hundred kilometer after the city of Sao Paulo the river looks nice and clean with fish and plants in a nearly perfect ecosystem.
In the last years we developed the "Green Liver System"which is based on several basic ecosystem function using aquatic macrophytes. One of these basic function is the ability of aquatic plants to take up and bioaccumulate xenobiotics as well as natural toxins. Comparing the metabolic possibilities of plants with human liver , a lot of similar pathways could be detected.

ET05 - Ecosystem exposure, toxicity pathways and adverse health outcomes

TH 079

Perfluorooctanoic acid induces peroxisome proliferation-associated activities in the liver of Japanese medaka

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Widespread contamination of perfluorooctanoic acid (PFOA) in the marine environment draws a great concern over its ecotoxicological impact on marine mammals and wildlife. In the present study, the male Japanese medaka (Oryzias latipes) was adapted to the seawater to mimic the marine environment and was then exposed to the nominal concentrations of 10, 50, 100 mg L-1 PFOA for 7 d. There were no impact on survival, relative liver and gonad size, and condition factor (measure of growth) at any concentration tested. Peroxisomal acyl-CoA oxidase (ACO) activity was elevated at the highest dose with a marginal significance ($P = 0.06$). The increase of ACO activity was paralleled by the significant upregulation of PPAR-α expression at the same dose. PFOA induced a significant inhibition of catalase (CAT) activity at high doses with no changes of superoxide dismutase (SOD) or glutathione peroxidase (GPx) activities in the liver. These results strongly suggest that PFOA may induce peroxisomal fatty acid oxidation and impose the oxidative stress through the alteration of cellular oxidative homeostasis in the liver. PFOA increased the mRNA levels of proinflammatory cytokines such as IL-6, TNF-α and IL-1β, suggesting that it may be involved in inflammation and tissue injury. This study may contribute to understanding the mechanism of PFOA-induced hepatic toxicity in Japanese medaka and assessing the potential risk of PFOA in marine fish and wildlife. In addition, the present results obtained at the high concentrations may provide important biological endpoints relevant to situations such as environmental spills.

TH 080

Sexually dimorphic behavior to characterize hormone-dependent effects of non-dioxin-like PCBs in rats

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Neurodevelopment and sexual differentiation of the brain are dependent on intrinsically formed sex steroids. We previously demonstrated that developmental exposure to a PCB-mixture which was reconstituted according to the pattern found in breast milk caused decreases in aromatase activity in newborn male rats and feminization of sweet preference (SP) as a sexually dimorphic behavior in adult males. This outcome may be due to dioxin-like (Ah receptor-active) or non-dioxin-like PCB congeners (NDL-PCBs). The aim of the present experiments was to determine if exposure to highly purified NDL-PCBs (to avoid any Ah receptor activity) also results in alteration of SP. Pregnant rats were orally exposed to PCB52 (6 dose groups, total doses of 0-3000 mg/kg bwt) or PCB180 (6 dose groups, total doses of 0-1000 mg/kg bwt). In another experiment, rat dams were treated with equimolar doses of PCB74 or PCB95 (total dose, 760 µmol/kg bwt - PCB74: 222 mg/kg or PCB95: 248 mg/kg). For the determination of SP, adult male and female offspring learned to drink from two bottles in the week prior to testing. Basal water intake was determined during this period. Thereafter, rats were given a choice between a bottle of saccharin solution (0.25%) and a bottle of tap water on 5 consecutive days. Amounts consumed of either fluid were measured daily. Control females consumed twice as much sweetened solution than control males, thus, demonstrating sexual dimorphism of this behavior. Signs of supernormal behavior were observed in female rats exposed to PCB180, with increased saccharin consumption at medium dose levels and dose-dependent increases in the percentage of saccharin to total fluid intake. PCB180 males were unaffected. Exposure to PCB52 caused an increase in the ratio of saccharin consumption (male to female intake) only at the highest dose level (3000 mg/kg), due to diminished saccharin intake in females of this group, while saccharin consumption was not changed in males. A similar result was obtained in rats exposed to PCB74. However, developmental PCB95 led to an increased ratio of saccharin intake in males to females, due to elevated saccharin consumption in males. This result indicates feminization of SP in males exposed to PCB95. It further demonstrates that feminization of sexually dimorphic behavior occurs in the absence of Ah receptor activity, but different NDL-PCBs exhibit differential effects on this behavior. (Supported by the EU; ATHON, FOOD-CT-2005-022923).

TH 081

Effects of solution chemistry on the interaction between triclosan and humic acid

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Increasing levels of dissolved organic matter (DOM) are produced in peat catchments due to climate change. These high levels of DOM in surface water can form a problem in the preparation of drinking water. The presence of DOM in the production of drinking water can cause the formation of toxic disinfection by-products and can mobilize polar organic contaminants such as pharmaceuticals, endocrine disrupting compounds, herbicides and other industrial chemicals. The sorption of polar organic contaminants in the aquatic environment is generally not predicted well from hydrophobicity parameters. The polarity and the charge also influence the degree of partitioning of these contaminants towards DOM and their removal by adsorption to activated carbon. A different mechanistic view is therefore required regarding sorption of polar organic contaminants to environmental sorbents. This research is focused on studying the sorption of different polar organic contaminants to DOM from different origins. The biocide triclosan is considered as an emerging contaminant in the environment and has been selected here as a representative compound for polar organic contaminants. Preliminary experiments with triclosan ($pK_a = 7.9$) show an effect of pH on the partitioning of triclosan to solid-phase micro-extraction (SPME) fibers and Aldrich humic acid. The experiments were performed in 25 mM phosphate buffer. Concentrations ranging from 1 to 100 mM of phosphate buffer did not affect partitioning of triclosan to SPME fibers. The logarithm of the fiber-water partitioning coefficients decreased from $\log K_f = 3.39$ to 2.62 by increasing pH from 6 to 9. Likewise, association of triclosan with Aldrich humic acid decreased from $\log K_{doc} = 4.05$ to 3.59. These results show that solution chemistry is an important variable that determines the freely dissolved concentration of triclosan. Further research is aimed at determining the relevant parameters to predict sorption of polar organic contaminants to DOM and activated carbon. The results of this research will be used in a joint coordinated effort to predict the removal efficiency of organic contaminants in the different treatment processes of drinking water production.

TH 082

Bioactivity of synergized neem (Azadirachta indica.A.Juss) and potentiated insecticides for the control of Culex quinquefasciatus Mosquito larvae

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Environmental pollution, poisoning accidents and insecticides resistance beside the high cost; had been the most emerging problems that restrict the continuous use of chemical insecticides for pests and vectors control. The use of plant extracts as alternatives for chemical insecticides has been a sustainable and ecofriendly approach for managing these problems. The neem tree (Azadirachta indica.A.Juss) proved to be one of the most promising among plant species.

The present work was set to test synergism in neem extracts against Culex quinquefasciatus larvae by mixing them with sesame oil and potentiation when mixing neem formulations with lower doses of standard insecticides to reduce the cost.

Results of experiments revealed that neem oil (No) was the most effective against Cx. quinquefasciatus. 3rd instar larvae than neem seeds water extract (NSWE). On the other hand mixing sesame oil with both (NSWE) and (No) produced synergistic effect significantly increasing the larval mortality of Cx. quinquefasciatus. Furthermore the synergized (No) was more effective than the synergized (NSWE).

Results also showed significant potentiation of standard mosquito insecticides Abate (temephos) and malation when mixed with NO and NSWE. Abate at half the concentration (0.0002%) + NSWE (0.0002%) and malathion at half the concentration (0.0002%) + NSWE (0.0002%) were equally effective against the tested animals as the insecticides alone at full dose (0.0004%). Similarly NO potentiated abate and malathion using half the concentration of each and produced an effect similar to that of the insecticides at full conc. (0.0004%) (reduction of cost).

The relative toxicity of neem oil, neem oil/sesame oil and Abate/neem oil mixtures was assessed in terms of LC50 and LC95 values.

TH 083

Interactive toxicity of alum and polyDADMAC to Newsealand rabbits

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Interactive toxicity of alum and polyDADMAC to Newsealand rabbits

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Abstract

Six heads of Newzealand rabbits were divided into 2 groups. Group 1 animals were the undosed controls. The test group was given 1% alum and 1% polydadmac at 1:2 for a period of 10 weeks after an adaptation period of two weeks during which the animals were under ideal experimental conditions.

Clinical signs were closely observed with postmortem and histopathological examinations. Chemical investigations included enzymatic concentrations of ALP, GOT, CK, GPT and LDH and metabolic changes of albumin, urea, total protein, cholesterol, bilirubin, glucose and creatinine. Fluctuations in electrolyte levels of Mg, Fe, Na, K, Ca and P were monitored together with hematological changes in Hb, PCV, RBCs and WBCs.

On evaluation of the above results, The interactive water treatment with 1% alum and 1% polydadmac at 1:2 was considered toxic to Newzealand rabbits at the dose rates tried .Practical implications of the results were highlighted and suggestions for future work were put forward.

TH 084

Response of Newzealand rabbits to drinking water treatment with alum and polyDADMAC in terms of change in electroytes

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Response of Newzealand rabbits to drinking water treatment with alum and polyDADMAC in terms of change in electrolytes

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Abstract

Neazealnd rabbits of both sexes were purchased ,clinically adapted,given doses of polydadmac and alum at dose rates similar to those given by Khartoum Water Plant at flood season for human consumption.Sera were analyzed for electrolyte prior to experiment and there after at a week interval.As for Neazealnd rabbits tested for polydadmac,Sodium levels investigations revealed significant (P< 0.05) increase in the group recieving the highest dose,where as the rest of the groups showed no significance (P>0.05) differences.For potassium, significant (P< 0.05 - 0.01) increases in different groups were observed.Calcium levels showed remarkable (P< 0.05) deviations from normal in the serum of most groups.Significant (P< 0.05 - 0.01) increases and decreases were highlighted when testing the undertest rabbits for their phosphorus levels, whereas the control rabbits showed normal values when tested for the above mentioned electrolytes the thing which can be attributed to the hjepatic damage induced by the chemical.Both test groups showed significantly low values (P< 0.05 - 0.01) in magnesium, iron, calcium, and phosphorus when compared to the control group in case of testing them fo alum. The control group animals showed normal electrolyte values.This can also be attributed to the direct effect of alum.Intestinal wall which was spotted with white (probably with alum causing focal enteritis) was greatly affected with the irritant alum and/or its metabolites. When the resin is precipitated by alum in its preparation, it is more apt to gripe; commonly the salt intensifies its action and the cream of tartar increases the hydragogue effect .This action was very clear on the congested mesenteric blood vessels and symptomatically, by diarrhea and salivation due to nausea.Similar picture was seen when using alum as atest material.Future suggestions were suggested and practical implications were forwarded.

TH 085

Evaluation of the toxicity of glyphosate and its different formulations using the cell line human HepG2.

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The herbicide N-phosphonomethyl glycine, commercially known as glyphosate, is one of the most widely used agrochemicals in the fields of Argentina and the world. The use of different glyphosate formulations for weed control in agriculture produces herbicide excess in the environment that may cause undesirable toxic effects in the biota. Although humans are not a direct target, they are in risk of glyphosate exposure. Because the molecular mechanism of gliphosate toxicity is poorly understood, this study will assess the possible adverse effects and the risk of human exposure to glyphosate. We studied the glyphosate toxicity in an in vitro model of human cells (hepatocytes cultures, HepG2). The human hepatoma cell line Hep G2 was cultured in minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 mg/ml streptomycin and 2.5 mg/ml amphotericin B. Cells were cultured at 37 ºC in a humidified atmosphere of 5% CO2-95% air and the medium was renewed twice a week. For all experiments, confluent attached cells were removed from cell culture dishes with 0.25% sterile trypsin, diluted with MEM/10% FBS and replated into 96-well plates (0.2 ml; 2 x104 cells/well) for 24, 48 and 72h for the MTT assay and into 6 well plates (3ml; 3 x 105 cells/well) for 24h for the caspase 3/7 activity assay, and lipid peroxidations as thioborbituric acid reactive substance (TBARs) level. We measured the cytotoxicity (LC50) of Roundup® and of Atanor® glyphosate formulations with or not the addition of Impact® adjuvant, and the technical grade glyphosate in its acid form on cell line HepG2. All parameters were measured at sub-agricultural doses with all formulations.

The LC50 of different glyphosate presentations at 24, 48 and 72h was determinate: 41.2 (37.6 - 45.1), 35.2 (34.9 - 35.4), 34.7 (34.1 - 35.3) mg/L for Roundup; 93.9 (75.0 -117.4), 40.4 (31.2 - 52.2), 29.9 (17.8 - 50.4) mg/L for Atanor® with Impacto®; 445.7 (416.4 - 477.1), and 418.7 (405.1 - 432.8), 383.5 (372.8 - 394.4) mg/L for Atanor®. Not significant effect was observed at sub-agricultural doses with technical grade glyphosate in its acid form. The caspases 3/7 are significantly activated with doses of Roundup® (35 mg/L) up to 240% in 24h. Not significant effect was observed at this dose for TBARs levels.

We conclude that cytoxicity and apoptotic effects were more dependent on the formulations than on the glyphosate concentration.

TH 086

Dissolution study of silver nanoparticles in toxicology media

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Currently, silver nanoparticles (AgNPs) are the most widely used nanomaterials, with particular applications as antimicrobial agents. Textiles, wound dressing, washing machines and cosmetics are some examples of consumer products containing AgNPs1,2. Due to their wide range application, exposure of AgNPs to the environment is likely3,4,5. Thus, risk assessment becomes more important.

Previous studies found that toxic effect of AgNPS was highly likely due to silver ions released from its NPs form6,7. As this study is part of larger study that will be looking at eco-toxicology of silver nanoparticles to daphnia magna, fish and algae, it is important to understand the solubility and dissolution kinetics of AgNPs in the toxicology media used for eco-toxicology study. Thus this piece of project will examine dissolution properties of silver nanoparticles with different physic-chemical properties in OECD toxicology media.

All the silver nanoparticles used in this study wese self-produced. Different techniques were implemented to generate particular size of NPs as well as capping agent. All of them were fully-characterised with several instrumentations such as UV-Vis spectrometer, DLS, Fl-FFF, TEM and AFM.

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TH 087

A comparison of methods for measuring the metallothionein induction in metal-exposed Chironomus tepperi.

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Biomarkers (changes in the biological functioning of an organism) have potential application in assessing the presence of pollutants in the environment including heavy metals. Metallothionein (MT) is a small, cysteine rich protein involved in detoxification and regulation of heavy metals in many species. It has previously been shown that exposure to metal contamination results in an increase in MT levels in a number of invertebrates. MT has therefore been suggested for use as a biomarker of metal contamination. This study compares three methods for the detection and quantification of MT in the non-biting midge *Chironomus tepperi*. The methods were Western transfer followed by immunoblotting using a specific antibody, a well established mercury saturation assay and the Brdicka differential pulse voltammetry (DPV) method. Tissue was collected from *C. tepperi* larvae following exposure to copper sulphate at sub-lethal concentrations in water, and controls exposed only to artificial water were run alongside exposed larvae. The immunoblotting method showed a clear difference in concentrations between two MT standards as well as in tissue from larvae exposed to different copper concentrations. As far as we are aware this is the first time a Western Blotting immunological method has successfully detected MT in chironomids. Results compared favourably with those from the well-established mercury saturation assay which uses mercury as a surrogate to MT. The Brdicka DPV method showed a clear difference between MT standards and was able to quantify MT in tissue at the higher contamination level, however polarographic peaks were indistinct. The immunoblotting technique may represent a relatively straightforward way of determining MT concentrations in aquatic invertebrates exposed to metals.

TH 088

Development of genitalia in the ivory shell (*Babylonia japonica*): observation of the specimens from wild populations and laboratory-reared juveniles

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We examined development of genitalia in the ivory shell (*Babylonia japonica*) with a histological method, using 2-year-old shells from wild populations and laboratory-reared juveniles for 0-1.6 year of age, in the chain of studies on analysis of induction mechanism of imposex caused by organotin compounds, such as tributyltin (TBT) and triphenyltin (TPhT) from antifouling paints, in gastropods. Parent ivory shells were collected in Miho Bay, Tottori, Japan by commercial fisheries and then reared in the tanks with flow-through systems and 60cm-glass aquaria with filtering systems, using natural seawater collected out at Sea of Japan and deep sea water collected at approximately 400 m in depth of Suruga Bay, Japan, respectively. Concentrations of TBT and TPhT in both of seawater were below the detection limit by gas chromatography with mass spectrometry (GC/MS). Egg capsules spawned by parent shells were rinsed by distilled water and then moved to other tanks/aquaria with flow-through systems. It took approximately 3 weeks until larvae hatched out. Veliger larvae were settled down at bottom of the tanks/aquaria within several more days. Settled juveniles were fed Antarctic krill (*Euphausia superba*) every day. Shells for approximately 6-month-old, 1-year-old, 1.2-year-old, 1.4-year-old and 1.6-year-old were removed for histological observation. After shell removal, soft tissues were fixed in Gendre's fluid (a 15:5:1 solution of saturated 2,4,6-trinitrophenol in 30% ethanol:35% formaldehyde:acetic acid) for 48h, and then embedded in paraffin. Serial sections were prepared using a microtome, stained with Hematoxylin-Eosin (HE) and observed with a light microscope. Two-year-old shells from wild populations in Miho Bay, Japan were also treated with the same method as described above. Differentiation of gonad (i.e., testis and/or ovary) was unclear before 2 years of age. Immatured vas deferens, however, was observed in juveniles for 1.2-year of age and older, although no penis was observed in those younger than 2 years old. Immatured oviduct was observed in 1-year-old juveniles in which vagina were not yet opened. Differentiation and development of genitalia preceded gonadal development in the ivory shell (*Babylonia japonica*), suggesting that regulatory mechanism of reproduction could be different between gastropods and vertebrates, in terms of differentiation and development of reproductive organs.

TH 089

Interactions between endogenous and exogenous antioxidants against Pb-induced oxidative stress in wild ungulates from a Pb polluted mining area

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Heavy metal mining and smelting commonly contaminates air, water and soil and this contamination is then transferred to biota through food chains. It has been widely suggested that certain physiologic disorders and diseases attributed to the exposure of lead (Pb) are related to the generation of oxidative stress. To protect from oxidative damage, organism rely on a complex antioxidant system composed of endogenously produced compounds such as glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPX); and dietary antioxidants such as vitamins A and E. The aim of this work was to study the interactions between both kinds of antioxidants and to evaluate the importance of dietary antioxidants to fight against Pb-induced oxidative stress in wild ungulates living in a mining area. Wild boar and red deer from the mining sites had higher bone and liver Pb levels than the controls. The results obtained in this work certify that important differences exist in the antioxidant cycling system between red deer and wild boar and their response to Pb pollution. Both red deer and wild boar exposed to Pb showed disturbed hepatic vitamin A and E homeostasis, which were closely related with the effects observed on endogenous antioxidants, therefore suggesting that dietary antioxidants are especially important for wildlife to fight against Pb-induced oxidative stress. Bone Pb in boar was essential to explain the main differences between control and mining area, therefore ratifying that long-term effects exposure on the antioxidant system are present in this species. We suggest that the management of these game species should consider a supplementary feeding with foods enriched with vitamins A and E when animals are suspected may be exposed to Pb pollution.

TH 090

Assessment of DNA adducts in Dreissena polymorpha exposed to benzo[a]pyrene

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Genotoxicity analysis is widely used in biomonitoring programs for the evaluation of biological effects of pollutants on marine organisms whereas similar investigations are not clearly envisaged for freshwater animals. In the present study, the relevance of DNA adducts as biomarker of genotoxicity related to PAHs contamination of urban water has been investigated using the freshwater mussels Dreissena polymorpha This zebra mussel is a very suitable sentinel species for pollution monitoring in view of its abundance and its capacity of accumulation of metals and organic micro-pollutants. Mussels were collected in a reference site and then exposed in the laboratory to various concentrations of benzo[a]pyrene ranging from 1 µg/L to 100 µg/L for 4 days. Afterwards, mussels were kept in running water for 28 days of recovery period and DNA adducts levels were subsequently measured on two different organs, the digestive gland and the gills, using the 32P post-labelling technique. Benzo[a]pyrene nominal concentrations and bioaccumulation in mussel tissues were measured by GC-MS (Gaz chromatography-Mass spectrometry). Results will depicted more precisely i) the dose response of DNA adducts formation following benzo[a] pyrene exposure and their relationship with nominal concentration and mussel benzo[a]pyrene accumulation, ii) the tissue differences in DNA adducts formation and iii) the persistence of DNA adducts. Ultimately, data converge towards the fact that DNA adducts represent a very promising biomarker for water quality monitoring.

TH 091

Flow cytometry methodology for measuring chemical induced cellular dead cell apoptosis and necrosis of splenic leucocytes in European bullhead, Cottus sp.

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In environmental immunotoxicology, leucocyte apoptosis and necrosis parameters may constitute pertinent biomarkers of immunological disturbance due to their modulations by numerous xenobiotics. In an ecotoxicological concern, the aim of this study was therefore to develop immune-related biomarkers using flow cytometry methodology in the European bullhead, Cottus sp., a current freshwater fish. In this context, spleens of bullhead were interesting due to sampling facilities and to their immune function. After spleen tissue disruption, leucocytes of each organ were isolated using density gradient centrifugation and cells were adjusted to 106 cells.mL-1. For each leucocyte sample, five aliquots were carried out. With the first aliquot, autofluorescence of cells were detected. YO PRO®-1 (100 µM) and Propidium Iodide (PI, 1.5 mM) were added in two different aliquots in order to obtain cellular fluorescence parameters indicating the presence of apoptotic and necrotic leucocytes, respectively. Both YO-PRO®-1 and PI were added in a double markedly method. Spleen leucocyte cellular aliquots were also treated with YO-PRO®-1, PI and quinoline alkaloid camptothecin (100 µM) which induce apoptosis. After incubation, cellular analysis were carried out with a CyanTM ADP flow cytometer connected to hypercyt®intellicyt (Beckmann Coulter). Apoptotic and necrotic dead cells showed green and red fluorescence, respectively. The camptothecin treated cells presented similar necrotic values and a higher percentage of apoptotic cells than those observed in controls. In consideration of these developments, dead cell apoptosis and necrosis were tested during in vivo controlled-laboratory experiments where European bullheads naturally infected with Saprolegnia sp. and Ichthyophtirius sp. were exposed to 25 µg.mL-1 of Copper. This exposure induced a decrease of cellular apoptosis and necrosis in contaminated organisms compared to controls. In conclusion, this work enabled a technical methodology to evaluate fish immune-physiological markers on spleen leucocyte of the sedentary bullhead, Cottus sp. For the assessment of wild fish health, further studies will be undertaken in order to characterize the potential and to validate the interest of these biomarkers in immunotoxicology studies.

TH 092

Integrated biomarkers responses in fish from active biomonitoring and whole effluent toxicity assessment of a mining polluted fresh water system

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An integrated biomarker response study was conducted on two fish species in the Blesbokspruit wetland subjected to water polluted primarily with mining effluent. This is a hydrological important wetland in the Gauteng region of South Africa and its ecological integrity is being continuously threatened by large volumes of effluent discharge. Previous studies were only conducted on metal accumulation. Active biomonitoring (ABM) was conducted by deploying laboratory reared Tilapia, Oreochromis mossambicus (n=20) in cages at five sites during the high flow period of 2009. After 4 weeks exposure whole samples of fishes were collected. In addition whole effluent toxicity (WET) testing was carried out in the laboratory on water collected from the respective sites using the standard fish test organism, Poecilia reticulata. Biomarkers of exposure: metallothioneins (MT), ethoxyresorufin-o-deethylase (EROD) and acetylcholine esterase (AChE) and biomarkers of effect: catalase (CAT), cellular energy allocation (CEA) and superoxide dismutase (SOD) responses were determined in all fish samples from the ABM and WET studies. Hundred percent mortalities were recorded in the ABM studies at sites 2 and 4. However the 96 h WET assessments revealed little or no mortality at all sites. Similar patterns in biomarkers responses were observed in fishes exposed both during the ABM and WET studies. For example, induction of MT and catalase stimulation both indicative of metal exposure and oxidative stress was observed during both times. EROD induction and AChE activity inhibition, which indicates organic and organometallic exposure was also observed. The WET exposed fish at sites 2 and 4 showed more altered biomarker responses. Integrated biomarkers studies were able to successfully demonstrate biological effects from toxicants in the system and allowed better understanding on organisms' responses to a combination of stressors other than just measuring mortality. The use of active biomonitoring as a reliable means of assessing environmental health is further established.

TH 093

Ex vivo evolution of kidney leukocytes immune parameters in European bullhead (Cottus sp.)

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In all living organisms, immunity is essential for body protection against biological pathogens. This physiological system is particularly sensitive to chemical contamination. Immune biomarker measurements can be used in laboratory experiments to assess the impacts of xenobiotics on cellular and humoral functions in an immunotoxicological concern. Moreover, these immune indicators can be also applied under field context to characterize the health of wild fish. In environmental immunotoxicology, analysis of immune parameters in native fish populations may be particularly difficult as immune cellular or biochemical responses have to be studied in fresh tissues with living cells. The aim of the present study was to determine in laboratory, required conditions to analyze cellular immune responses from kidney tissue samples in European bull-

head (Cottus sp.). For this purpose, eight bullheads were sacrificed and kidneys were immediately dissected out in order to study some immune markers. Each immune response was analyzed either immediately after tissue homogenization (T0) or 12 and 24 hours after tissue kidney treatment. Oxidative burst activity was measured by a chemiluminescence technique. Cellularity, cell mortality (apoptosis versus necrosis and leukocyte phagocytosis activity were performed by flow cytometry. Results obtained from analysis realized 12 or 24 hours after fish dissection were compared with those obtained when analysis were done immediately after kidney dissection and homogenization. In this context, no significant variation in kidney leukocyte composition was observed at 12 or 24 hours. Cellular mortality and phagocytosis activity were reduced 12 hours after kidney dissection whereas the oxidative burst activity decreased only 24 hours after tissue sampling. Simultaneously, leukocyte viability was increased 12 hours after kidney treatment. The sampling procedure and tissue treatment may be responsible for stressful conditions for isolated leukocytes which must be taken into account for the following cytometric procedure. Under field conditions, kidneys must be homogenized immediately after dissection but cellular analysis and immune marker measurements may be done only 12 hours after kidney sampling procedure. So, in the field context of environmental immunotoxicology, it is not necessary to analyze immune cellular responses directly in field and a time of 12 hours is useful to obtain low stressed-isolated leukocytes suspensions.

TH 094

Litter degradation of transgenic corn, a potential pathway of Bt-toxin in streams

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Corn (Zea mays) has been genetically changed to produce the toxin Cry 1Ab against the lepidopteran European corn borer (Ostrinia nubilalis). Studies on the effect of Bt-corn on non-target organisms were mainly focused on terrestrial species. However, the exposure pathway of chaffed plant biomass into adherent ditches and creeks has so far hardly been taken into account. Data on half-live of the Cry 1Ab protein in decaying submerged corn material is up to now not available. Therefore a fate study by use of decaying plant material in microcosms was performed. The concentration of Bt-toxin in corn leaves was measured over a period of 23 days along with other components (lignin, cellulose, polyphenoles, C:N) of the leaves. Even after 23 days a relevant share of initial concentration of the toxin was detectable. The results will be discuss in the context of Bt-toxin fate and restricted decay under submerged aerobic conditions.

TH 095

Health risk of heavy metals (lead, cadmium) in game species

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One of the most important aspects of environmental pollution for humans is the intake of toxic elements in the diet. There is an increased interest among consumers in meat from animals kept in natural conditions, as in game occurs, because its high nutritional values, special sensory properties and it is considered a free-chemical food. However, these species often show higher levels of contaminants which can constitute a health/toxicological threat. In the European Union, the Commission Regulation 1881/2006 sets maximum levels (MLs) for certain contaminants (including lead and cadmium) in food derived from domesticated and wild organisms, but no level has been set for wild game.

In this study, concentrations of Cd and Pb in the muscle of free-living wild boar (n = 310) and red deer (n=205) were analysed by stripping anodic voltamperometry after acid digestion. Samples were taken from hunting activities in several places of Extremadura (East of Spain).

There were no important differences between the sampling places and between the two species. Levels of metals (mg/kg) in muscle of red deer ranged as follows: Cd: <LD-0.389 (mean: 0.016), Pb: <LD-11.557 (mean: 0.546). Samples with a concentration <LD were 45.8% for cadmium and 13.2% for lead. In muscle of boar these levels were: Cd: <LD-0.293 (mean: 0.011), Pb: <LD-20.678 (mean: 0.780); and samples <LD were 53.9% for Cd and 19.7% for Pb.

Metal concentrations exceeded the permitted values for domestic animals (Cd 0.05; Pb 0.1 mg/kg) in 10.73% and 3.23% of samples for Cd, 49.3% and 49.7% for Pb in red deer and boar respectively. However, these LMs can not be applied directly to game muscle since its consumption is quite lower than that of meat from domestic animals.

Another way to stablish the public health risk of these meats is the comparison with the PTWI (Provisional Tolerable Week Intake) set by the WHO. Our results show that the intake of these game meats represents <0.5% of the PTWI for both metals and species. A extreme situation is when the game meat is the only meat consumed (as in some consumers like hunters). In this case consumption of red deer meat could be 4.08 and 38.86% of the PTWI for Cd and Pb, respectively, and boar meat could be 2.86 and 55.79 % of the PTWI for Cd and Pb, respectively. Lead concentration in game animal, particularly wild boar, represent a serious health risk for this group of consumers.

TH 096

Development of tissue-based mammalian toxicity reference values for use in Ecological Risk Assessment

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Wildlife exposure to environmental contaminants is typically expressed as average daily dietary dose, where the total dose may consist of fractional ingestion of contaminated prey items, soil/sediment, or drinking water. Less frequently, wildlife exposure to contaminants is quantified in target tissues where contaminants exert toxic effects. Measurement of target tissue contaminant residues allows for estimating exposure at the site of action and serves as one line of evidence for evaluating potential risks to ecological receptors; however, tissue-based toxicity reference values (TRVs) are not typically available in the scientific literature, as is the case for dietary-based TRVs. Mammalian TRVs for kidney and liver tissue were developed for a subset of metals. The TRV development procedure consisted of three primary steps: (1) mechanism of action review; (2) a literature search for appropriate and applicable studies; and (3) TRV selection. Standard toxicology databases were reviewed to identify chemicals of interest (COIs) that have mechanisms of action relevant to kidney and liver tissue. The following COIs exhibit toxic effects in kidney and/or liver tissue: cadmium, chromium, copper, lead, and zinc. A literature search was performed to identify studies that met the following two primary criteria: (1) measured toxic effects that have been shown to produce potential population-level effects such as reproductive or growth impairments and survival; and (2) quantified kidney and/or liver COI tissue residues. A total of 348 toxicity endpoints from 212 studies were reviewed. Those studies that reported kidney and/or liver tissue residues that could be clearly associated with no observed adverse effect level and lowest observed adverse effect level concentrations were included in TRV development. Tissue-based TRVs were developed using a weight-of-evidence approach similar to the procedure described in the U.S. Environmental Protection Agency's Guidance for Developing Ecological Soil Screening Levels.

TH 097

Effects of polycyclic aromatic hydrocarbons (PAHs) by lipid oxidation in avian embryos

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In this study we present a new avian assay for the study of chemicals effects on lipid homeostasis. The input of PAHs into the atmospheric, aquatic and terrestrial system has been accelerating since the beginning of early industrialization. PAHs are well known to be potentially carcinogenic to humans and ecotoxicological effects of PAHs have been shown in a diverse suite of studies, including microorganisms, reptiles, birds and mammals. Relatively few studies address questions concerning the health impact of PAHs in avian species, but some laboratory studies show that PAHs are highly embryotoxic to birds when injected into eggs. We have designed a method to investigate the effects of PAHs on the β-oxidation of fatty acids in hepatic embryonic tissue from chicken (Gallus domesticus). Using this assay previous results suggest a small but significant increase in β-oxidation of fatty acids in chicken embryonic liver tissue after in ovo exposure to some PFCs. The focus for this study is exposure to 16 PAHs injected on day 4 into the air cell. This is then followed by in vitro incubation of the embryonic livers on day 10 with a tritiated fatty acid mixture (palmitate 16:0). The fatty acids undergo oxidation and repetitive cleavage of carbons before entering the tricarboxylic pathway and cycles of β-oxidation. As a result of this lipid oxidation tritiated water is produced. The radioactive water (3H2O) is collected and measured. Using our assay the effects of PAHs on the enzyme function regulating the hepatic fatty acid metabolism can be studied in vitro. This approach could be a very useful trying to find out possible mechanisms behind avian toxicity including disturbance of fatty acid oxidation and lipid homeostasis. Other compounds than PAHs will be tested. To the best of our knowledge, this is the first time such study has been performed.

TH 098

In vitro effects of pesticides and metals on biomarkers in Cerastoderma edule tissues (Bivalvia: Cardidae)

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Several studies have been showed that metals and pesticides in water can affect the activity of enzymatic biomarkers in bivalves. However there is little information on the mechanisms and pathways involved in the toxicity of these contaminants that can often affect non-target organisms. In this perspective the objective of this study was to evaluate the activity of glutathione-S-tranferases (GST), carboxylestarese (CaE) and cholinestarase (ChE) of *Cerastoderma edule* tissues exposed in vitro to mercury, cadmium, dimethoate and carbofuran. Cockles (20-30mm) were collected in July/10 from a site commonly used as reference in toxicity studies at Ria of Aveiro, Portugal (Barra). Digestive gland extracts, used for GST and CaE analyzes, and adductor muscles extracts, used for ChE analyzes, were prepared from three animals exposed to several concentrations of each chemicals (50, 25, 12.5 and 6.25 mM) during 30 minutes at 25°C. This study will help to understand the range of the in vitro effects of metals and pesticides on the biomarkers before the whole metabolic process by organisms.

TH 099

Ifluence of radiation on prolificity and viability of stable strains Drosophila melanogaste

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The pollution of biosphere with radioactive substances, heavy metals, organic pollutants initiates mutational changes. It is known the stable mutations, potential changes arise in chromosomes under mutagenic factors. These allterations may be restored to initial position for chromosomes or transfered to new mutations that would depend on external conditions.

Any changes of genetical material's metabolism (DNA, enzymes of replicating complex, synthesis of DNA precursors) will lead to alteration of mutational frequency. The mutations of definitive genes may arise damages in structure or function of enzymes. Those enzymes would participate in the processes of repairation, replication and recombination of genetical material.

The new stage in investigation of mutagenesis and recombination is bound with mobile genetical elements that were revealed in different representatives of each taxonomy group (including human). The research of mobile genetical elements (MGE) in pro- and eukaryotes have revealed their participation in realization not only mutations, but different adaptive and regulatory processes. Mobile elements are integral part of genome making its own correlation in manifestation morphological, physiological, biochemical characteristics of organism. In ordinary surroundings mobile genetical elements keep silence in cellular mechanisms, they occupy their constant sites of localizations in genome without moving. However, MGEs would be activated under unsuitable conditions such as radiation, heat shock, chemical mutagens. In its turn, this process leads to induction such indexes as increasing level of the mutational chromosomal rearrangements, alteration of gene expression, cell's death. That activation becomes the reason of new phenomena and signs, non-typical for individual organism.

The frequency of spontaneous mutations is sufficiently low and makes up 10 -5 - 10-7 range. The frequency of MGE's transpositions which are the basic reason for spontaneous mutagenesis is the same. Under different conditions the rate of transpositions of MGE may increase in a few orders. The reasons of increasing transpositional levels may be conditioned by genetical mechanisms or by activity of unfavourable external factors, to which heat shock, radiation, toxins are related .

TH 100

A watershed analysis of anthropogenic disturbance and fish species sensitivity in South Carolina, USA

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In 2009, the population of the State of South Carolina, USA was over 4.5 million people and has increased at a rate of around 13.7% during the last decade. While the population density per sq. mile is relatively low compared to other US states, it seems certain the population will further increase in years to come. Increasing population density leads to urbanization, which usually results in an increase in impervious surfaces, such as roadways, parking lots, and building roofs. This changing land use can have dramatic effects on smaller streams and creeks which form the upper

reaches of watersheds. In eastern North America, the development of once forested land causes habitat loss and interferes with natural processes and cycles especially in aquatic systems. During storm and rainfall events metals, polycyclic aromatic hydrocarbons (PAHs), and other contaminants are washed into lakes, rivers, and streams instead of being absorbed into the ground. These chemicals are being found in drinking water which is often prepared from surface water. Aquatic ecosystems are sinks for many contaminants. Despite the fact that waterways can be fragmented, watersheds encompass the entire drainage area of a locale. An investigation was conducted in 2009 to determine anthropogenic disturbances on watersheds throughout South Carolina, USA. Fish biomarker, fish species/diversity, water column metals, and land use data were used to determine endpoints of fish species sensitivity and resilience. The results from this investigation indicate the disappearance of fish species as well as reduced species diversity may be influenced by land development.

TH 101

Influence of radiation on prolificity and viability of stable strains Drosophila melanogaster

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The pollution of biosphere with radioactive substances, heavy metals, organic pollutants initiates mutational changes. It is known the stable mutations, potential changes arise in chromosomes under mutagenic factors. These allterations may be restored to initial position for chromosomes or transfered to new mutations that would depend on external conditions.
The new stage in investigation of mutagenesis and recombination is bound with mobile genetical elements that were revealed in different representatives of each taxonomy group (including human). The research of mobile genetical elements (MGE) in pro- and eukaryotes have revealed their participation in realization not only mutations, but different adaptive and regulatory processes. Mobile elements are integral part of genome making its own correlation in manifestation morphological, physiological, biochemical characteristics of organism. MGEs would be activated under unsuitable conditions such as radiation, heat shock, chemical mutagens. In its turn, this process leads to induction such indexes as increasing level of the mutational chromosomal rearrangements, alteration of gene expression, cell's death. That activation becomes the reason of new phenomena and signs, non-typical for individual organism.
The frequency of spontaneous mutations is sufficiently low and makes up 10 -7 - 10-8 range. The frequency of MGE's transpositions which are the basic reason for spontaneous mutagenesis is the same. Under different conditions the rate of transpositions of MGE may increase in few orders. The reasons of increasing transpositional levels may be conditioned by genetical mechanisms or by activity of unfavourable external factors, to which heat shock, radiation, toxins are related.
In some cases correlation between behavior of retrotransposons and changes of quantitative and adaptive signs are observed. In other cases the selection on dicreasing adaptive characteristics have lead to changing sites of MGE. The selection on increasing adaptive level have lead to special insertions in the previous sites. Such behaviour of MGE provides genotypical adaptations to harmful factors.

TH 102

Haematological effects of 1% Alum to Newzealand rabbits

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Newzealand rabbits were purchased form Balsam pharmaceutical factory, weighed and divided into groups. Group 1 animals were the undosed controls. Test groups were given alum at dose rates of 1% for a period of 10 weeks after an adaptation period of two weeks during which the animals were under ideal experimental conditions. .
Clinical signs,postmortem and histopathological examinations were closely observed together with hematological changes in Hb, PCV, RBCs and WBCs.
On alum challenge, Newzealand showed inappetance, nervous signs and were finally recumbent. The mortality rate was 100 %. On atomic absorption only the lungs kept residual alum , while the livers washed- out the substance ,may be via bile . Notably oral dosing with alum caused congested liver with white spots, stiff-greenish lungs and inflammed empty intestines. The un-dosed group 1 goats showed a normal picture.
On histopathology, alum-dosed goats showed necrosis in the cortex and medulla of the kidney in one group member, emphysema in the lungs and necrosis in the hepatocytes and congestion in the liver . Practical implications of the results were highlighted and suggestions for future work were put forward.

TH 103

Copper exposure increases histamine levels in marbled crayfish (Procambarus sp.)

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The kinetics of 0.05 mg Cu /L and 0.5 mg Cu /L in the form of CuSO4 to marbled crayfish were studied. Marbled crayfish exposed to copper individually in aerated glass jars for 14 days and then transferred to copper free water for another 14 days. At different sampling times the animals of each treatment were dissected to collect their organs. The copper uptake and elimination in the crayfish organs (hepatopancreas, gills, muscle, ovaries and exoskeleton) were evaluated with a one compartment model ; and the levels of histamine (neuromodulator) present in the heptopancreas and muscle over the exposure time were determined. The results showed that the copper accumulated mostly in the hepatopancreas where the uptake rate (223 ± SE 114 L.kg-1.d-1) was much higher than the elimination rate (0.008 ± SE 0.032 L kg-1.d-1). In the other organs, the copper concentrations were strongly regulated due to their efficient excretion. The histamine concentrations found in the hepatopancreas significantly increased with the duration of exposure. Animals treated with copper 0.5 mg/l contained higher level histamine than animals treated with copper 0.05 mg/l; in hepatopancreas were 13.8 ± 5.93 W.B. µg/g and 5.39 ± 1.01 µg/g W.B. respectively while in muscle were 1.46 ± 0.31 µg/g W.B. and 1.36 ± 0.02 µg/g W.B. respectively. The conclusion is that excess copper in water will be accumulated in the hepatopancreas and that the organ contained higher copper concentration had higher histamine level.

ET07 - Endocrine disrupting chemicals in the environment

TH 107

Testing the effects of environmental levels of xenoestrogens as found in the Douro River estuary (Portugal) on the maturation kinetics of fish gonads - A stereological study using the zebrafish

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A wide range of chemicals, both natural and man-made, do cause endocrine disruption. Particularly, some these compounds are contaminants that evoke biological responses mimicking those caused by female sex steroids. This event is occurring at many locations worldwide, and both field and experimental data point that negative consequences exist not only for individuals but also for populations. The mechanisms by which those compounds exert final effects are being disclosed, but doubts remain, including on impacts in the gametogenesis kinetics. Also, since in natural environments fish populations are exposed to many potential xenoestrogens, understanding impacts of mixtures continues to be of great interest. The main objective of this study was to understand if and how an environmental relevant mixture of xenoestrogens that we found in the Douro River, by chemical screening, can disrupt the normal gametogenesis in fish. Our hypothesis is that physiological disruption can end up disturbing the normal amounts of gametogenic compartments at breeding. For this purpose, adult zebrafish of both sexes were exposed during 21 days to an environmental mixture (MIX) of eleven xenoestrogens of diverse sources. A 100 ng/L ethynylestradiol positive control was added. Vitellogenin mRNA expression, evaluated by QT-RT-PCR, was greatly increased in both treatment groups, confirming the success of the experimental exposure setup and the estrogenicity of the mixture of xenoestrogens at concentrations observed in the Douro River estuary. A quantitative (stereological) analysis was made in the gonads, at light microscopy, targeting both the relative and the total volumes of the gametogenic stages. Point counting and the Cavalieri's principle were used. Preliminary data point that the estrogenic stimuli induced changes in structural compartments, and particularly in the positive control; with decreasing trends for the advanced maturation stages. There was a trend for a greater amount of interstitial connective tissue. This kind of effects was never structurally quantified in fish gonads. Despite the ultimate consequences of such disruptions are unknown, it could be logically argued that reduction / slow-down of the most mature cohorts and/or eventual interstitial fibrosis can adversely count when breeding is at stake. The findings add further explanatory bases to understand the negative impacts of xenoestrogens.
Acknowledgements: FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).

TH 108

Ethinylestradiol exposure of brown trout hepatocytes in primary culture can induce the emergence of smaller peroxisomes, mimicking new estrogenic effects uncovered in vivo

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Pharmaceuticals are biologically active compounds often found as environmental pollutants of aquatic systems. Estrogens are one of these pollutants and are able to promote substantial morphofunctional changes in liver peroxisomes. Our group discovered a seasonal pattern of peroxisome modifications in the trout model. There is a negative correlation between ovary maturation and the size of hepatic peroxisomes. There was found in vitellogenic females under endogenous estradiol induction a decrease in the peroxisomal volume and in its enzymes activities. This pattern can be reproduced by experimental *in vivo* exposure to waterborne estrogens. We aim to better establish the details of peroxisome morphofunctional kinetics under estrogenic influences and so herein we tested an experimental model, based on trout primary hepatocyte cultures exposed to ethynylestradiol (EE2). This study has a biomedical interest, namely when concerning all the potentially hidden impacts existing from the use of birth controlling pills, estrogen therapy or drugs that can achieve the environment and evoke peroxisome changes in reference models. Juveniles of brown trout (*Salmo trutta*) were used. The hepatocytes were isolated by means of a perfusion technique using collagenase and were seeded in a density of 5x105 cell/ml in a 24-well plate coated with poli-L-lysin, with white L-15 medium. After 24 h in culture, cells were exposed to 1, 10 and 100 µM of EE2 and to 0.1% of ethanol (solvent control). After 48 h and 96 h of exposure, cell pellets were submitted to catalase cytochemistry and then processed for transmission electron microscopy. Manual stereology was used on microscopic photographs for estimating relative volumes, surfaces and numbers of peroxisomes. Their mean surface, volume and spherical equivalent diameter were secondarily derived.
The preliminary results show a decrease in mean surface, volume and spherical equivalent diameter of peroxisome with the increase of EE2 concentration, however without modifications in their number. Despite this trend, only for 100 µM the results were statistically significant. In brown trout, similarly to what was described *in vivo*, there is *in vitro* a regulation of hepatic peroxisomes by estrogenic stimuli. In this way the *in vitro* test proved to be a good model for getting mechanistic insights about effects of estrogenic compounds in hepatic peroxisomes of brown trout.
Acknowledgements: FCT Project PTDC/MAR/70436/2006 (POCI2010, FEDER).

TH 109

Synergistic androgenic effects of an engine oil caused by the joint action of at least three chemically different compounds

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In previous studies, we showed that a series of crude oils and refined petroleum products generally caused (anti-)estrogenic and/or anti-androgenic effects in recombinant yeast and mammalian in vitro assays. As such, oils may represent an important source of EDCs in the environment. Interestingly, one oil (a BP engine oil; EO) caused a clear dose-dependent synergistic effect when combined with testosterone (T) in an androgenic (AR) yeast assay. As there is only little knowledge on synergistic effects and compounds, in particular for the androgenic system, the objective of the present study was to investigate this oil in more detail. To this end, the EO was fractionated into the so-called SARA fractions: saturates (i.e. aliphatic compounds), aromatics, resins (i.e. more polar compounds), and asphalthenes (i.e. very high molecular weight compounds). When dosing the AR yeast assay with T combined with the separate SARA fractions, no synergistic effects were however observed anymore. When pooling the fractions again, the synergism returned. From subsequent exposures of the yeast to combinations of two or three SARA fractions it appeared that at least the resin and the aliphatic fraction were required for the synergistic response. These results indicate that the mixture effect (synergism) was caused by a mixture of at least three compounds, presumably having different chemical properties: testosterone, a resin compound, and an aliphatic compound. They add to the growing body of evidence on complex mixture effects of EDCs, indicating that an addition assumption may be a too simplistic approach.

TH 110

Chemical and biological monitoring of EDCs in Italian drinking waters: a case study

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EDCs are routinely detected in surface waters. Due to the large number of suspected ECDs and the potential hazard to human health, there is a growing interest in their presence in drinking water and their fate during drinking water treatment. Both conventional and more specific treatment processes may be partly ineffective in completely removing selected EDCs, removal efficiency also depending on the water quality.

A pilot study was carried out in Italy (2008-2010) to detect the presence of estrogens and estrogenic EDCs and the estrogenic activity in raw and drinking water samples from 6 Italian waterworks (ACSM - Como, Acquedotto Pugliese-Bari, Publiacqua-Firenze, Hera-Bologna, Mediterranea delle Acque-Genova, SMAT-Torino). The project (www.edinwater.com) involved the Italian Istituto Superiore Sanità (ISS) for chemical analyses, and the Universities of Pisa and Genoa for biological analyses. Samples of raw and treated waters from 5 different sampling campaigns were analysed for the presence of 6 representative estrogenic compounds (17beta-estradiol, 17alpha-ethynylestradiol, estrone, Bisphenol A, 4-octylphenol, Nonylphenol) and for their estrogenic activity by two in vitro assays, the YES (Yeast Estrogen Screen) and the E-screen assay (human MCF-7 cell proliferation).

Chemical data indicate that relatively low concentrations of the chosen analytes are present in raw water samples (<LOR - 1914 ng/l) and that treatment processes are generally effective in removing these compounds (<LOR - 621 ng/l). Chemical data were supported by biological data of estrogenic activity obtained with estrogenic assays (EEQ low ng/l). A fair correlation was observed between data obtained by the two different assays utilising yeast cells and human cell lines. The present study is the first example of chemical and biological monitoring of EDCs in drinking water in Italy. The results obtained represent and important basis for comparison with similar surveys in other European countries.

TH 111

EDCs as Obesogens: effects of Bisphenol A (BPA) and Tetrabromobisphenol A (TBBPA) on lipid accumulation and PPAR expression in rat hepatoma cells

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The development of obesity and related disorders (fatty liver disease, type 2 diabetes, insulin resistance) in western countries has been associated with exposure to environmental chemicals. According to the 'Environmental Obesogen' hypothesis, obesogens are chemicals that can affect lipid homeostasis in various tissues by interfering with nuclear receptors (such as steroid, thyroid, retinoid receptors and Peroxisome Proliferator-Activated Receptors-PPARs) involved in regulation of lipid metabolism. Potential candidates are Bisphenol A, phthalates, brominated flame retardants, organotin compounds.

Bisphenol A (BPA), a potential estrogenic EDC, has been recently shown to promote adipogenesis. However, little is known on the direct effect of BPA on lipid homeostasis in the hepatocytes. In this study, the effects of BPA were investigated in the rat Fao hepatoma cell line. BPA induced lipid accumulation, as evaluated by Oil-Red-O staining and determination of triglycerides (TAGs). This effect was accompanied by down-regulation of all the three PPAR subtypes (PPARalpha, gamma and delta, that play key roles in lipid homeostasis. Moreover, down-regulation of the antioxidant genes catalase and superoxide dismutase-SOD was observed. The results support the hypothesis that BPA directly affects lipid homeostasis and redox balance in the hepatocytes. On the other hand, the flame retardant TBBPA (tetrabromo bisphenol A) did not affect the hepatocyte lipid content; however, TBBPA reduced TAG accumulation in fat enriched Fao cells and modulated the expression of different PPARs. The lipid-lowering effects were comparable with those observed with equimolar concentrations of thyroid hormones.

Our results demonstrate that different EDCs can directly interfere with lipid metabolism in the hepatocyte. The effects of obesogens on activities and expression of enzymes crucial in metabolic pathways (glycolysis, fatty acid oxidation, Lipid droplet formation) are under investigation.

TH 112

Ligand-independent activation of the Aryl hydrocarbon receptor (AhR) by the imidazole fungicide Prochloraz

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Microcystin is a toxic substance released by cyanobacteria, among which stands out *Microcystis aeruginosa* (MA). In the natural environment, blooms of cyanobacteria (FC) where MA is present alone will rarely happen. Normally, we can find in cyanobacteria blooms different cyanotoxins and substances that together to microcystins may increase or decrease the effects of this toxin on the aquatic organisms. In this study we evaluate whether microcystin-LR (MC-LR), present in lyophilized FC and MA, is able interfere on detoxification enzymes (glutathione S-transferase-GST) and on antioxidant defenses (superoxide dismutase-SOD, catalase-CAT, glutathione peroxidase-GPx and reduced glutathione, GSH) and cause DNA damage. Juveniles of *Prochilodus lineatus* were exposed to solutions containing 1 $mg.L^{-1}$ of lyophilized FC or MA or only water (control) for 24 and 96 h. After exposure, aliquots of water were collected for quantification of MC-LR and the fish were anesthetized for blood sampling, and then killed to remove the gills and liver. Biochemical analyses were run in gills and liver samples and the comet assay (CA) was performed using red blood cells. The analysis of water containing FC and MA showed 0.9 µg.L-1 MC-LR. When compared to controls, fish exposed to FC for 24 h showed a significant increase on liver SOD, and after MA exposure there was an increase on SOD, CAT and GPx in the gills and GST, CAT and GSH in the liver. After 96 h exposure, the exposure to FC lead to an activation of SOD and GPx in gills and GSH in the liver, while after MA exposure, there was an inhibition of GSH in gills and CAT in liver. DNA damage was detected only after 96 h exposure to MA. In summary, these results show that after 96 h exposure to MA there was inhibition of antioxidant defenses leading to oxidative stress and DNA damage of erythrocytes. Thus, it appears that exposure to lyophilized MA was more toxic to the P. lineatus than the exposure to lyophilized FC, although the two materials have the same concentration of MC-LR. This variation is a sign that, in addition to MC-LR, there are other cyanotoxins and/or other substances in lyophilized FC that inhibited the effect of MC-LR on the fish species studied in this work.

TH 113

Effects of Decabromodiphenyl ether (BDE-209) exposure on mRNA transcription of thyroid hormone pathway and spermatogenesis associated genes in Chinese rare minnow (Gobiocypris rarus)

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Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants, which are ubiquitous environmental contaminant found in both abiotic and biotic environmental samples. Deca-BDE (BDE-209) is the principal component, which is currently used worldwide. In this study, the effect of BDE-209 on the mRNA levels of thyroid hormone related genes and spermatogenesis associated genes were determined from larvae and adult rare minnow (*Gobiocypris rarus*) exposed to concentrations 0.01, 0.1, 1, and 10 µg/L for 21 days. The results showed that the type II deiodinase (*dio2*) and sodium iodide symporter (*nis*) mRNA levels were significantly up-regulated in the larvae at 10 µg/L treatment. In adult, histopathological observations showed that liver of female fish were degenerated at 10 µg/L treatment, and inhibition of spermatogenesis were observed in testis in male fish. In addition, the *thyroid hormone receptor α* (*trα*), *dio2*, and *nis* mRNA levels in the liver of male and female fish were significantly up-regulated, whereas *dio2* and *nis* mRNA levels were significantly down-regulated in the brain. These results indicate that exposure to BDE-209 could result in tissue-specific alternations of TH-related genes expression in adults. Moreover, the mRNA levels of the testis-specific apoptosis genes, the *spermatogenesis-associated 4* (*spata4*) and * spermatogenesis-associated 17 (spata17), were* down-regulated in 10 µg/L treatment in testis of male fish. Our results suggest that BDE-209 may pose threat to normal thyroid and reproductive function.

TH 114

Acute and short-term effects of 2-Chloro-4-Phenylphenol on mRNA expression of endocrine hormone receptor (TR, ER, and AR) in Chinese rare minnow (Gobiocypris rarus)

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In aquatic environment, phenols are a large group of organic contaminants because of their high solubility in water, strong reactivity, and poor biodegradation. They are toxic, persistent and bioaccumulative in animal and vegetable organisms and may pose serious threat to public health. 2-chloro-4-phenylphenol is one of PCBs metabolites, whereas quantitative data on the toxicity are not available. In this study, the acute toxicity tests of 2-chloro-4-phenylphenol to Chinese rare minnow (*Gobiocypris rarus*) were carried out by using Karber method. The results showed that the LC_{50} value of 2-chloro-4-phenylphenol on fish in 96h was 1.29 mg/L. And histopathological observation showed that mass cell apoptosis were observed in liver of die fish. Moreover, to determine the endocrine disruption effects of 2-chloro-4-phenylphenol, adult fish was exposed to concentrations 0, 0.1, 1, 10, and 100 µg/L for 21 days. The results showed that no effects on growth, gonadosomatic index (GSI), and hepatosomatic index (HSI) were observed between treatment and control. However, histopathological observations showed that inhibition of spermatogenesis were observed in testis in male fish. In addition, the *thyroid hormone receptor α* (*TRα*), *estrogen receptor* (*ER*), and *androgen receptor* (*AR*) mRNA levels in the liver of male and female fish were significantly down-regulated. These results indicate that exposure to 2-chloro-4-phenylphenol had antiandrogenic activity in male, antiestrogenic activity in female, and negative effected on thyroid system in male and female. Therefore, 2-chloro-4-phenylphenol could be a potential endocrine disruptor.

TH 115

Study on multi-generational effects of perfluorooctanesulfonate (PFOS) in Japanese medaka (Orizias latipes).

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Multi-generational effects of perfluorooctanesulfonate (PFOS) on medaka (Orizias latipes) were investigated. The present study was carried out according to the Draft Guideline of Medaka Multi-generation Test (MMT) which was prepared by National Institute for Environmental Studies in Japan. All adult fish or eggs used in MMT were maintained in test medium at 26 ± 1°C and fed exclusively the brine shrimp. PFOS (CAS 1763-23-1) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PFOS (10, 30, 100, 300, 1000 ppb) and controls were exposed during the exposure phase for 25 weeks in the flow-through system. For the first generation (F0), adult spawning fish were exposed various concentrations of PFOS for 4 weeks. Three pairs (female-male breeding pair) were placed in each test tank containing 5L test medium (duplicate). An adult pair and 10 sub-adult fish in the second and third generations (F1 and F2) were exposed PFOS in 2L test medium (6 replicates). Endpoints including survival rate, growth (body length and weight), time to hatch, hatching rate, fertility (reproduction), sexual development (secondary sex characters and gonad morphology), and hepatic vitellogenin concentrations were continuously measured during exposure phase. Chemical analysis of PFOS in the test medium was carried out once/every-week using high performance liquid chromatography (HPLC)-mass spectrometry (MS).

In the F0 generation, eggs were counted to investigate fecundity and fertility. PFOS had no effect on fecundity of F0 adult medaka, whereas statistically significant decrease in fertility was observed in 300 and 1000 ppb PFOS groups. Eggs were collected from breeding pairs to propagate the next generation. In the F1 generation, PFOS had no effect on time to hatch, hatchability and mortality of eggs. Adverse effects on fecundity and fertility were not observed in F1 sub-adults. In the F2 generation, PFOS influenced mortality of egg-sac fry depending on concentrations. NOEC (the no observed effect concentration) about all effects by this test showed 100 ppb. We are planning to report the additional data such as the gonad histopathology.

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TH 116

Occurrence of endocrine disruptors in Garda Lake and Mincio river (Italy): chemical analyses, in vitro assay and risk assessment

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An extensive characterization was conducted on the presence of endocrine disruptors in water and sediments from Garda Lake and its effluent Mincio river. Investigated compounds include selected natural and synthetic steroid hormones, alkylphenols, bisphenol A, triazine herbicides, polycyclic aromatic hydrocarbons, dioxins and polychlorinated biphenyls. The attention was also focused on the influent and effluent of a wastewater treatment plant which is located at the bottom of the lake and discharges treated water into the Mincio River.

Chemical analysis by liquid chromatography tandem mass spectrometry and gas chromatography mass spectrometry were integrated by biological assays to estimate the effects of the EDCs mix-

ture on MCF-7 cells. This in vitro model is an estrogen receptor (ER) positive cell line derived from a human breast adenocarcinoma. To determine the relative proliferative potency of organic extract from the waters, the E-SCREEN test was applied. This bioassay compares the cell yield between cultures of cells treated with estradiol, as positive controls, and cultures treated with different concentrations of extracts suspected of being estrogenic.
Our results showed low contamination of water and sediment by the monitored contaminants with levels below the quality standards defined for selected chemicals by the current Italian legislation. The effluent of the wastewater treatment plant contained estrone and estradiol in the range of 2-42 ng/L and nonylphenols, bisphenol A and tert-octylphenol in the range of 14-218 ng/L. The presence of estrone was also identified in the water from Mincio river.
Results from chemical analyses will also be analyzed applying the guidelines for human and ecological risk assessment in order to evaluate the adverse effects on human health and ecosystem induced by the levels of EDCs in the water.

TH 117

Evaluation and identification of estrogenic activity with the Yeast Estrogen Screen and the HPLC DAD of some Tunisian WWTPs

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In the present study, the yeast estrogen screen (YES) has been used to assess the estrogenic activity in effluent and influent of some WWTPs and surface waters of a coastal region in Tunisia. In order to assess the estrogenic activity, a recombinant yeast-based assay has been used. Two sampling campaigns have been carried out in 2004 and 2010 to measure the estradiol equivalent concentrations of fourteen different stations in Tunisia. Dose-response relationships for selected substances and their relative estrogenic potencies expressed as estradiol equivalent factors (EEFs) were determined. EEFs together with previously obtained concentration data based on liquid chromatography tandem mass spectrometry measurements (HPLC-DAD) were used to calculate estradiol equivalent concentrations. Solid-phase extraction C18 SPE was carried out on approximately 1l water samples. From the final extract volume, aliquots were used for the measurement of estrogenic activity and a other one for chemical analysis, which was performed by liquid chromatography coupled to high performance liquid chromatography HPLC-DAD. From 14 samples, water samples and suspended matter showed an estrogenic response. Based on the activity of 17-_-Estradiol in the YES assay, we estimated the estrogenic activity. The response in the YES was expressed as measured estradiol equivalents (EEQs). Samples from Wastewater Treatment plants located near an industrial or tourist area showed high estrogenic activities. Data obtained on high performance liquid chromatography (HPLC-DAD), confirmed the presence of various estrogenic compounds (17 _ estradiol, ethynilestradiol, estrone and estriol). The main contributors to the overall estrogenic activity were synthetic and natural hormones. Finally, the results obtained by biological and chemical analysis were compared.

TH 118

Tournament of bioassays: Comparing different in vitro systems for detecting endocrine activity in environmental samples

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Due to the ever-growing universe of synthetic chemicals, environmental pollution has reached a considerable level of complexity. This not only concerns the sources, fate, and groups but also the biological effects of man-made chemicals introduced in the ecosphere. With regard to the latter, the potential of chemicals to induce endocrine disrupting effects is of special concern because it might impair reproduction and development of diverse biota and, therefore, affect the integrity of wildlife populations.
Besides environmental chemistry, bioanalytical techniques (i.e. in vitro bioassays) add value to the investigation of the endocrine potential of complex environmental samples because they provide a direct measure of the relevant biological effect, e.g. estrogenic activity of sediment extracts or effluents of sewage treatment plants. The application of bioassays for environmental monitoring has two main advantages: First, bioassays integrate the joint effect of complex mixtures of chemicals and, therefore, realistically characterize mixture toxicity. Second, bioassays include the effects of so-far unidentified compounds that are missed in traditional chemical analysis. In that sense bioassays are a valuable tool to tackle the complexity and, thus, provide a more holistic picture of the universe of environmental chemicals.
To facilitate the use of bioassay data in regulatory practice, standardized assay systems and procedures are needed. In that context, we focus on estrogenic endpoints, for which not only a broad base of scientific data but also an array of in vitro bioassays exists. This includes reporter-gene assays based on yeast (e.g. Yeast Estrogen Screen) or mammalian cell lines (e.g. ER-CALUX) as well as proliferation assays (e.g. E-Screen). Within the scope of a project funded by the German Federal Environment Agency (FKZ 3710 26 323) we compile information on different in vitro methods that are commonly used for the quantification of estrogenic potentials. Here, we will compare several systems in terms of sensitivity, reproducibility, and applicability and discuss advantages and disadvantages with respect to different test matrices (waste water, surface water and drinking water).

TH 119

The development of a weight of evidence framework for the evaluation of endocrine activity

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Over the past 15 years, the "endocrine disruptor hypothesis"has been the subject of attention in the scientific community and the media. The hypothesis postulates that certain substances act like natural hormones, causing adverse effects by interfering with the endocrine systems of wildlife and humans. A component of the process is risk characterization, integrating all credible scientific information on hazard, dose-response, exposure and uncertainty to reach a scientifically defensible risk determination. To integrate available data into risk characterization, 'weight of evidence' (WOE) approaches are often used to examine and prioritize results. Hazard identification and risk characterization for endocrine active chemicals has been presented in various documents, including reports from ECETOC and the US EPA's two-tiered Endocrine Disruptor Screening Program. In the US program, chemicals selected for screening are subjected to a battery of 11 assays to determine their potential to interact with the endocrine system through androgen, estrogen, or thyroid pathways. Data integration is necessary, beginning with screening assays that identify potential interactions with the endocrine system, to testing for adverse effects in long-term reproductive and developmental toxicity assays, to mode of action analysis and risk characterization. We have formulated a framework for conducting WOE evaluations to help meet these objectives. For evaluating results of screening studies, null hypotheses are constructed for each of the three targeted hormonal modalities to test the premise that the chemical does not interact as an agonist or antagonist in estrogen, androgen, or thyroid pathways or with the aromatase or steroidogenic enzyme systems. Data for evaluating each hypothesis are weighted based on published recommendations for reliability and validity. Reliability is assessed on the basis of transparency and completeness of data reporting. Validity is assessed according to the degree to which three fundamental tenets are met, including that measurements are authentic and verifiable within an acceptably small degree of error, that accuracy is not compromised by extraneous uncontrolled factors and influences, and whereby results using the method are replicable by independent scientists. Each hypothesis is tested by comparing data from the screening battery versus the response produced by the appropriate prototype hormone and positive and negative controls.

TH 120

Endocrine disrupting potential of six major organophosphate flame retardants in H295R cells

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Flame retardants have been widely used in construction materials, furniture, plastics, electronic equipments, textiles, and other materials. Among the flame retardants globally used, organophosphate (OP) flame retardants are the third important group of flame retardants next to alumina trihydrate and brominated flame retardants. Compare with brominated flame retardants, OP flame retardants have received little attention with regards to potential health effects. Because of the ban of polybrominated diphenyl ethers (PBDEs), production and use of OP flame retardants has surpassed that of PBDEs in Europe. OP flame retardants have been detected in the house dust, surface water, fresh water biota, human urine and milk, at the levels similar to or greater than those of PBDEs.
In the present study, we evaluated the potential risk of OP flame retardants using H295R cell. The test compounds include tris-(2-chlorethyl) phosphate (TCEP), tricresylphosphate (TCP), tris-(2-butoxyethyl) phosphate (TBEP), TPP, tris-2-chloroisopropyl phosphate (TCPP), and TDCPP. The cytotoxicity of H295R cells by using MTT assay showed that TCP and TPP have highest cytotoxicity (1 mg/L), followed by TCEP, TBEP, TDCPP (10 mg/L), and TCPP did not show cytotoxicity even at 100 mg/L. In the H295R steroidogenesis assay, we found that all of these chemicals could increase 17β-estradiol (E2) concentrations. The E2 concentration significantly increased when exposed to 0.001 mg/L of TCP, 0.01 mg/L of TDCPP, 0.1 mg/L of TCEP and TBEP, 1 mg/l of TPP, and 100 mg/L of TCPP or greater. Concentration of testosterone also increased when exposed to concentration of 0.1 mg/l of TCP, TBEP, TDCPP, 1 mg/l of TCEP, and 10 mg/l of TCPP or greater. Mechanisms of endocrine disruption and the consequences should be further investigated.

TH 121

Assessment of in situ estrogenic effects in the Black chinned Tilapia (Sarotherodon melanotheron) using vitellogenin measurement by ELISA method

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The aim of this study was to develop an enzyme linked immunosorbent assay (ELISA) to quantify vitellogenin in the Black chinned Tilapia (Sarotherodon melanotheron). Tilapia vitellogenin T-vtg) was first purified from plasma samples of estradiol-induced fish using a two-step chromatographic method. The T-vtg was characterized with an estimated molecular weight of 482 kDa under native conditions. Polyclonal antibodies against T-vtg were raised in rabbits and the specificity of the antibodies to recognized T-vtg was verified by Western blotting and indirect ELISA experiments on blood samples. By using these antibodies and purified T-vtg, a competitive ELISA was set-up and validated. This assay appears sensitive, with a detection limit of 1.81 ng/ml, and specific, as shown by the competition curves, obtained by serially diluting blood of females, that were parallel to the T-vtg standard curves. The ability of the T-vtg ELISA to quantify vtg induction was achieved by in situ experiment. For this purpose, adults males and females tilapia were collected in 8 West African sites and circulating vtg concentration was measured. The present study shows that the developed T-vtg ELISA is a useful tool to assess the effect of estrogenic contamination in wild Black chinned Tilapia.

TH 122

Effects of EDCs in a wild gudgeon population (Gobio gobio L.) in Luxembourg

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For many decades human activities have dispersed toxic chemical substances in the environment. Over the last years new xenobiotics such as endocrine disrupting chemicals (EDCs), antibiotics or nano-particles have appeared. In order to assess the health of river ecosystems in relation to this increasing pressure, twenty sites contaminated at different levels were sampled by electrofishing across the Luxembourg hydrographical network. The status of fish communities was characterized according to an index of biotic integrity (IBI). Furthermore, on six sites, population structure, reproductive parameters and biochemical assays were performed on gudgeon (*Gobio gobio*) as sentinel species. Stress factor responses were observed at different levels of biological organization: from molecule up to fish communities. At one site, male gudgeons exhibited high levels of plasmatic VTG. Histological examinations revealed high alterations of the gonads: feminization of the testes and high incidence of atresia in ovary. No significant difference in the level of plasmatic VTG was noticed between intersex and non-intersex fish. Conversely, the incidence of atresia seems to be linked with VTG synthesis in female gudgeons. Weight index of the liver also highlighted an exposure to EDCs. More surprisingly, no impairment of the gudgeon population was pointed out. This study provides interesting data concerning endocrine disruption and their higher-level effects of the biological organisation in a wild fish population inhabiting Luxembourg's rivers. Factors potentially leading to alterations of the reproductive tract and more generally to a reduced fertility seem to be diverse and varied, and not exclusively VTG-dependent. By using a multiparametric and multiscale approach, a better understanding

of response mechanisms to pollution in fish may be achieved and help improving the ecological status of river ecosystems.

TH 124

Assessing the endocrine disrupting potential of Bacillus thuringiensis israelensis based insecticides

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Synthetic insecticides have always played an important role in ecotoxicological research. The excessive use of insecticides such as dichlorodiphenyltrichloroethane (DDT) has lead to crucial hazardous impacts on wildlife. Several indications also implicate that humans are affected. In response, a protein isolated from *Bacillus thuringiensis israelensis* (*Bti*) has been used as a specific biological control agent against mosquitoes since 1981. So far no adverse effects on the environment have been observed. Hence *Bti* has been increasingly used over the past decades.
However, recent studies implicate that *Bti* may act as an endocrine disruptor. To assess a potential threat to the environment, four different *Bti* formulations have been analyzed in the lyticase yeast estrogenic screen (LYES assay). The LYES assay is used to investigate estrogen receptor mediated endocrine activity. Three *Bti* formulations have shown estrogenic activity in the LYES assay with estradiol equivalent quotients (EEQ) ranging from 9.2 ng/l to 22.8 ng/l.
Furthermore, to assay the effect of *Bti* on the steroid genesis surface water samples are currently analyzed in the H295R Steroidogenesis assay (H295R assay). The samples have been collected before and after the application of *Bti* and were extracted using solid phase columns.

TH 125

Endocrine disruption effects of 3,3',4,4'-tetrachlorobiphenyl (PCB-77) on juvenile zebrafish

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The main evidence suggesting that exposure to environmental chemicals can lead to disruption of endocrine function comes from changes seen in a number of wildlife species. Persistant organic pollutants (POP), such as the PCBs (polychlorinated biphenyls), are of major concern for human and ecosystem health due to their high degree of persistence and bioaccumulation in the food web. PCBs are a type of industrial chemical that might cause endocrine changes, in terms of estrogen-regulated processes, such as, vitellogenesis - vitellogenin (Vtg) production. Commercial PCBs exists as mixtures of several congeners, among them, the PCB congener 77 (3,3',4,4'-tetrachlorobiphenyl) is one of the most toxicologically important PCBs, representative of a class of PCBs, as a di-ortho substituted non-coplanar congener. Zebrafish (*Danio rerio*) has been used as model organism to study endocrine-disrupting compounds (EDCs) effects and their mechanisms of action and to make the functional link between environmental effects and human health level disturbances. With the aim to assess the effects and potential mechanisms of reproductive toxicity of PCB-77 to zebrafish during gonadal differentiation, juveniles (30 days post hatch) were exposed through water to PCB-77. The LC50 for 96h was determined and fish were then exposed to sub-lethal concentrations during 14 days, using a semi-static test design with test medium renewal each three days. A control solvent and a positive control (100 ng L^{-1} 17β-estradiol) were also included in the test. Results will be discussed based on endocrine (gonad histology, Vtg induction) and adverse effects endpoints (survival and growth, sex ratio, gonadosomatic index (GSI) and hepatosomatic index (HSI)) recorded at the end of exposure.

TH 126

Concentrations and space-temporal trends of polybrominated diphenyl ethers (PBDEs) in Eurasian eagle owl (Bubo bubo) unhatched eggs from Southeastern Spain

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Since the 1960´s PBDEs have been added to plastics, textiles, foams and electronic products to reduce fire risks, becoming ubiquitous in the environment. Because PBDEs are considered endocrine disruptors, it is essential to get more insight in temporal and spatial trends of their environmental levels. In Southeastern Spain, the Eurasian Eagle Owl could be considered a suitable sentinel species for monitoring local pollution of PBDEs. Our study area is relatively large and was divided in two sub-areas. In the Northern subarea, citrus and dry farming predominate, and rabbits, the Eagle Owl's main prey, is very abundant, while in the Southern some nests are located around 16 Km far from Cartagena, an important industrial city, there are some irrigation orchards and rabbits are less abundant. Hence the Eagle Owl's diet is completed with birds, rats and hedgehogs. Between 2004 and 2009, 58 unhatched eggs were collected from monitored nests. A subsample from each homogenized egg was mixed with anhydrous Na_2SO_4 and extracted with n-Hexane using Soxtherm. Extracts were cleaned up by GPC and congeners 28, 47, 99, 100, 153, 154 and 183 quantified with a GC-MS. General linear models applying a log-link function were used to analyse spatial and temporal trends in levels. The PBDE profile was characteristic of terrestrial birds and dominated by BDE 99, 47 and 100, which coincides with the Little Owl eggs from Belgium. Concentrations were very low (median ∑PBDEs 19.58 ng/g lipid weight) compared with most terrestrial birds, including owls. These levels are about 1000 times lower than the PBDEs LOEL (32 µg/g lw) in kestrels eggs. When analysing spatial and temporal trends, a significant interaction between space and time was found ($F_{prob\ overall\ analysis}$<0.001, t_{prob interaction term year*subarea}=0.007, $t_{prob\ year}$=0.198, $t_{prob\ subarea}$=0.007). While concentrations in the Northern decreased, levels tended to increase in the Southern. This apparent increasing trend could be due to the release from PBDE containing products used in Cartagena. A shift in the Eurasian Eagle Owl diet could also explain the trend, as this has been previously reported as a cause for changes in the organochlorine loads in raptors. Supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS), Seneca Foundation (08758/PI/08) and WIMEK. Thanks to Autonomous Community of Murcia Region for sampling permission. Special thanks to E. Pérez and J.A. Lacalle for sampling efforts.

TH 127

Where to cut-off? - Endocrine hazard profiles of EBI fungicides.

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The new EU pesticide regulation states: "An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible." (EC 1107/2009, Annex II, 3.8.2). Thereby, a paradigm shift in the environmental risk assessment and decision making was introduced for substances with this specific mode of toxic action: The risk-based approach as required by the precedent directive 91/414/EEC is now changed for a hazard-based approach. I.e., the risk that may arise from the intended use of the plant protection product under consideration is not characterized quantitatively by a comparison of the expected exposure of non-target organisms with established (no) effect thresholds. Instead, only the proven presence or assumed absence of endocrine disrupting properties shall be decisive for a (non-) approval of the respective plant protection product. However, the debate on specific scientific criteria for such a "cut-off"decision making regarding endocrine disrupting properties in wildlife has only just started. As a contribution to this debate, a case study was conducted to characterize and compare the endocrine hazard profiles of a selection of ergosterol biosynthesis inhibiting (EBI) fungicides. Active Substances of this heterogeneous chemical group (triazoles, imidazoles, pyrimidines) share a similar pesticidal mode of action: inhibition of ergosterol synthesis in fungi. The target enzymes in fungi is partly analogous to enzymes involved in the production of steroid hormones in vertebrates as well as in other animal classes with sexual steroids, what is why EBI fungicides are known to harbor an intrinsic endocrine hazard potential. Based on available mechanistic evidence and observed adverse effects endocrine hazard profiles of the selected active substances were developed with special emphasis on vertebrates (mammals and fish). The resulting endocrine hazard profiles are compared, with the results being discussed regarding endocrine "cut-off"decision making.

TH 128

In vitro screening of estrogens in waters pointed out another possible source of EDCs pollution than waste water treatment plants

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Waste Water Treatment Plants (WWTPs) have been widely discussed as one of the main sources of compounds that are able to disrupt endocrine systems of organisms and numerous cases of feminized male fish downstream of WWTPs have been reported. In the Czech Republic (CZ) majority of WWTPs contain mechanical treatment and activated sludge systems, therefore this type of WWTP was investigated in our studies. Reporter gene assay was used to evaluate efficiencies of estrogen removal at 7 municipal WWTPs in CZ. The efficiencies ranged from 92 % to more than 98 % and levels of estrogenic potential of treated effluents varied from less than 0.5 ng/l EEq to 2.0 ng/l EEq. Considering proportional contribution of the effluents to the recipient rivers, levels of estrogens detected in these effluents would not be most probably fully sufficient to cause fish feminization observed at some locations. In another study Polar Organic Integrative Samplers were used to sample surface water at 7 relatively unpolluted areas of CR to (i) obtain approximate background levels of estrogenic potential and (ii) evaluate the impact of 7 WWTPs of smaller towns which are the only substantial source of anthropological pollution in these studied areas. Estimated background levels of estrogenic potential of surface water (samples upstream from the WWTPs) varied from below 0.1 to 0.3 ng/l EEq with most values around 0.2 ng/l EEq. The same rivers downstream of WWTPs contained approximately 2 times higher levels of estrogenic potential compared to upstream locations but in two out of seven cases, the estrogen potential was approximately 8 times higher and reached values around 2 ng/l EEq. At the locations with the highest EEq levels, the contributions of WWTP effluents to the overall flux of the rivers were 30 % and 10 % respectively. This means that if the effluents were the only source of estrogens, they would contain approximately 7 and 20 ng/l EEq. Relatively low levels of estrogenic potential found in the first study in WWTP effluents and considerably enhanced EEq levels detected in two out of seven rivers downstream of WWTPs (second study) brought up important question about other sources of estrogens e.g. discharging non-treated waters to the recipient waters due to insufficient capacity of WWTPs. These studies have been supported by the projects ENVISCREEN (Ministry of Education C.R. No. NPVII 2B08036) and CETOCOEN (CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

TH 129

U-shaped responses of fish zona pellucida protein and vitellogenin gene expression after octylphenol waterborne exposure

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The endocrine disrupting chemicals have become ubiquitous in the environment and affect development and physiology by interfering with normal endocrine functions of human and wildlife. Octylphenol (OP) is a degradation product of alkylphenol ethoxylate surfactants (mainly used in industrial applications, agrochemicals, cleaners among others) which can mimic natural estrogens and cause reproductive impairments. Our previous results showed that in the cichlid fish Cichlasoma dimerus, zona pellucida proteins (ZP) that form the eggshell, and vitellogenin (VTG) the precursor of egg yolk, are both synthesized by the liver of mature females under estrogenic control but are normally not produced by males. The aim of the present study was to assess the effect of environmentally relevant concentrations of OP on ZP and VTG gene expression in C. dimerus males, which were exposed to 0.15, 1.5, 15, and 150 µg/L OP for 28 days. The expression of a putative housekeeping gene, Na+/K+-ATPase, was measured. Estrogenized female livers were used for the standard curve to normalize data. Exposure of male fish to OP caused down-regulation of Na+/K+-ATPase expression except for the lowest concentration of OP. However, exposition to 0.15 and 150 µg/L OP caused significant up-regulation of both forms of ZP (β, γ) and VTG while the other two concentrations of OP (1.5 and 15 µg/L) caused minor non-significant effects. These U-shaped dose-response curves or hormesis provide new evidences for the need of risk assessment of toxicants with a wider dose range. Conventional way of risk assessment of low dose might not be accurate by simple extrapolation based on the traditional theory of the threshold or linearity. It has been suggested that U-shaped responses appear as an over compensatory response to maintain homeostasis or take place by the combined contribution of different receptor subtypes with different affinity, accessibility or signaling cascade. Our preliminary data support the latter explanation.

TH 130

Endocrine disruptor in humans: a longitudinal study of exposure and effects

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Bisphenol A (BPA) is a base chemical compound widely used as a plastic monomer and plasti-

cizer. BPA is suspected to have estrogenic properties and endocrine-modulating ability. Due to the ubiquity of this compound, human exposure to BPA is virtually universal.
Associations have been reported between urinary BPA concentrations, heart disease and diabetes in adults. Given this evidences, further human studies on the effects of low levels chronic exposure are needed.
We present the first longitudinal study on the daily exposures to BPA in a population representative sample of European adults. Measures of daily intake rely on two different time-points (the baseline of the study and nine years after). Epidemiological analyses were performed to investigate BPA endocrine disrupting activity (circulating hormone and metabolite concentrations).
A Genome Wide Analysis study was carried out and targeted replications were performed to validate the results.
Study design: Analysis were performed on the baseline stage of the InCHIANTI follow-up study, an Italian population based cohort. Participants were 1453 adults aged more than 20. BPA excretion rates were measured by liquid chromatography mass spectrometry (LC-MS).
Results: Demographic differences in UER BPA and BPA daily intake estimation will be presented. Possible mechanism of actions of BPA resulting from the G.W.A. study will be shown.

TH 131
Estrogenic active substances in bottled mineral water?
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Two recent studies in Germany and Italy found estrogenic activities up to 75 ng 17β-estradiol-equivalents (EEQ)/L in bottled mineral waters which were packaged in PET- and glass bottles (Wagner & Oehlmann 2009; Pinto & Reali 2009). These results have caused public concern and raised the question about the safety of beverage containers, particularly PET-bottles. The purpose of this study was to investigate if the findings for German and Italian bottled mineral water also hold true for Switzerland. A screening study with 31 mineral waters (22 brands of 15 manufacturers) with high market-share in Switzerland was conducted. Fifteen mineral waters showed very low estrogenic activities (mean 4.84±1.59 pg EEQ/L; maximum value 8.0 pg EEQ/L) in the ER-CALUX® assay, just above the limit of quantification (LOQ) at 3.6 pg EEQ/L. No significant differences could be determined between waters from PET- and glass bottles as well as between carbonated and non-carbonated waters. The measured estrogenic activities are around 1'900- to 3'700-times lower than the EEQ measured in bottled mineral waters in the two studies from Germany and Italy. According to current knowledge the very low estrogenic activities in Swiss mineral water samples do not pose a health risk.

TH 132
Assessing estrogenic activities of sediment samples from Laguna Lake, Philippines and fractions obtained by Effect directed analysis in the LYES-Assay
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Due to the increasing use of several diverse chemicals by modern society and the large-scale production triggered through the ongoing industrialisation in all parts of the world, nearly every living space is affected by numerous known and unknown chemicals of anthropogenic origin. A large range of these chemicals have shown to act as endocrine disrupting compounds. The present study was conducted to further investigate the state of contamination level of sediment samples from Laguna Lake. It is the second largest freshwater lake in Southeast Asia and the largest lake in the Philippines, it is also part of the Living Lakes Network. In this study the LYES-Assay (Yeast estrogenic screen assay assisted by enzymatic digestion with Lyticase) was performed to screen for estrogenic active fractions in sediment samples from Laguna Lake. Based on preliminary results, the present study selected sediment samples of two different sites for fractionation and effect-directed analysis: Central Bay and East Bay. While the watershed around the Central Bay is urbanized and industrialized, that around East Bay is basically residential and agricultural area. The sediment samples have been extracted by using an accelerated solvent extraction method. The fractionation of Central Bay and East Bay extracts was conducted using an automated multistep fractionation method developed by Lübcke van Varel et. al. (2008). Each fraction was tested in seven different dilution steps. Furthermore five different Blanks, a Crude Extract and a Fraction Summation from both study sites were tested. In the sediment samples from East Bay four fractions showed a significant endocrine effectiveness at the one fold concentration (fraction 11, 15, 16, 18). The estrogenic activity ranged from 8.43 ± 4.37 ng/L at fraction 18 to 10.79 ± 5.28 ng/L at fraction 15. Fraction 16 also revealed a significant endocrine effectiveness at the ½ fold concentration of 12.59 ± 9.91 ng/L. Only one fraction indicated a significant endocrine potential from sediment samples of Central Bay. Indeed fraction 18 already showed the first significant endocrine effectiveness at the 1/8 fold concentration of 8.80 ± 2.29 ng/L up to 27.32 ± 18.39 ng/L at the one fold concentration. The data was statistically analysed by a Kruskal-Wallis One Way ANOVA on Ranks and a following post-hoc test according to Dunn. Overall the sediment samples did not reveal a high estrogenic impact as it can be observed in some European sites.

TH 133
How to deal with endocrine disruptors - a joint strategy for different chemical regulations
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While being identified as of special concern in the environment since decades, in the regulatory context, endocrine disruptors were not treated differently to substances acting via other toxic modes of actions until recently. Now, taking into account the current revisions of the Pesticides and Biocides directives, endocrine disruptors are at least explicitly mentioned in all substance regulations (REACH, Pesticides and Biocides directives, Pharmaceuticals). However, the way how substances with endocrine disrupting properties are identified, the outcome of the identification and the regulatory consequences differ between the individual regulations.
While disturbance of the endocrine system is a toxic mode of action among others, it may result in adverse effects in the environment difficult to predict with our current set of laboratory tests. Although for some species (e.g. fish) tailored test systems and test strategies are available, similar approaches for other organism groups like invertebrates are still missing.
A common understanding on how to identify substances of special concern due to their endocrine disrupting properties for the environment among different regulations was developed by the German Federal Environment Agency (UBA). UBA proposes to differentiate between three groups a) substances for which endocrine disrupting properties are the basis of the pesticide or pharmaceutical mechanism of action in target organisms b) substances where endocrine disrupting properties are decisive for the overall side effects on non target organisms and c) substances where endocrine mechanisms of action are identified but are not relevant for the overall risk assessment. UBA proposes to focus special regulations on the second group. Some general thought on how to identify these types of substances e.g. for the identification as a substance of very high concern under REACH or as a "cut-off - candidate"for pesticides will be presented.

TH 134
A transcriptomic approach for the fish embryo test with zebrafish and medaka to identify endocrine disruption
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The endocrine system is susceptible to disruption by various chemicals occurring in the environment. The current testing approach in regulatory ecotoxicology for endocrine disrupting chemicals (EDCs) relies on long-term reproductive, animal intensive studies. These labour intensive tests entail high costs and are ethically disputable. The need for alternative test strategies for EDCs is urgent, and zebrafish and medaka embryos have good potential as alternative test approaches for EDCs. Relevant endocrine pathways are already developed in the embryos, and endocrine related genes have shown to respond similarly than in reproducing fish. Both species have their particular advantages in this context. The rapid development of zebrafish is beneficial for screening applications, whereas the longer embryogenesis of medaka may alleviate the prediction of chronic effects. To compare medaka and zebrafish, we used embryos of both species to study adverse effects of endocrine active compounds like genistein, linuron and methylparaben. 48h zebrafish were analysed by microarrays and 7-day old medaka by qPCR, to gain insights into the specific transcriptional modes of action. Sets of responsive genes specific to each chemical were found of which several indicate effects on endocrine pathways. In both species, genistein and methylparaben enhanced mainly the expression of estrogenic genes; linuron on the other hand, repressed androgen-dependent enzyme genes. Simultaneously, we found a considerable intersection of steroidogenic genes regulated by all tested substances. Some of these genes show good promise for ED marker gene candidates and are now being validated for a potential screening application. The goal is to establish qPCR arrays for EDC-specific suites of genes suitable to screen specifically for EDCs in fish embryo and larvae of zebrafish and medaka. Such a transcriptomic fish embryo assay approach could become a valuable component of an alternative EDC testing strategy.

TH 135
Validation and interpretation of the amphibian metamorphosis assay
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In response to these new data requirements our laboratory has developed and internally validated test systems for the conduct of the amphibian metamorphosis assay OECD 231 and OPPTS 890.1100. The amphibian metamorphosis assay (AMA) was conducted with stage 51 xenopus laevis tadpoles using a chemical known to interfere with the normal function of the hypothalamus-pituitary-thyroid (HPT) axis. The amphibian metamorphosis assay is considered to provide a well-studied, thyroid-dependent process which responds to substances active along the HPT axis, and it is the only proposed assay that assesses thyroid activity in a species undergoing morphological development. Four days after fertilisation the best spawn is selected based on hatching success and tadpoles (Stage 45/46) were transferred to glass tanks at a density of 10 tadpoles per litre and develop to stage 51 within 12 days (approx. 17 days after fertilisation). The test was conducted with a control group and three test concentrations containing four replicates per treatment in a randomised block design.
The results of daily observations of sub-lethal effects, malformations, abnormal behaviour and mortality. Body weight, hind limb length, snout to vent length and developmental stage after 7 and 21 days exposure are reported. At test termination (21 days) five animals (stage-matched) from each replicate were sampled for histological assessment of the thyroid gland e.g. thyroid gland hypertrophy/atrophy, follicular cell hypertrophy, follicular cell hyperplasia , follicular lumen area etc.

TH 136
The short term fish reproduction assay
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The fish short-term reproduction assay was conducted with fathead minnows using a chemical known to induce endocrine disrupting effects. A control and three test groups were prepared each including four replicate tanks containing two males and two females. The test was designed to detect changes in spawning, morphology, gonadal size/architechture and specific biochemical endpoints that reflect disturbances in the hypothalamic-pituitary-gonadal (HPG axis), including oestrogen agonists/antagonists and vitellogenin induction in males. Collectively, the endpoints observed are intended to allow inferences to be made with regard to possible endocrine disturbances involving the estrogen hormonal pathway and, thus, provide guidance for further testing. The test was conducted under continuous flow conditions in 18.0 L glass tanks with a test media volume of 10 L and a 2.7L/hr flow rate. The concentration range was selected using the maximum tolerated concentration (MTC). Environmental conditions were 16h:8h light:dark photoperiod @ 540 to 1080 Lux, temperature was maintained at 25 ± 1°C, pH 6.5 to 9.0, Dissolved oxygen >60% ASV. Test media concentrations were determined on a weekly basis.

TH 137
Contraceptive choices and the resultant estrogenic load on the environment
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More than a decade of evidence suggests that the estrogenic content of wastewaters being released to the environment feminizes fish and that such feminizing effects could potentially comprise the reproductive capacity of the effected fish. Environmentally-relevant estrogens can be broadly categorized in two classes; namely, those that are endogenously produced and those that are exogenously consumed. Of the two, the excretion and therefore the subsequent environmental presence of endogenous estrogens can be considered to be omnipresent since they are a fixed characteristic of our biology. When considering exogenously consumed estrogens used for human contraception, the most important by far in terms of amounts consumed and environmental relevance is the female birth control pill, containing the synthetic estrogen ethynylestradiol as its active ingredient. The pill was first approved 50-years ago as a form of contraception and is

currently used by approximately 11 million women in the USA for this purpose. Since the use of ethynylestradiol, and hence the estrogenic content released as a consequence, is a contraceptive choice made by members of society, a logical question arises: When evaluated from an environmental perspective, should the use of ethynylestradiol be mitigated or even eliminated? And if so, do greener alternatives exist to this contraceptive? How does one go about making such an evaluation? Further, the mere suggestion that society's contraceptive choices be altered to mitigate environmental impact should be analyzed in broader context. Are contraceptive choices that are greener also clinically and personally preferred? Would such a switch to more greener contraceptive choices also be financially feasible? This study will present a first attempt at answering these questions.

TH 138

Morphological changes in Girardia tigrina induced by atrazine

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Atrazine is applied to control weeds in different plantations and is among the most used herbicides in the world. This herbicide is also an endocrine disruptor and has been found in several water bodies worldwide therefore exposure of animals from different trophic levels is expected. Laboratory tests can help in the hazard evaluation of those substances to non target organisms. Among them, planarians, such as *Girardia tigrina*, are interesting because of they are metazoan, present sexual and asexual reproduction, are able to regenerate and are easy to be cultivated and maintained under laboratory conditions. The objective of this work was to evaluate the effects of pure and commercial atrazine in *G. tigrina*. Atrazine p.a. and a commercial product containing 50% of atrazine were used. Atrazine p.a. was tested from 10 to the maximum water solubility (33 mg/L). The commercial product was tested from 10 to 100 mg/L (doses expressed in atrazine). Acute toxicity tests were performed with young specimens using two vessels per dose. Seven healthy *G. tigrina* with maximum age 10 days were exposed to 50 ml of each test solutions in 100-ml vessels. Morphological alterations and mortality were observed under a stereomicroscope after 24, 48, 72 and 96 hours of exposure. Both atrazine p.a. and the commercial product presented similar results. The 96-h LC_{50} was only calculated for the commercial product (34 mg/L expressed in atrazine). It was not possible to calculate the LC_{50} for atrazine p.a. because of limited solubility. But both LOAEL (Lowest Observed Adverse Effect Level) were similar: 20 mg/L to p.a. atrazine and 25 mg/L to commercial herbicide. Morphological alterations were observed in the survivors of the tests performed with commercial and atrazine p.a. Some animals showed retractable pharynx permanently exposed, changes in the shape of the pharyngeal region and loss of the posterior part of the body. The affected regions are the ones responsible for feeding and reproduction. Therefore, the exposure of atrazine at subletal concentrations can cause adverse effects in those animals. More studies are necessary for understand how atrazine causes these alterations in *G. tigrina*.

TH 139

Assessing potential risks to benthic organisms exposed to bisphenol A

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Bisphenol A (CAS RN 80-05-7) may enter the environment via permitted effluent discharges. In surface water, bisphenol A will rapidly biodegrade and some will become entrained in suspended particles and deposit onto sediment. Currently, the assessment of potential risks to benthos in sediments containing measurable concentrations of bisphenol A is based on aquatic toxicity chronic values and equilibrium partitioning to provide estimates of sediment toxicity. A recently completed risk assessment for bisphenol A in the European Union used this approach to assess risks to sediment dwelling organisms. To support the hazard assessment of bisphenol A in sediment, chronic toxicity tests have been performed using three sediment dwelling species representing different feeding and living conditions: an endobenthic freshwater annelid (the oligochaete, Lumbriculus variegatus), an epibenthic insect (the midge Chironomus riparius), and an estuarine crustacean (the amphipod Leptocheirus plumulosus). The no observed effect concentrations (NOEC) reported for the three species ranged from 22 to 54 mg/kg-dw. Predicted no effect concentrations (PNEC) were calculated using current European Chemicals Agency guidance by dividing the lowest chronic NOEC for sediment organisms of 22 mg/L by an assessment factor of 10 for freshwater and 50 for marine sediments. The freshwater and marine PNEC are 2.2 mg/kg-dw and 0.44 mg/kg-dw, respectively. PNEC were then compared to freshwater and marine surface sediment concentration data from monitoring programs reported from Europe and North America. Risk characterization ratios were calculated using median and upper 90th or 95th percentile concentrations of bisphenol A in European and North American sediments. The risk characterization results will be used to update and refine sediment risk assessments in these regions.

ET11 - Mechanistic modelling for risk assessment: sub-lethal responses and population-level effects

TH 147

Bayesian calibration and evaluation of agent-based models: a case study of a skylark population model for pesticide risk assessment.

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European regulations concerning pesticide risk assessment focus on protecting populations rather than individuals [2]. As field experiments at a population scale are necessarily very expensive and may pose risks to wildlife populations, population models for higher tier risk assessment have recently become more widely used. Especially agent-based models seem to solve best the problem of extrapolation from the individual scale of laboratory data to the population scale necessary for risk assessment. They incorporate knowledge about individual behaviour and pesticide effects and population characteristics emerge from simulations. To use those models in practice, however, their credibility has to be ensured. For that a proper calibration and validation need to be provided.

We are currently developing a skylark (Alauda arvensis) agent-based model and at the same time a set of calibration and validation tools. We have chosen skylarks as they are often used as focal species in risk assessment due to their arable habitat and diet. The validation and calibration methods we are applying are based on the Bayesian paradigm and on the work of Mark Beaumont on Approximate Bayesian Computation [1]. We present those procedures we are performing for the skylark model together with the separate data sets we have acquired for the purpose. We hope they are a step in the direction of getting risk assessment models more widely accepted and used by risk assessors and managers.

[1] Beaumont M. 2010. Approximate Bayesian Computation in evolution and ecology. Annual Review of Ecology, Evolution, and Systematics, 41: 379-406.
[2] European Food Safety Authority EFSA. 2009. Risk assessment for birds and mammals. EFSA Journal 7(12).

TH 148

Dynamic energy budget theory meets individual-based modelling: a generic and accessible implementation for population risk assessment

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Dynamic energy budget (DEB) theory provides a quantitative framework for modelling the acquisition and use of resources for organisms over the entire life cycle. It thereby generates a quantitative explanation for the time patterns of life history traits such as growth and reproduction. DEB theory has been widely used in the field of ecotoxicology. The process-based approach used in the DEB analysis of toxicity experiments differs from standard ectoxicological analysis (LC50, ECx, ect.) in that it links the effect to internal concentration of the chemical and takes into account the biological characteristic of the test individual used. This is of great advantage in a population context because it allows one to extrapolate the effect of the chemical to among different size classes and to differing environmental conditions and exposure scenarios. So far, however, DEB theory has only occasionally been used in the population context. We therefore implemented an individual-based population model (IBM) that is based on DEB theory. Our program is designed as a tool for risk assessment of a wide range of species and substances. It allows for a relatively simple extrapolation from the individual effects determined from the analysis of standard OECD tests to population level. As an example, we present our initial results using this approach with *Daphnia magna*. We discuss the potential of DEB theory and our implementation of this theory in an IBM to serve as a standard tool for ecological risk assessment of chemicals.

TH 149

Effects of uranium in Daphnia magna exposed over three successive generations: extrapolation of DEBtox analyses to the population level

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As part of the ecological risk assessment associated with radionuclides in freshwater ecosystems, toxicity of waterborne uranium was recently investigated in the microcrustacean Daphnia magna, exposed over three successive generations. Toxic effects on daphnid life history and physiology, increasing over generations, were demonstrated at the individual level under controlled laboratory conditions. Nevertheless, response to toxicant at higher levels of biological organisation, in terms of demographic toxicology, is now recognized as more relevant in a broader ecological context than toxicology at the individual level.

Extrapolating effects to the population level requires considering key ecological factors, like life cycle characteristics. Concomitantly the complexity of relationships between individuals and populations makes necessary the use of modelling approaches, especially when ecotoxicological outcomes are expected to be predictive and not only descriptive. Uranium effects at the individual level were previously described with a DEBtox modelling approach based on the dynamic energy budget (DEB) theory, which describes how organisms acquire energy from food and allocate it towards survival, growth, maturity and reproduction. Here, we extrapolated effects from the individual to the population level through multigenerational Leslie matrix models.

DEBtox models were fitted to experimental data for each of the successive generations with Bayesian inference, a statistical method combining prior information (expert knowledge or literature) and data to provide parameter estimates as posterior probability distributions reflecting uncertainty. Matrix model inputs were calculated from the individual level models with parameter sets sampled from those distributions. This approach allowed us to echo DEBtox parameter uncertainty to the population level and to predict the population dynamics over the three generations as a function of the uranium exposure concentration. The population extinction probability is suggested as a mathematically convenient and ecologically relevant toxicological outcome for risk assessment.

TH 150

Modelling harpacticoid copepod populations; matrix and individual based modelling

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To increase the ecological relevance in the risk assessment process several authors have suggested that there should be an increased focus on the population level. The use of population models is a way of achieving this; and toxicity tests including development and reproduction provide data suitable for this kind of modeling. Copepods are important test species since they are a significant food-source to fish, and therefore provide an important ecosystem service. A life cycle test for copepods is also suggested for standardization (e.g. Amphiascus tenuiremis). The aim of this study was to compare the output of simple matrix- and individual based models, which were both calibrated using the same data sets that were generated in life cycle tests with harpacticoid copepods.

Two data sets with harpacticoid copepods were modeled: Nitocra spinipes which had been exposed to different sewage effluents and Amphiascus tenuiremis which had been exposed to Lindane (unpublished data from validation of OECD test method at Stockholm University).

An individual based model (IBM) that was developed for harpacticoid copepods was compared to a more traditional Lefkovitch matrix model. The matrix model included survival, developmental proportions, and fecundities. The IBM included survival, developmental proportions and rates, sex ratios, latencies (time from mating to first brood), time for embryo development, brood size and brood success. Both models used the same data sets for each of the test species.

The main effect that sewage effluent had on the copepods was reduced proportions of animals developing to the next stage. Lindane however, mainly affected the rates at which development took place; e.g. development was shifted. Changes in proportions are easily captured in matrix models, but changes in rates are not. In the IBM, rates are inherently modeled.

Simple individual based models do not require more data than simple matrix models. Individual

based models are more powerful tools compared to matrix models since they inherently incorporate rates, not only proportions.

TH 151

Population models in Gammarus (Crustacean) and Potamopyrgus (Gastropod): depicting the seasonal variability in demographic sensitivities to toxic stress by sensitivity analysis

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Today there exists a large consensus that ecological models with populations rather than individuals as targets to protect have a high potential to be implemented into ecotoxicological risk assessment [1]. Indeed, they revealed particularly useful to solve the challenging issue of extrapolating toxic effects measured at the individual level towards possible contamination impacts on populations. Nevertheless, with the perspective to project or predict population answer, the environmental relevance of these models has to be improved, accounting for variability in population vulnerability and making them transferable with native species. In this way, the extrapolation process could be also used within a diagnostic framework.

Our work was focused on two phylogenetically and ecologically contrasted species (the Crustacean Amphipod Gammarus fossarum and the Mollusk Gastropod Potamopyrgus antipodarum), widely present in European rivers and for which ecotoxicological (sub)individual biomarkers are available. We first developed environmentally realistic size-structured population models including both in situ caging and laboratory data, and relating individual-level demographic parameters (survival, growth, reproductive activity, fecundity, size at maturity) to the dynamics of reference native populations all along a year.

Second, this methodology was coupled with in situ biotests or laboratory bioassays, in order to allow a population-level assessment of water quality or chemical compound toxicity. We showed thus the relevance to take into account the phenology of population dynamics (seasonal variability), by illustrating how it strongly controls the demographic sensitivity to toxic alteration of individual fitness traits in native species. More precisely, the poster will exemplify how sensitivity analyses of population endpoints were able to improve the comprehensive evaluation of possible contamination impacts on ecologically relevant biological levels.

TH 152

Decomposition analysis of LTREs may help to design short-term ecotoxicological tests: population modelling approach

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A Life Table Response Experiment (LTRE) lasting 42 days was performed to investigate toxic effects of cadmium (Cd) and imidacloprid on reproduction and survivorship of aphids (*Acyrthosiphon pisum* Harris). Daily life table data obtained from the experiment described by Laskowski (2001) were used to construct and develop an age classified matrix model to estimate population growth rate (λ) at each treatment level. Decomposition analysis was performed to examine the relative contribution of changes in each life-history trait on $\Delta\lambda$, that is the difference between the control and a treatment.

Results of the decomposition analysis indicate that the contribution of differences in survival to $\Delta\lambda$ was most important at the 1st and 2nd weeks, while the contribution of differences in fertility to $\Delta\lambda$ was the greatest at the 3rd and 4th weeks. The two toxicants exhibited different actions though. Cadmium affected $\Delta\lambda$ mostly by impairing fertility at the age of three weeks and survivorship from 2nd to 3rd week. On the other hand, imidacloprid affected mostly the survivorship at the 1st and 2nd weeks of the age.

This LTRE analysis clearly indicates the shortcomings of traditional ecotoxicological assays, which usually concentrate on the traits that are most sensitive to toxicants. As shown by this study, the population-level interpretation of individual-level effects requires information on both the effects of a toxicant on vital rates and the demographic sensitivity of life stages. LTREs with decomposition analysis, as shown in this study, can be used as an important analytical tool to fill this gap by combining effects of toxicants with demographic sensitivity of the organism.

TH 153

Correlated random walk as mode of short distance dispersal for Asellus aquaticus - an experimental approach for model parameterization

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Current EU legislation on ecological risk assessment (ERA) of pesticides mainly focuses on individual-level laboratory toxicity tests whereas envisaged protection goals mainly aim at populations of non-target species. Within the context of pesticide ERA, ecological models are rarely recommended in official documents but are sometimes applied by researchers to estimate risks to populations, communities and ecosystems. Some of the reasons for the hesitation to use ecological models in official ERA studies are a lack of case studies showing real model application and validation data that make models more trustworthy to describe real, natural systems.

An individual-based model, MASTEP (Metapopulation model for Assessing Spatial and Temporal Effects of Pesticides), has been developed at Alterra (Wageningen, The Netherlands) and was used to estimate the combined allogenic and autogenic recovery of Asellus aquaticus after exposure to an insecticide. In the case of A. aquaticus, and other species also (e.g. Gammarus pulex), recovery by immigration of individuals from uncontaminated sites is an important factor for re-establishing population densities after pesticide exposure. However, not much information on movement patterns of aquatic macroinvertebrates is available. In the submodel on dispersal in MASTEP, correlated random walk (CRW), as a relatively standard way to model animal movement, is applied for short distance dispersal of A. aquaticus individuals. The parameters for this submodel have been estimated from previous observational studies. However, such studies did not sufficiently account for habitat quality or density effects on locomotion of asellids.

Therefore, we performed video tracking experiments to derive information on movement behaviour of individual A. aquaticus, specifically to obtain relevant parameters for the CRW, such as preferred turning angle range, resting times, and movement velocity. The experiments tested for different population densities and habitat qualities (e.g. presence of food or shelter) as different locomotion activities were assumed under varying conditions. The next step will be to use this information to parameterize and adjust the dispersal submodel in MASTEP to enable for more realistic estimations of population dispersal in different environments.

TH 154

The importance of maternal environment in determining offspring size in Daphnia magna population models

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This study is in the context of a Marie-curie training Network (CREAM Project) aiming to develop population models for risk assessment of chemicals. Population-level ecotoxicology has recently gained much interest and its relevance for risk assessment is now well documented. The impact of toxicants on populations depends on exposure, toxicity and also on the life-cycle of the species as well as abiotic and biotic interactions. Individual-based models were proven to provide an excellent methodology for population modeling, allowing to account for such interactions and to consider the individual variability within the population. Amongst the species used in modeling, *Daphnia magna* is an ideal organism that has been adopted in many studies due to its well-studied biology.

In 2009, Preuss *et al.* developed an individual-based model (IDamP) predicting the population dynamics of *Daphnia magna* under different feeding conditions. In this work, we use the IDamP model to describe the effects of sub-lethal exposure to toxicants on *Daphnia magna* populations. One adopted chemical is known to shift the reproductive strategy of the species by producing larger broods but smaller and less fit offspring. Offspring size is a key life history trait in *Daphnia* as it is intimately associated with important individual traits on the population level such as growth rate, maturation and reproductive allocation. It is subjected to variation with different maternal conditions like size, density and food level. Thus, it is crucial to consider these conditions when modeling *Daphnia* populations, and this has not been included in IDamP or other *Daphnia* population models yet.

In this poster, we provide a mathematical description of the offspring size relation to maternal environment. This relation was achieved by means of multiple regression analysis of data under toxicant-free conditions.

Preliminary analysis revealed a linear relation between offspring size and mother size; an exponential decay function related offspring size to density, and finally maternal food level negatively affected offspring size. In this context, laboratory experiments were conducted to determine the equation that best fits the data.

Toxicity is integrated using a relation derived from toxicity data of the chemical. Therefore, the population dynamics and size structure of *Daphnia* will be simulated in the IDamP model under control conditions and in the presence of a toxicant with a defined mechanism of action.

TH 155

Modelling toxic effects on Chaoborus populations under field conditions - an individual-based simulation study

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For the application of mechanistic population models in ecological risk assessment, a sufficiently high ecological complexity and an accurate description and prediction of the effects is recommended. This study presents a modelling approach for the extrapolation of toxic effects from individuals in the laboratory to populations in the field.

As a model organism for emerging insects, we used the multivoltine aquatic phantom midge Chaoborus crystallinus, which is a common pelagic invertebrate predator in ponds and is known as a frequently very sensitive organism to pesticide exposure.

We used an existing mechanistic individual-based population model for Chaoborus crystallinus, which was formerly validated with experimental data from aquatic outdoor mesocosms. In this model the individual chaoborids are explicitly modelled and population dynamics emerge directly from their individual life-cycles.

In order to predict ecotoxicological effects on the population dynamics of the chaoborids precisely under field conditions, the Chaoborus population model was coupled with a toxicokinetic-toxicodynamic (TKTD) model following the General Unified Theory for Survival (GUTS). The TKTD model was parameterised by data derived from toxicological experiments with individual Chaoborus larvae exposed to triphenyltin (TPT) in the laboratory.

The results of the advanced coupled model were validated using data from aquatic outdoor mesocosms treated with TPT that caused pronounced adverse effects on chaoborids, daphnids, copepods and rotifers. Due to the fact, that TPT is toxic to Chaoborus as well as to its zooplanktonic prey, a combination of direct toxic effects on Chaoborus and indirect effects caused by reduced food availability was taken into account in this modelling approach.

The poster will show results of simulated control populations, acute effects and recovery time.

TH 156

Ecological risk assessment of pesticides on wood mouse populations using individual-based models

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The wood mouse (*Apodemus sylvaticus*) is the most common and widespread wild rodent species on agricultural land in much of Europe. It is commonly used as a focal species in higher tier risk assessment of pesticides. The aim of the project is to construct an ecological model in support of population level risk assessment of pesticides for the wood mouse. An individual-based model (IBM) is being developed to simulate and predict the population dynamics so as to assess the risk of pesticides with different potential toxic effects, e.g. - acute effect on survival, sub-lethal effects on reproduction and somatic growth.

Here both a control model and an experimental model are presented, the former showing the population dynamics without pesticide application and the latter showing some preliminary runs with a hypothetical pesticide. Both models included life history traits (e.g. breeding pattern, dispersal, *etc*) and behaviour (e.g. foraging and territoriality) of the wood mouse; whilst the experimental model also tested the effects of the pesticide application patterns (i.e. where, when and how much) as well as interactions with other agricultural practices (e.g. ploughing, tillage and harvesting).

TH 157

SAM-X (R) - R implementation of an algae population model for potential application in the risk assessment of pesticides

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The environmental risk assessment of pesticides for aquatic organisms in the European Union is a

comparison of effect data from laboratory studies and model-predicted environmental concentrations (PEC, using the FOCUS models).
The FOCUS exposure patterns often show peaks of different height and duration on a variable time-scale. The effects of such pulsed exposure patterns at population level (*e.g.* algae populations), including the ability and time to recover of affected populations, are important issues. Population models are promising higher-tier risk tools to assess the effects of time-variable pesticide exposure on representative aquatic organisms (*e.g.* green algae). A recently developed and verified simulation model describes the growth of algae species depending on variable environmental conditions (nutrients, light, and temperature) and the effects of chemical substances on these algal populations. The model consists of four compartments described by differential equations and uses standardised parameter sets of the physiological species properties.
In the context of the regulatory acceptance of population models for pesticide risk assessments, transparency and reproducibility of simulated processes are highly desirable. This presentation reports an implementation of the SAM-X algae model in the open source software environment R. Advantages over alternative implementations using commercial software (*e.g.* Matlab©) include 1) the free availability of the underlying R software for all major operating systems, 2) the possibility to inspect the code of R.
The model was used to describe experimental laboratory data from algae population capacity and competition tests. The simulation results were compared with results from an alternative implementation of the model.

TH 158
A case study of aquatic population modelling for pesticide regulatory risk assessment using the MASTEP model: chlorpyrifos
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Chlorpyrifos is an organophosphate insecticide which is highly toxic to aquatic invertebrates and has a Regulatory Acceptable Concentration (RAC) of 0.1 µg/L, based on EU review of a number of outdoor mesocosm studies in north and south Europe. Application rates of chlorpyrifos of up to 1000 g/ha in apple orchards and 2400 g/ha in citrus are registered. The combination of the low RAC coupled to a high exposure potential from spray drift onto edge-of-field water bodies makes the aquatic risk assessment challenging. However, the mesocosm studies on which the RAC is based are short-term static systems, whereas recovery may be possible in moving water, larger water bodies, or over longer timescales; and the exposure calculations are simplistic. It is not possible to test all possible eventualities and processes in reality. However, modelling offers the opportunity to theoretically test options. When based on realistic parameters, the model offers some degree of realism in its predictions. The individual-based population model MASTEP (Metapopulation model for Assessing Spatial and Temporal Effects of Pesticides) has been used to evaluate the recovery potential of four aquatic invertebrates known to be sensitive to chlorpyrifos, with differing life histories, using real toxicity data, in various water bodies, with input from various crops, and with various risk mitigation measures. Model runs deliberately included scenarios that cause prediction of strong effects, to indicate its sensitivity. Then various mitigation measures were tried to determine their influence on recovery. The methods and results to date will be presented and discussed. Toxicokinetic/toxicodynamic considerations are also planned.
This case study on chlorpyrifos is designed to explore whether population modelling can provide valuable information to risk assessors and, in particular, to risk managers to support decisions on realistic risk mitigation possibilities.

TH 159
Spatially-explicit risk-assessment: what's the appropriate spatial scale for observing recovery?
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When applying spatially-explicit individual-based population models for pesticide risk assessment in a realistic situation as defined in FOCUS scenarios, several spatial scales become relevant: the typical scale of the species set by its movement attributes, of the exposure pattern and of the structure of the habitat. Taking population recovery as an endpoint in risk assessment, we have to choose the most appropriate spatial scale of observation. Do we have to estimate time to recovery for the total population in the system or just for the population living in the (most) exposed part? How far should the total system extend, and which part of it may we consider as 'the landscape context' that may potentially act as a source of migrants for the exposed part?
We investigate these questions using simple individual-based models (MASTEP) for four aquatic species with different life-history and mobility, roughly representing Asellus, Gammarus, Chironomus and a mayfly species. We assume a constant environment (no seasonality driven by e.g., temperature), linear aquatic habitat (ditch) and for all species equal sensitivity to a hypothetical pesticide defined by a single dose-effect relationship for acute mortality.
The landscape context is set by the length of the ditch: 200 to 1000m. A fixed part (100m) is directly exposed (spray-drift); the remaining part has exposure exponentially declining with distance. Only peak-values of exposure are considered. We calculate recovery times for the 100m part as well as for the whole system population and compare these to the ones expected for a well-mixed population recovering in absence of density-dependent limitation. Expected recovery time is determined by population growth rate λ, which we obtain by solving a discrete version of the Euler-Lotka equation.
Simulated total population recovery times that are longer than expected, indicate that the total system cannot be treated as well-mixed: the species operates at a smaller spatial scale and heterogeneity in exposure at this smaller scale does matter. Simulated recovery times at the 100m scale that are shorter than expected, indicate that the 100m part is too small to be considered in isolation: the species operates at a larger spatial scale and exposure outside the 100m zone has an impact on recovery in the 100m zone. Comparing outcome for different total system size we are able to identify for each species the most appropriate spatial scale for recovery.

TH 160
Development of ecological risk assessment methods for space- and time-varying herbicide exposure
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Ecological risk assessment methods to analyze risk from herbicides under space- and time-varying exposure were developed. First, we analyzed spatial variations of risk from herbicides by creating ecological risk maps in Japan. The hazard ratio of maximum environmnetal concentration predicted by G-CIEMS (Geo-Referenced Multimedia Environmental Fate Model System) simulations to ecotoxicity data (EC50) was used as a risk index at a site level. The number of the sites in which the harzard ratio exceeed one was used as a risk index at the national level. Second, we analyzed ecological risk under time-varying herbicide exposure by combining time series variations in environmnetal concentration predicted by G-CIEMS simulations and dynamic energy budget toxicity models. The predicted total loss in the biomass of algae population over an exposure period was used as a risk index. We analyzed the risk from pretilachlor, butachlor and symetrin. Our analysis suggested that pretilachlor was the predominant risk factor among these herbicides.

TH 161
An IBM to explore the effect of local variability in soil contamination levels on population dynamics of Folsomia candida
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The abundance and diversity of Collembola have been widely used to assess the environmental impact of pollutants on soils, and a standard test guideline has been developed for the parthenogenetic springtail *Folsomia candida* for this purpose. However, the guideline assumes homogenous contamination of the soil matrix, while recent evidence from different biological systems, modelling approaches, and perturbation types indicates that local variability in habitat conditions has an impact on population dynamics.
An individual-based model will be presented that simulates the avoidance behaviour of *F. candida* and the effect of heterogeneously contaminated soil on population dynamics. It has been implemented using the NetLogo software platform. In the model, springtails are characterized by their position, age, time that passed since reaching adulthood and the number of days they spend on contaminated patches of soil. Spatial units of soil are characterized by their concentration of toxicant and the collembolan's avoidance probability. Individuals are assumed to sense and avoid contaminated patches with a certain probability, which depends on contamination level. Movement is random if the patch ahead of the animal is not contaminated. Model rules and parameter are based on data from the literature and are evaluated against experimental data sets.
Average age-mortality curve of individuals in simulated populations was in good accordance with laboratory observations. Specific laboratory experiments to test the avoidance behaviour of *F. candida* have also been designed, and the results utilised to calibrate model parameters related to the toxicant effects. Preliminary simulation results show a clear influence of the level of heterogeneity on population dynamics of *F. candida*. Implications of these effects for standard tests based on *F. candida* and for risk assessment of chemicals in general will be discussed.

TH 162
Modelling population response to toxicants considering multi-species interaction
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Chemical risk assessment is getting more and more complex as ecological realism is increasingly included. However, not each and every field situation can be tested in the risk assessment. Therefore, ecological models offer the opportunity of virtual experiments to investigate a wide range of field-relevant situations. Additionally, modelling approaches can be used to investigate the combined effect of different processes identified in the laboratory.
Biological interactions are an essential part of ecological systems. Thus, also individuals that are confronted with chemical stressors are affected in nearly every situation by predators, synergists and / or competitors of the own and other species. Such biological interactions are, however, rarely taken into account in chemical risk assessment.
In this study we therefore present a generic, individual-based model (IBM) of two competing species. We investigate how lethal and sub-lethal effects of toxicants measured on the individual level are translated to population level response. The model incorporates variables that are measured as endpoints in standard individual tests (acute and chronic mortality, reproduction).
To conclude, competition strongly influences the effect of toxic stressors on the population level. Additionally, the effect mode of a toxicant on the individual is an important factor determining the response. Hence, biological interactions as well as the life cycle parameters that are influenced by the specific toxicant should be taken into account to improve the realism and therefore accuracy of toxicant risk assessment.

TH 163
Why a population model of the cyclopoid copepod Mesocyclops leuckarti for the ecological risk assessment of chemicals is absolutely necessary
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Copepods are important species in aquatic systems, quick to colonize and arguably the most dominant zooplankton. However copepods are mostly under-represented and not studied well with respect to ecotoxicology despite them being organisms of interest in this matter owing to their sexual life cycle, different developmental stages and prominent switch in food strategies during development. Thus they are well suited to study the traits essential for characterization of both lethal and sub-lethal effects of toxicants. A fair amount of work has been done on harpacticoid copepods (bottom dwelling) in this area, mostly marine. Yet, cyclopoid copepods are certainly more abundant and relevant for freshwater ecosystems and there is a great need to study them in the light of chemical risk assessment.
Out of the three freshwater free-living copepod orders namely Calanoida, Cyclopoida and Harpacticoida, the cyclopoid copepods are mostly planktonic. A detailed literature review on the distribution of freshwater cyclopoid copepods revealed their prominence in almost every freshwater habitat. Cyclopoid copepods are relatively easy to handle and maintain in the laboratory. They are also sensitive to environmental changes and can be good indicator organisms for ecotoxicological studies. Summer species of cyclopoid copepods exhibit the phenomenon of diapause in winter and dominate freshwater habitats in summer. These species show a relatively short life cycle compared to the winter species. Therefore they are the preferred organisms for full life cycle studies.
We will present the importance of copepods for environmental risk assessment and the culmination of the search for a relevant species of cyclopoid copepods for chemical risk assessment. The search was carried out by intense literature review and discussion with experts in the field of copepod ecology. The species finally selected for the study is *Mesocyclops leuckarti* which is widespread in occurrence. This species shows takes approximately 21 days to grow from naupliar stage to adult stage. The adults on average live for 20 days and the whole life cycle is completed in approximately 40 days. Based on life-cycle and population tests we aim to develop a mechanistic

population model of this species applicable in the risk assessment of chemicals.
The study is a part of the **EU Project CREAM (Mechanistic Effect Models for Ecological Risk Assessment of Chemicals)**.

TH 164

Screening for realistic worst-case species of freshwater fish for pesticide risk assessment in edge-of-field water bodies in the EU

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Fish are considered under all EU directives and regulations that are related to the environmental risks of chemicals. The assessment of potential effects of chemicals on fish is usually based on ecotoxicological tests on the level of the organism (e.g. effects on survival, growth, reproduction). However, because the environmental protection goals are, in most cases, related to the sustainability of populations, there are two types of extrapolation which might improve risk assessment for fish: from sub-lethal effects on individuals to the population and from test species to ecologically relevant species. There are several ecological models developed for fish available in the open literature. However, so far, none is used within the regulatory framework for plant protection products to refine the standard risk assessment for fish. Therefore, we intend to develop a population model of a realistic worst case fish species which is applicable to the EU. A comprehensive dataset on the EU freshwater fish species is filtered by habitat (excluding riverine, lacustrine, and brackish species), and by range (excluding endemic species and species of small range relative to the 3 zones of mutual recognition according to the new EU pesticide regulation). Then, the species are ranked for population sustainability under stress based on their life history traits. To achieve this, a toxic effect is considered as a disturbance in the life-history of a species, and thus vulnerability is assigned to a species based on the dependence of its population growth rate on changes in its life-history parameters. This dependence is determined via elasticity analysis using age- or stage-based matrix models. Based on the results of the elasticity analysis, as well as the availability of information on life cycle traits, density dependence, and population dynamics in the field, we intend to select one species for the analysis of potential pesticide effects in more detail by means of an individual based model.
In this poster we present the screening approach and the resulting list of the worst-case species.

TH 165

Modelling data from field trials of rodenticides in anticoagulant-resistance areas: are resistance-breakers better for the environment?

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Anticoagulant rodenticides (ARs) are used globally to control pest rodent infestations. Resistance to ARs first appeared against warfarin and diphacinone in Scotland in 1958. In the wake of this more potent ARs were produced but resistance has since developed to some of these. Surveying carcasses of predatory and scavenging animals in the UK has shown that ARs may affect a range of non-target species, including some of high conservation value. The risk to wildlife depends on several factors, including AR concentrations in individual rats, total AR residue in rat populations, and probably also rat population densities, as predators are known to be attracted to high concentrations of prey. In this paper we show that resistance also plays a part because AR-resistant rats are not controlled by some AR baits and this can lead to rat infestations carrying substantial residues of ARs that are available in the environment. Field trials of three ARs (brodifacoum, bromadiolone and difenacoum) were conducted on farms in the Münsterland region in North-west Germany where resistance to bromadiolone and difenacoum in Norway rats (Rattus norvegicus) is conferred by the tyrosine139cysteine mutation. Rat population censuses were carried out pre- and post-baiting and bait applications were conducted according to product labels. Pulsed-baiting was used for brodifacoum and surplus baiting for bromadiolone and difenacoum (all baits contained 50ppm of active substance). Here we present a model that utilises the data obtained from these trials to predict the amount of AR in different environmental compartments through the course of the trials. Baiting with brodifacoum resulted in the lowest levels of AR entering the rat population during the treatments, because it was used in smaller quantities and was effective against the resistant rats. Residues were also short-lived because the rat infestations were quickly reduced. Bromadiolone and difenacoum resulted in markedly higher and potentially long-lived burdens of AR in the rat populations because they were used in greater amounts and were less effective. The use of compounds that are not 'resistance-breakers' results in greater uptake of AR into the rat population and long-lived AR residues available to wildlife. Thus, resistance influences the total amount of AR available to non-targets and should be considered when dealing with rat infestations, as resistance-breakers may present a lower risk to wildlife.

TH 166

Use of the Comprehensive Aquatic Systems Model for Atrazine (CASMATZ) to estimate potential changes in primary producer community structure based on intensive stream monitoring chemographs.

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In 2003, the US EPA required an intensive stream water exposure monitoring program to determine the potential impact of atrazine on the aquatic primary producer community structure. The Comprehensive Aquatic Systems Model (CASM) was parameterized to use atrazine laboratory toxicity data and region-specific characteristics to translate daily atrazine exposure concentrations generated from the stream monitoring program into estimated potential effects on individual model populations of aquatic primary producers and consumers in a generalized 2nd-3rd order Midwestern U.S. stream. CASM_Atrazine (CASMatz) is a bioenergetics-based approach that describes daily production dynamics for a user-specified aquatic food web. It considers the combined implications of varying exposure concentrations/durations, changing environmental conditions, differential population sensitivity to atrazine, and grazing/predator-prey relations in estimating any potential direct and indirect risks posed by site-specific measured atrazine chemographs The generic stream producer community consists of populations of phytoplankton, periphyton, and macrophytes while populations of zooplankton, benthic invertebrates, decomposers, and fish constitute the consumers. In reference simulations, individual population biomass (carbon) values vary as nonlinear functions of daily values of light, temperature, nutrients (N, P, Si), current velocity, water depth, and complex grazing and predator-prey interactions. CASMatz estimates of magnitudes and temporal patterns of population biomass in the absence of other stressors are similar to those reported for 2nd/3rd-order streams. CASMatz provides the flexibility to use alternative methods to estimate exposure-response based on an extensive database of atrazine laboratory toxicity studies and the resulting modelled changes in producer population biomass for varying atrazine exposure scenarios compare favorably with effect and recovery results measured in 33 atrazine microcosm/mesocosm experiments. This generic stream version of CASMatzdemonstrates that potential population-level responses to time-varying herbicide chemographs can be characterized for risk assessment using bioenergetics-based models. Importantly, CASMatz provides ecological complexity commensurate with more realistic modeling of potential changes in aquatic primary producer community structure and consumer communities.

TH 167

Estimating costs of chemical impacts on populations: a case study on peregrine falcon exposed to PBDEs

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Besides contributing to human welfare, the production and application of chemical substances may generate significant side effects in terms of adverse ecological impacts. To quantify the trade-offs between these benefits and risks, monetary evaluation methodologies can be used. To estimate ecological costs caused by chemical pollution, the following steps are involved: 1) quantifying changes in ecosystem attributes resulting from a chemical impact, and 2) translating these changes into monetary values. So far, very few studies in ecological risk assessment have integrated these two parts. Here, we present an approach to assess the costs associated with chemical impacts on animal populations. We used a model in which population dynamics are related to environmental contaminant concentrations by adjusting survival and reproduction rates in accordance with substance and species-specific concentration-response curves. The model was extended by including introduction of captive-bred individuals to compensate for declines in the population caused by contaminant impacts. In the second step, we calculated the costs associated with the introduction of these captive-bred individuals. We applied the model in a case study aimed to assess the costs associated with the impacts of PBDEs on peregrine falcons (Falco peregrinus) in California. Our approach illustrates that incorporating actual replacement costs in an impact assessment model enables the calculation of actual monetary estimates, circumventing the limitations associated with more subjective valuation methods like 'willingness-to-pay' surveys. In our presentation we will outline our modelling approach, present the main results of our case study, and discuss benefits and limitations.

TH 168

Analyzing in vitro-in vivo genotoxicity gap: mechanistic models for in vivo liver genotoxicity and in vivo MNT

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In vivo tests measure the toxic effects of chemicals in the whole organism, where the "logic"of the metabolism is different, since it is "organized"towards detoxification. The investigation of *in vitro-in vivo* gap with respect to genotoxicity at different levels of biological organisms is the main goal of the current work. This allowed better understanding the nature of *in vivo* detoxification effects in terms of *in vivo* bioavailability. The latter is assumed to be the primary difference between *in vitro* and *in vivo* effects whereas the reactivity factor acts in same way in *in vitro* and *in vivo* interactions. In difference with the *in vitro* generated metabolites, which are freely available to interact with macromolecules, the metabolites in *in vivo* detoxification pathways are "trapped"being engaged in enzyme complexation (channeling effects) and subsequently unable to interact with DNA and proteins. A classification scheme interrelating the different levels of genotoxic effects has been created on the basis of a large amount of quality assured documented data. The relationships established in the scheme allowed development of models for *in vivo* liver genotoxicity and *in vivo* MNT which are the other aims in this work. These mechanistic models are of practical importance given that genotoxicity test bateries commonly comprise two *in vitro* (AMES, CA, MLA, SCE) and one *in vivo* tests (UDS, Comet, MNT). The two *in vivo* models are implemented in the TIMES platform (http://oasis-lmc.org) and could be used for predictive purposes. The scheme is a good basis for in-depth investigation of the *in vitro-in vivo* gap with respect to genotoxicity and building reliable mechanistic models for these effects.

HM02 - Impact and remediation of wastewater

TH 172

Determination of SSRIs in sewage sludge by hollow fiber liquid phase microextraction

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Pharmaceuticals are emergent contaminants in the aquatic environment, wastewater treatment plants and municipal sewage treatment plants (STPs). Selective serotonin reuptake inhibitors (SSRIs) are new antidepressants used for psychiatric illnesses. These compounds are becoming the most-prescribed drugs due to their selectivity. SSRIs include citalopram, paroxetine, sertraline and fluoxetine which is metabolized to norfluoxetine. Few studies dedicated to the determination of SSRIs in sewage sludge have been reported. The complexity of the matrices and the trace amounts of pharmaceuticals make the application of extraction techniques for the enrichment and cleanup necessary steps.
The most common technique applied to the extraction of organic contaminants from sewage sludge is pressurized liquid extraction (PLE) combined with solid phase extraction (SPE). However, this technique needs a considerable manipulation and the co-extraction of some interferences is not avoided. Liquid phase microextraction (LPME) is an alternative technique for the selective extraction, clean-up and enrichment of organic pollutants. The most important advantages are that LPME minimizes organic solvent and reagent consumption, gives an efficient clean-up and selectivity, short time of analysis and low cost.
Three phase hollow fiber liquid phase microextraction (HF-LPME) has been applied for the determination of concentration of SSRIs in sewage sludge. Enrichment factors between 250 and 900 have been obtained in water samples. Slurry samples spiked with different contents of sludge were analyzed and an increase of enrichment factor was observed. By relating this increase of the concentration in acceptor phase to the spike levels, initial concentrations of SSRIs in sludge can

be calculated. Concentrations found in sludge samples from Källby WWTP (Lund, Sweden) were 530 ng g^{-1} for citalopram, 37 ng g^{-1} for paroxetine, 210 ng g^{-1} for fluoxetine, 472 ng g^{-1} for norfluoxetine and 251 ng g^{-1} for sertraline.

TH 173

Analysis of perfluorinated compounds in sludge by pressurized solvent extraction followed by liquid chromatography-mass spectrometry

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Perfluorinated compounds (PFCs) are widely used in everyday life and one of the main recipients of these compounds were waste water treatment plants (WWTPs). Due the structure and physicochemical properties of PFCs, these compounds could be redistributed from influent water to sludge or other WWTP treatments. The present work was focused to develop a useful extraction procedure for 18 PFCs from sludge samples based on pressurized solvent extraction (PSE) followed by anionic solid phase extraction (SPE) clean-up, analytes separation by liquid chromatography and analysis in an hybrid quadrupole-linear ion trap mass spectrometer (LC-MS/MS) working in a triple quadrupole mode.
During optimization process of PSE extraction, 3 different solvent mixtures including: water:methanol (90:10), water:methanol (50:50) and methanol (100 %) were tested. In parallel, 3 different temperatures were studied (70, 100 and 130ºC). The final method was established at 70ºC, 100 bar, 2 cycles and methanol as solvent extraction. The method was validated using a blank sewage sludge fortified at different concentration levels. The method limits of detection were ranging from 15 to 79 ng/Kg, with exception of perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA) and perfluorobutanesulfonate (PFBS) which were higher (831µg/Kg, 161µg/Kg and 219µg/Kg, respectively). The limits of quantification were from 50 to 264 ng/Kg for most PFCs. These values were comparable to the decision limit (CCα) and the detection capability (CCβ), which were 17-1134ng/Kg and 18-1347ng/Kg, respectively. The percentage of recovery, for the different fortification levels, was from 79 to 111% in the most cases. Finally, the repeatability of the method was in the range 4% (perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA)) to 25% (RSD%).
In order to evaluate the applicability of the method, 5 sludge samples from WWTP were analyzed. The results showed that the 18 PFCs were present in all samples. However, the concentrations for most of them were below the limits of quantification. The compound present at higher concentrations was PFOS, which was in concentrations from 53.0 to 121.1µg/Kg. The other PFCs were at concentrations between 0.3 and 30.3µg/Kg.

TH 174

Solid phase extraction combined with large volume injection-gas chromatogrophy-mass spectrometry for the determination of a wide variety of priority and emerging pollutants in wastewater samples

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In the complex process of harmonizing laws, directives and actions within the European Union countries, the European Water Framework Directive (WFD, 2000/60) is probably the most important international legislation introduced for many years in the water management and protection field [1]. According to the WFD, the good state of the aquatic bodies is obtained when the concentration of the priority substances in water, sediments and biota are below the established Environmental Quality Standards (EQSs), which have only been fixed for water. However, the WFD does not specify the analytical methods that have to be used, so there is an urgent need to develop monitoring tools and analytical methodology able to provide improved chemical and biological data at a lower cost in order to respond to the challenges of the various tasks involved in each type of monitoring [2].
In the present work a multi-class method is proposed for determination of a variety of organic pollutants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), pesticides, hormones, alkylphenols (APs), phthalate esters (PEs), bisphenol A (BPA) using solid phase extraction (SPE) combined with large volume injection-gas chromatography-mass spectrometry (LVI-GC-MS) in wastewater samples. Variables affecting the extraction such as the nature of the sorbent, the sample pH, the ionic strength, the methanol addition, the sample volume and the elution solvent were studied. In the case of the LVI, injection solvent, injection speed, cryofocusing temperature were considered. During LVI, the feasibility of the direct derivatisation of hormones, BPA and APs during the injection using N,O-Bis(trimethylsilyl)triufloroacetamide with 1% of trimethylchlorosilane (BSTFA+1 %TMCS). The matrix effect in both the extraction and analysis steps was also studied. Samples from three different WWTPs from the Basque Country (Spain) were analysed.
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TH 175

Multi-trace analysis of pharmaceuticals in wastewater samples by GC and GC-MS techniques - optimization of derivatization step

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In recent years, there has been a continuously growing interest in the occurrence, fate, and possible effects of human and/or veterinary drug residues in the environment. During analysis of pharmaceuticals in wastewater samples there are often interfering elements. Due to mentioned above and low concentrations of studied analytes the extensive knowledge must be gathered by the means of elaboration of sensitive and selective analytical methods. GC-based analyses, although often require derivatization of polar pharmaceuticals, they still offer the highest chromatographic resolution. Moreover, the most recent trend in analytical methods for these analytes is the development of multi-component methods among several classes of drugs, too.
The main aim of our investigation is to elaborate the multi-trace analysis of drugs derived from four different classes of pharmaceuticals including non-steroidal anti-inflammatory drugs, hormones, β-blockers and β-adrenomimetics in wastewater. Special emphasis was laid on optimization of derivatization step in these analyses. Several derivatization agents (e.g. N-methyl-N-trifluoroacetamide (MSTFA), N-trimethylsilylimidazole (TMSI), the mixture of N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA+TMCS)) were used to prepare the appropriate derivatives. Parameters such as the volume of derivatization reagents, time of the reaction, temperature of the reaction were tested. Additionally, different GC conditions were applied in term to obtain the highest chromatographic resolution and the lowest limits of detection. The results of this investigation will be shown and discussed.
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TH 176

Matrix effect during the determination of endocrine disruptor compounds in wastewater samples during membrane assisted solvent extraction

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EDCs, defined as "exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)-populations"[1], can have synthetic and biological origin and include hormones, insecticides, phytoestrogens or industrial chemicals. Such EDCs are often detected in waste water treatment plants (WWTPs) effluents and their receiving environments since not all micropollutants are completely removed in such plants [2,3]. In fact, effluents from several WWTPs have been reported to be estrogenic to fish [4].
The determination of EDCs such as alkylphenols (APs), hormones and sterols requires of a preconcentration step in order to analyse target analytes at low ng/L level. During the last decade the use of sample preparation techniques that minimise solvent consumption have been proposed as alternatives to classical techniques. Membrane-assisted solvent extraction (MASE) guarantees low solvent consumption since the acceptor phase is protected by a non-porous membranes. Many variables are studied during MASE development and matrix effect should always be included when complex samples such as the influents and effluents of wastewater treatment plants are studied. In the present work matrix effect during the determination of a variety of EDCs in wastewater was studied both during the extraction and the detection steps. For the detection both liquid chromatography-tandem mass spectrometry (LC-MS/MS) and large volume injection-gas chromatography-mass spectrometry were considered.
ACKNOWLEDGEMENTS
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TH 177

The fate of pharmaceuticals during biological nutrient removal sewage treatment

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Biological nutrient removal is becoming a widespread sewage treatment technology in the UK and Europe. The reduction in nutrients such as nitrogen and phosphorus from water bodies are key strategies outlined in the Water Framework Directive (2000/60/EEC). Sewage treatment plant (STP) effluents are identified as the principal contributors of these nutrients, which if left untreated can lead to eutrophication. Therefore in many sensitive areas STPs are designed to include a step to remove such problematic nutrients. With conventional activated sludge plants decreasing in numbers the aim of this research is to understand the potential underlying benefits of biological nutrient removal (BNR) for removing pharmaceuticals from wastewater.
During BNR the sewage passes through areas of differing redox potential, increasing the opportunity for alternative degradation pathways. Laboratory batch tests have been designed to reproduce the redox conditions necessary for BNR. These tests will assess the biodegradation outcome of five radiolabelled pharmaceuticals; salicylic acid, caffeine, diclofenac, carbamazepine and propranolol.
Nitrifying (aerobic) redox conditions are relatively simple to achieve by adding ammonium to the activated sludge biomass and aerating. Denitrifying (anoxic) conditions are attained by adding a source of nitrate with no aeration and anaerobic conditions by purging the vessel headspace with nitrogen. The reference compound ^{14}C PEG 400 was dosed at a concentration of 0.1 mg/l into aerobic and anoxic vessels, under each condition the compound was mineralised by over 75%. The reference compound demonstrated the viability of the activated sludge. In the next phase ^{14}C propranolol was dosed at 0.01 mg/l under the same conditions. Mineralisation was 50% for aerobic conditions and 27% for anoxic conditions. These results demonstrate the potential for anoxic conditions to mineralise ^{14}C propranolol suggesting when redox conditions are linked together in a flow through system representative of full scale plants total mineralisation could be higher than during aerobic only treatment. Further work will focus on removal during a flow through system.

TH 178

Biodegradation of pharmaceuticals by active heterotrophic biomass in activated sludge and its link to sludge age

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Microbial biomass is a key factor for the biological elimination of polar pharmaceutical active compounds (PhACs) during activated sludge treatment. It is usually addressed by lumped param-

eters such as volatile or total suspended solids (Xss) that do not consider differences in microbial activity or consortia. This can lead to biased estimations in fate prediction or in the determination of biodegradation rates. In this context, only little attention has been paid to identify responsible PhAC-degrading microorganisms as well as to the question of weather they can be actually characterized (cost-effectively) as a sum parameter or estimated from other parameters such as the sludge retention time (SRT). The latter has been suggested as a predictor parameter for slow growing bacteria resulting in increased pharmaceutical removal efficiency which is, however, controversially discussed in the literature.
Investigating this, the presented study focused on the active heterotrophic biomass Xbh that governs COD removal suggesting a potential determining factor for biological PhAC removal as well. Activated sludges from two wastewater treatment plants that clearly differed in size, operation, SRT and active biomass were investigated. The biodegradation rates of the five polar PhACs caffeine, paracetamol, sulfamethoxazole, diclofenac and carbamazepine were determined using a combination of biodegradation batch experiments and respirometry tests. The latter allowed the simultaneous estimation of the active heterotrophic biomass Xbh.
Fractions of Xbh / Xss varied significantly between the sludges showing that Xss contains little information if any concerning heterotrophic activity. Notedly higher removal rates of the selected compounds were observed in sludge with low SRT and a high Xbh fraction.
The variability of biodegradation rates could be largely explained by using the Xbh in pseudo first-order reactions. It suggests that Xbh greatly governs the removal of the selected pharmaceuticals which is consistent with the fact that high Xbh fractions are linked to low SRTs. The selected substances are all (heterocyclic) aromatic carbohydrates and are assumed to follow similar breakdown pathways as other aromatic carbohydrates present in wastewater and therefore being subject to non-specific enzyme cleavage. Naturally, due to the broad variety of xenobiotic compounds in domestic wastewaters, testing for other substances with different molecular structures is required.

TH 179

Heavy metals mass balance and efficiency of removal in three wastewater treatments plants

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The presence of heavy metals in wastewater is one of the main causes of pollution in water and soil [1]. There has been a growing concern over the effects of heavy metal due to their biomagnifications [2] and, in this way, metal production has decreased due to legislation, as in the case of Hg.
The behavior of heavy metals in wastewater treatment plants has been extensively studied in recent years [3]. The removal of these metals in the water line of the plants generates accumulation in sewage sludge. The destination of sewage sludge is one of the problems of the wastewater treatment plants as they have high concentrations of metals. The agricultural use of the sludge according to the spanish regulation RD 1310/1990 is one of the alternatives to minimize the impact on the environment [4].
This work analyzes the behavior, mass balance and efficiency of removal of 12 metals (Al, Ni, Cd, Zn, Hg, Fe, Cu, Mn, Sn, Cr, As and Pb) in three wastewater treatment plants in Bizkaia (Basque Country, Spain). These plants are located in the towns of Bakio, Gernika and Galindo and they present different characteristics due to the size of population they serve and to economical activity.
The analysis of the obtained results concluded that the behavior of these three plants is quite similar in spite of their different characteristics. The efficiency of removal for the majority of the metals is around 85 %, except for Cd, Mn and Hg. The analysis of the sludge concluded that they are useful for agricultural use according to RD 1310/1990.
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TH 180

Degradation of PPCPs in activated sludge-comparison between degradation kinetics obtained from sludges from four different WWTPs under normalized conditions

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The presence of pharmaceuticals and personal care products (PPCPs) in wastewater treatment plant effluents have been an emerging issue in environmental science, thus more knowledge on removal kinetics and mechanisms is needed.
In this study we focused on comparing the biodegradation kinetics of PPCPs in different activated sludge samples collected from four different wastewater treatment plants (WWTPs) under normalized conditions. Naproxen, Ketoprofen, Fenoprofen, Carbamazepine and Triclosan were analysed in the experiments. The 1st order degradation rates (normalized to sludge biomass) differed significantly (e.g. for Triclosan from 0.0025 to 0.0082, for Fenoprofen from 0.007 to 0.081) with huge consequences on potential removal efficiencies due to biodegradation in different activated sludges under aerobic conditions. Furthermore, the possible treatment plant-dependent factors influencing PPCPs removal, such as sludge concentration, sludge retention time, hydraulic retention time, temperature, pH and oxygen concentration were investigated.

TH 181

Can laboratory activated sludge simulations be used to predict the fate of propranolol in sewage treatment plants?

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Propranolol is a non-selective beta blocker mainly used in the treatment of hypertension. It was the first successful beta blocker developed in the late 1950s and therefore it has been widely used for many years. It is excreted mainly as metabolites, but published data for sewage effluents and sewage treatment plant (STP) removal rates show that propranolol is discharged into the environment. This makes propranolol a good compound for comparing laboratory derived data with sewage treatment plant (STP) monitoring data.
The activated sludge (AS) simulation used 14C propranolol and was conducted following a modified OECD 303A approach. The study was dosed at 0.01 mg L-1 for the first 40 days, thereafter this was increased to 0.1 mg L-1. In the first 120 days liquid scintillation counting showed that there was up to 37% removal of propranolol from the effluent. By the end of the study (250 days), HPLC analysis of the effluent showed that radioactivity was not the parent compound indicating primary degradation of propranolol. The AS simulation mass balance data provided further information about the fate of propranolol. This showed that most of the radioactivity was in the effluent, and not adsorped to the sludge solids, <1% was trapped as 14CO2, indicating limited mineralisation in the first 120 days.
The laboratory simulation data is very similar to field STP data for the first 120 days. Data published in the last four years shows that propranolol is removed by up to 35% in modern STPs. However, the latter stages of the simulation show higher removal than field data. Future laboratory studies should include both LSC and HPLC analysis at each sampling point to remove any ambiguity when interpreting the data. AS simulations that run for long periods (>120 days) would benefit from microbial analysis to determine if there are any shifts in population dynamics. This would allow adaption and/or acclimation of the microbial communities to be evaluated. AS simulations would benefit by including tertiary treatments such as sand filtration, ozonation or/and activated carbon as these have been shown to be good at removing pharmaceuticals and are used frequently in STPs.

TH 182

State of art of lanthanum-modified clays: concerns and evidence

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Eutrophication is a common process of many lakes, rivers wetlands and estuaries caused by an abnormal proliferation of algae. The main cause of this large proliferation is the high concentration of nutrients, particularly phosphorus and nitrogen compounds, derived from natural and anthropogenic sources (such as urban and industrial discharges, use of fertilizers and detergents). The most important effects of eutrophication are: development of toxic species of phytoplankton, increased water turbidity and odor, decrease in the amount of oxygen dissolved and decline of biodiversity.
In recent years a variety of restoration techniques have been employed. The novel lanthanum-modified clays (generally bentonite) water treatment technology seems to be effective in remediation of eutrophic water system.
The active ingredient in these products is lanthanum (La^{3+}) that can bind phosphate from the water column and stabilize sediment in order to prevent possible resuspension phenomena.
The main concerns derived from the use of these products are linked to the possible environmental effects, especially for lanthanum ions released from bentonite to aquatic organisms after the application into the environment.
A fair number of studies and technical reports are available about toxicity, eco-toxicity and bioavailability of modified-clays and of lanthanum.
Most of these studies reported no significant evidences about lanthanum-modified clays toxicity to aquatic organisms and humans at suggested applied dose rate. It's also evident that the results of the toxicological tests are influenced by the conditions test (in particular by the physical-chemicals properties of the medium) and by tested species.
Available reports also suggest that the amount of La^{3+} released from bentonite following the application in water system, is very low. Once released it would be almost immediately captured by the humic acids present into the natural waters, becoming not bioavailable to the organisms.
The informations currently available therefore suggest that the use of lanthanum-modified clays could be an effective and generally safe solution against eutrophication.

TH 183

Concentration profiles and environmental load of emerging pollutants from Swedish sewage treatment plants

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In the society today, an ever-increasing number of chemicals are used. Some of these chemicals are eventually released into the waste streams handled by municipal sewage treatment plants (STPs). The purpose of this study was to perform an extensive screening of persistent organic pollutants (POPs), pharmaceuticals and personal care products (PPCPs) and other organic contaminants (including many emerging pollutants) both in effluent water and sludge from Swedish STPs. The STPs use conventional methods for treatment of the sewage, including mechanical, chemical and biological processes. Solids that are removed from the water is then anaerobically digested or aerobically stabilized and dewatered. The sampling campaign occurred in September and October 2010 when the weather conditions were approximately average of the time of year (average temperature: 9.4 ± 2 °C). Composite (n = 7) effluent samples were flow proportionally sampled in dark 2.5 L bottles (totally 17.5 L) and stored in a dark cold-store prior to chemical analysis. Sludge samples (1000 g wet weight) were collected as grab samples in dark bottles and stored in a freezer at -18 °C until chemical analysis.
In order to be able to estimate the environmental load, mass flows of the pollutants at each STP were calculated from their measured concentrations and estimates of water and solid mass flows at the time of sampling. The environmental load of the pollutants, as well as concentration profiles, did not differ very much between the STPs. The distribution between the water and solid fractions for the pollutants can, at least partly, be explained by their chemical and physical properties. The treatment process and load of the STPs (number of inhabitants, catchment area, geographic location, industry etc.) have also been taken into consideration during data evaluation. Lipophilic POPs are, as expected, separated to sludge while semi-persistent water soluble pollutants, in general, seem to pass through STPs relatively unaffected of the treatment process. PPCPs are highly presented in the sludge and also recovered in large proportions (about 40% in average) compared to the annual national consumption (based on Swedish national statistics). In conclusion, the overall pattern of the pollutants within the investigated STPs, as well as the environmental load, is rather similar.

TH 184

Effects of the wastewater treatment plants on remediation of a closed gulf

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The level of pollution in Patraikos Gulf, Greece, was evaluated at two time intervals, in 1992 and eighteen years later, in 2010. Metals and Polycyclic Aromatic Compounds (PAHs) were determined in sediments, marine organisms and seawater. Sediment Quality Guidelines (SQGs) and Contamination Factors were applied to assess the contamination status. The main difference between the two time periods is that in 1990s no wastewater treatment plants existed to treat the municipal wastes produced by about 250000 residents living in the city of Patras and contiguous areas, while the adjacent industrial area was equipped with a secondary treatment plant discharging its effluents into a river (Pirros). The river's mouth is located at the center of the south coast of the Gulf. In the early 2000s two wastewater treatment plants were constructed and served the whole population while two submarine outfall pipelines started to discharge the treated industrial and municipal effluents with a dilution factor of about 1: 200.
Results. Metals that originate from the geological structure of the basin did not show any difference during the two time spans. On the contrary, metals reflecting anthropogenic activities, i.e. Cu, Zn, Cd, Pb and partially Ni, as the factor analysis revealed, were found at significant lower concentrations at the second sampling compared with the first one. As for PAHs, their mean values were close to the detection limit at both time periods. The indigenous marine organism Pinna squamoza was chosen as a living indicator during the first monitoring due to its specific characteristics. Significant decreases were observed in their metals' content during the second sampling reflecting the improvement of the situation. Seawater samples showed no significant variations in the metals' concentrations. Contrary, phosphorus has been significantly reduced.
Conclusions. The construction and operation of wastewater treatment plants, in compliance with the relative European directive, especially in coastal areas that were receiving wastewater effluents seems to improve the pollution status and contribute to the ecosystem reclamation. This fact proves that even closed gulfs that have been polluted, can be remediated so as to preserve the beneficial use of the sea and its product.

TH 185

Heavy metals in intertidal rocky shore species: effect of sewage discharges

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The discharges of sewage effluent treatment plants have a major impact on coastal communities. Not only by the organic and nutrient enrichments but also due to heavy metals contamination. We can estimate the pollution caused by heavy metals on aquatic systems by analyzing water, sediments and marine organisms.
This study examines the influence of sewage pollution on the concentration of trace metals (Cu, Zn, Pb, Ni, Co, Cr, Fe, Mn) on intertidal rocky shore communities, quantifying those metals on water, sediment and the most abundant species.
The study was conducted on the Peniche peninsula, on the western Portuguese coast. We chose three shores: one received the effluent of a sewage treatment plant (impacted area) and the others were used as references.
The specimens were collected randomly by hand on the intertidal shores, and then placed in polythene bags and frozen at -20ºC. Metals concentrations were determined by atomic absorption spectrophotometry (GFAAS) after acid digestion.

TH 186

First evidences of polycyclic synthetic musk compounds in surface water systems in Italy: galaxolide, tonalide and celestolide concentrations in the Molgora river (Lombardia Region, Northern Italy)

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Polycyclic synthetic musk (PCMs) compounds, are used as fragrances in a wide array of personal care products. In view of their yearly global use, such substances are classified as High Volume Product Chemicals in Europe. For instance in 2000, at EU level, it has been estimated a global usage of 1427 and 343 tons of galoxolide and tonalide respectively. The removal of PCMs during municipal sewage treatments process has been estimated at approximately 40-60%. In view of their broad form of use and biological stability it is not surprising to find these compounds as contaminant of aquatic environments. As matter of fact, in the last few years there is an increasing number of publications indicating the presence of PCMs in surface water systems and in the aquatic biota, with concentrations ranging from ng/L to µg/L and µg/kg to mg/kg lipid weight respectively. Surprisingly, literature data, reporting the presence of PCMs in the Italian aquatic systems, are very scarce and jeopardized even if the very large use of personal care products. To partially contribute to fill the gap of knowledge in our country a monitoring survey of galaxolide, tonalide, traseolide and celestolide started in 2010. The survey was conducted along the Molgora river located in a very densely populated area of Lombardia Region, Northern Italy. Water, river water and sediment samples were carried out seasonally in 7 monitoring stations. The first one located in a natural protected area was used as background level of pollution. The other monitoring stations were located up and downstream of the three sewage treatments plants (STPs) present along the river. The results obtained indicated in all cases an increase of PCMs concentrations immediately after the 3 STPs with the highest contamination in the last sampling station. Concentrations found are in agreement with literature data with galaxolyde > tonalide >> celestolide. Finally, no significant differences were found in samples collected seasonally in the same location indicating a continuous input of PCPs during the year.

TH 187

Contribution and risk of antibiotics from hospitals in urban areas: the case of Lausanne, Switzerland.

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Antibiotics are substances commonly consumed by the population under medical prescriptions. These compounds may reach the environment through human excretion as they are partially eliminated in WWTPs. The occurrence of antibiotics in the aquatic environment is therefore of particular concern as they can present a risk for both ecosystems and human health. Furthermore, they have the potential to foster bacterial resistance.
One source of antibiotics in the environment can be linked with hospitals activities. In this study, antibiotics were measured in the effluent of the main building (650 beds) of the biggest hospital of Lausanne city (CHUV, 1200 beds), Switzerland. In parallel, the entry and the outlet of the WWTP of the city as well as the receiving waters (lake Geneva) were also investigated, collecting samples of wastewater or lake water. Nine antibiotics (azithromycin, ciprofloxacin, clarithromycin, clindamycin, metronidazole, norfloxacin, ofloxacin, trimethoprim, sulfamethoxazole) were analyzed with SPE extraction followed by LC-MS/MS analysis. Results showed high concentrations in the range 2.3 - 32'000 ηg/L at the outlet of the hospital that may represent a risk of bacterial resistance induction. The mass of antibiotics measured at the outlet of this hospital, expressed in nanograms per bed, was extrapolated to all clinics and medical centers of the city (representing 3540 beds in total) and used to compute the total production of antibiotics from hospitals for the all city. The total antibiotic mass at the entry of the WWTP of all hospitals and medical centers represent a small fraction of the total measured amount of antibiotics (< 5%) except for ciprofloxacin (15,1%) and sulfamethoxazole (23.6%). Similar results were obtained in a study at the hospital site in Baden. For some antibiotics (azithromycin, metronidazole, norfloxacin), concentrations at the entry of the WWTP were higher in comparison with hospital concentrations. This demonstrated an important consumption of antibiotics by the population outside of medical centers. After treatment in the WWTP, concentrations of antibiotics were reduced but were still bigger than PNEC values for clarithromicyn and sulfamethoxazole. Finally, after dilution in Geneva Lake, the measured concentrations are below effects values. However, the risk of multiresistant bacterial discharged from hospitals is another issue that needs consideration.

TH 188

BOD5 and COD of leachate and piezometric water from semi controlled, non sanitary landfill in Novi Sad, Serbia

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The absence of landfill liner systems means that disposal of solid waste often leads to water pollution. In such cases, water percolating through the waste and dissolving various materials is considered the most serious environmental impact of landfill and causes surface and groundwater pollution. Landfill leachate is one of the most complex problems of municipal solid waste landfills. Leachate is generated as a result of the percolation of water through landfill body and the squeezing of the waste due to its weight, and it is contaminated with dissolved and suspended organic and inorganic compounds with different characteristics. Since all natural waterways contain nutrients and bacteria, their enzymes will initiate biochemical reactions of almost any waste compounds that are introduced into such waterways. Any oxidizable material present in a leachate will be oxidized both by biochemical (enzymatic) or chemical processes. The result is that the oxygen content of the water will be decreased. Both the BOD_5 and COD tests are a measure of the relative oxygen-depletion effect of a waste contaminant. In August and September of 2008. research campaign was conducted to determine composition and quantity of waste that is disposed at landfill in Novi Sad. The landfill in Novi Sad is semi controlled non sanitary, municipal waste landfill without impermeable bottom liner to prevent impact contamination of soil and groundwater with leachate. The biggest part of waste from households and commercial sector is biodegradable organic waste that is decomposed by microbiological and chemical mechanisms in landfill body. The leachate samples and samples from piezometers were collected from collecting channel and 6 piezometers in municipal solid waste landfill in Novi Sad in January and May of 2010. From obtained results of BOD_5 and COD it can be concluded that the leachate from landfill in Novi Sad is contaminating soil and groundwater in continuus. The most contaminated piezometer is the one that is located downstream from landfill body and suffers most of the contamination. The groundwater level in this part of landfill is very high so there is justified concern for spreading of contamination. Because leachate is very toxic (high content of heavy metals, organic matter and pathogenic microorganisms) it is necessary for landfill to be sanitary in order to prevent further soil, groundwater and contamination of surrounding environment.

TH 189

Elimination of micropollutants using engineered constructed wetlands - a laboratory scale study

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In Switzerland, a new standard for wastewater treatment plants (WWTP) - 80% reduction of micropollutants from raw sewage - is under consideration. Five substances have been proposed as target compounds: Diclofenac, Carbamazepine, Mecoprop, Sulfamethoxazole and Benzotriazole. Although very efficient and reliable, advanced treatment technologies for traditional WWTPs - such as ozonation - are expensive and difficult to implement on relatively small WWTPs, of which there are many in Switzerland. To tackle this problem, we propose to develop a promising technology for supplemental treatment of wastewater: engineered subsurface flow constructed wetlands (ECWs).
Classical constructed wetlands (CW) are passive systems, using natural processes to transform and remove contaminants from wastewater. In this work, we have developed and proposed a different design paradigm, i.e., to combine in a single ECW both natural processes (as in CWs) and ad hoc engineered treatments. The resulting system - an ECW - will combine the strengths of both natural and engineered processes, that is, it will be efficient and reliable, with low maintenance and running costs. However, there is still a lack of detailed knowledge of the factors influencing micropollutant degradation and, ipso facto, in the ability to optimize the conditions for contaminant effective elimination.
A laboratory scale study was performed to develop a combination of treatments able to achieve the target elimination rate for micropollutants. Based on a literature review, two processes - direct photolysis and adsorption by light expanded clay aggregates (LECA) - were expected to remove more than 80% of all target compounds. Batch experiments were conducted to test the removal efficiency of each process alone. Direct photolysis removed 60 to > 95% of four compounds while adsorption by LECA showed only moderate elimination. A laboratory-scale ECW combining the two removal treatments with biodegradation was subsequently performed to evaluate whether the combined processes could enhance the degradation rate. It was found that removal of just three target compounds was enhanced. A conceptual model was developed and will be used as starting point for modeling micropollutant removal in ECW's in future studies. Based on the measurements and insights gained from the model, possible reasons for the relative poor performance observed will be discussed, and possible improvements presented.

TH 190

Removal of selected endocrine disrupting chemical during on-site wastewater treatment using a constructed wetland

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Significant research has shown that domestic and industrial wastewater can be a source of endocrine disrupting chemicals (EDCs) to the environment. Much of this research has focused on municipal and industrial centralized wastewater treatment plants. These plants have been shown to be a significant source of both natural and synthetic hormones and alkylphenolic compounds to

receiving systems. However, in the US, about 25-30% of wastewater is treated using decentralized wastewater treatment technologies. These technologies (septic systems, constructed wetlands, etc) have not been adequately characterized for their capacity to manage these emerging contaminants. The focus of this study is to characterize both the seasonal loading of these compounds to a constructed wetland receiving domestic wastewater and to characterize the removal capacity of the wetland treatment unit.
The research was conducted over two years at a subsurface constructed wetland designed to treat domestic wastewater. The loading to the system is primarily from a retreat center in southwestern Ohio that receives a low flow (8-12 people) of domestic waste the majority of the time with periodic high flows (greater than 50-100 people).. The influent and effluent flows were characterized for hormones (7 natural and 1 synthetic), alkylphenol ethoxylates (including the octylphenol ethoxylates and nonylphenol ethoxylates and their respective metabolites), nutrient (species of N and P), TOC, and pathogens over a two year period to evaluate season loadings and treatment capacity of the wetland. Results have shown, to date, that the EDC are treated to varying degrees dependent on loading and seasonal condition of the wetland. Evaluations of loading rates, removal rates, and season relationships will be presented.

TH 191

Hot flushes and hormones - can microwave technology reduce endocrine disrupting activity?

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Wastewater effluents and storm-water runoff are now thought to be among the major sources of endocrine disrupting chemicals discharged to the aquatic environment. Many studies have reported endocrine disrupting effects on fish located near wastewater outfalls and some EDCs once applied to soils can be taken up by plants. Environmentally relevant concentrations of a few selected EDCs have been reported but the concentration of many implicated EDC compounds in municipal sludge remains largely unknown.. Therefore, municipal wastewater sludge disposal in landfills and land application of biosolids may pose a risk to aquatic life in receiving waters through runoff and leaching processes.
Microwave processes have been widely used for diverse applications throughout the food industry. More recently, microwave technology has been applied and investigated for sludge drying, pasteurization, solids reduction and phosphate recovery. Uniform heating and precise temperature control are the key primary advantages of the microwave process. Non-ionizing electromagnetic radiation from the production of microwaves are thought to produce both athermal and thermal effects in sludge at a molecular level and this, in turn, could offer potentially valuable wastewater treatment options.
In this poster, we describe the application of microwave processes with pre- and post-digested sludge from a municipal sewage treatment plant utilizing conventional sludge digestion (mesophillic anaerobic) to investigate and demonstrate the viability of this innovative wastewater treatment technology to reduce whole estrogenic activity in municipal sewage sludge. Using GC/MS chemical analyses and bioluminescent yeast estrogenic screening assays, a mass balance approach was adopted to assess whole estrogenic activity in the municipal wastewater treatment plant sludge.

TH 192

Electrocatalytic degradation of anions used in ionic liquids

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Ionic liquids (ILs) have been area of great interest owing to unique properties and unlimited resources for designed properties by variation of cation or/and anion. Among the several applications foreseeable for ILs in the chemical industry such as solvents in organic synthesis, as homogenous and biphasis transfer catalysts, and in electrochemistry, as extraction media. However, from the environmental point of view despite the excellence of their technical performance the release of these chemicals could be a problem for the environment. When effective regeneration or recovery at a reasonable cost is not possible, utilized ionic liquids should be destroyed using suitable advanced oxidation processes (AOPs). They are designed to generate hydroxyl radicals (-OH) as a primary, non-selective oxidizing agent. A few advanced oxidation technologies have been proposed as a consequence of some ILs being refractory to biological treatment. Among them, a particularly attractive treatment option for non-biodegradable ILs is electrochemical oxidation using so called "non-active"electrodes (BDD, PbO_2). The electrochemically oxidation of imidazolium cations showed the influence of their side chain structure on efficiency treatment and pathway of degradation process.
The main goal of the presented investigations is to shield more light on the electrochemical degradation processes of anions typically used in ILs. Degradation of 1-butyl-3-methylimidazolium salts with different anions (Cl^-, Br^-, PF_6^-, BF_4^-, $CF_3SO_3^-$, $C(CN)_3^-$) was carried out on "non-active"BDD and "active"IrO_2 electrode in Na_2SO_4 water solution. The obtained results are discussed and intercompared.
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TH 193

Towards understanding of ionic liquid (IL) fate in the degradation processes: investigations of ILs interactions with O2-• radicals

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In recent years ionic liquids have gained a broad of interest, because of their great potential to be used in industrial possesses based on different tuneable properties. Concerning the chance of wide applications of ionic liquids in the industry there appears a real risk of finding them soon in industrial wastewater and finally in the environment. Therefore, there is a need to look at their properties from the point of view of their ageing in industrial processes and through wastewater treatment, before they reach the surface- and groundwaters.
Electrochemistry plays an important role in many areas of science and technology. On the one side, some of IL could be very good electrolyties e.g. in batteries. From the other side the electrochemically methods successfully used for degradation of toxic and not readily biodegradable organic pollutants, could be applied for degradation of ILs in wastewater. Depending on conditions of electrooxidation process, active entities such as $^{\cdot}OH$, $O_2^{\cdot-}$, $S_2O_8^{2-}$, ClO^- etc, could be generated on electrode surface. The identification of these entities, determine their reactivity towards the organic pollutants. Additionally they present information about degradation pathway and are very important for estimation of efficiency of the degradation method. From the other side it has been shown that these oxidizing entities may be present in all oxygen-metabolizing organisms and its toxicity has been recognized.
The investigation concern the interaction of superoxide ion with ionic liquids contains an imidazolium headgroup in cation. Some of the alkyl side chains were substituted by functional groups such as hydroxyl or phenyl residues. They were studied by the cyclic voltammetry technique. The generated intermediates were identified and the degradation pathway of IL by $O_2^{\cdot-}$ radicals is presented.
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TH 194

The degradation of ionic liquid on the electrodes with different electrocatalytic properties

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Industrial effluents containing toxic and recalcitrant organic compounds lead to serve environmental problem. Traditional treatment methods, including biological, physical and chemical treatment are ineffective, and thus a number of alternatives have been researched. Electrocatalytic oxidation is one of the alternatives for efficiency degradation of these compounds. It is suitable for a low-volume application, versatility, energy efficiency, amenability to automatization and environmental compatibility method. Electrode material is one of the most important factors influent on the efficiency of degradation process and cost effectiveness. Ionic liquids (ILs) (alternative solvents, extrahents, electrolytes, etc.) characterized by wide applicability which is mainly based on their beneficial physico-chemical properties and their tuning possibilities. Thus ILs become more popular and they may pose a real risk of being present in technological wastewater, aquatic environment and soil. The impact of ILs on aquatic ecosystem is highly important since some ILs have a high solubility in water. Therefore there is a need to look at possibilities of their degradation in effluent, before they enter environmental systems.
The presented investigations are focused on the electrochemical oxidation of 1-butyl-3-methylimidazolium chloride on anodes with different electrocatalytic properties such as: BDD, PbO_2, IrO_2 and Pt/Ir. Cyclic voltammetry experiments were carried out to get a better understanding of IL electrooxidation process. The investigations were also focused on the identification of main oxidants species and intermediates generated by electochemical process.
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TH 195

Elimination of micropollutants in wastewater treatment plants : ozonation or activated carbon? Conclusions of a pilot project over one year.

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Many organic micropollutants present in wastewater, such as pharmaceuticals and pesticides, are poorly removed in conventional wastewater treatment plants (WWTP). To reduce the release of these substances into the aquatic environment, advanced treatments are necessary and will be soon mandatory in Switzerland. Two advanced treatments were tested in pilot systems over more than one year at the municipal WWTP of Lausanne, Switzerland. The first pilot involves the ozonation of the effluent to oxidize organic substances. It is followed by a sand filtration (SF) to remove the readily biodegradable reaction products. The second pilot consists of a powdered activated carbon (PAC) addition into the effluent to sorb micropollutants. It is followed either by a membrane filtration (ultrafiltration) or a sand filtration to separate the PAC from the treated water. A selection of 58 potentially problematic substances (pharmaceuticals, pesticides, endocrine disruptors) were regularly measured at different stages of treatment by ultra performance liquid chromatography coupled to a tandem mass spectrometer, and a large battery of ecotoxicological tests (16 in vitro and 9 in vivo assays with different organisms) were performed to evaluate the toxicity of the effluents. The results showed that most compounds were removed over 80% with an average ozone dose of about 5.5 mgO_3/l or a PAC dose between 10 and 20 mg/l. Only some single compounds, such as X-ray contrast media, were only partially eliminated in both cases. A clear reduction in toxicity was also observed after the two treatments in most of the cases. These two processes (ozonation and PAC addition) are therefore effective to reduce the release of micropollutants into surface waters. Ozonation-SF and PAC-SF proved to be feasible in terms of implementation and operation at large-scale in WWTP, for relatively similar investment and operation costs (about 0.1 to 0.15 € per m^3 treated based on local costs). Each technique has its advantages and disadvantages. Therefore the method selection should be made case by case for each WWTP depending on the local constraints (e.g. space, security, energy costs, existing treatment processes, sludge disposal process, need for disinfection, etc.), the quality and the quantity of incoming water and the desired output water quality.

TH 196

Performance of coupling an heterogeneous photocatalysis (TiO2/UV) with activated sludge reactor for removing the color of an industrial wastewater.

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Generally, wastewaters can be easily treated by biological reactors with a good efficiency regarding organic matter removal. However, the process can be impaired in the presence of compounds that are toxic or have low biodegradability. Advanced Oxidation Processes (AOPs) may be a choice to oxidize these compounds and minimize their toxicity and/or increase biodegradability. AOPs are based in the "in situ"generation of strong oxidants that reacts with the organic matter. The most important oxidant agent generated by AOPs is the hydroxyl radical (-OH), as they have a high oxidation potential. The aim of this work is to study, in laboratory scale, the decolorization achieved by the combination of a heterogeneous photocatalysis (TiO2/UV) with activated sludge reactor, in the treatment of a wood's industry wastewater, optimizing catalyst concentration, temperature, and pH. The Hydraulic Retention Time (HRT) of the activated sludge reactor was 3 hours, with an interruption in the aeration of 30 minutes (same time used in the industry). Photocatalysis was carried out in a jacketed reactor with the irradiation of a medium pressure

mercury lamp (250 W), away 20 cm from the wastewater surface (effective volume of 100 mL), magnetic stirred at 450 rpm. The biological reactor removed almost all COD of the effluent (85-90%), although color was not removed. When photocatalysis was used as a pre-treatment with 60 minutes of irradiation, there was a color removal of 60% and the ecotoxicity to lettuce seeds did not change, with the variables optimized pH 5.7, 25°C, and 0.42 g L-1 TiO2. However, color removal increased to 90% with an irradiation time of 5 hours and 30 minutes. The photocatalytic decolorization kinetics followed a first order model, with a R2= 0,992 and a of (2.60 ± 0.24) ´ 10-2 u.a. min-1. Photocatalysis is a good choice for removing the color of this effluent.

TH 197

Degradation of selected sulfonamides in the wastewater using heterogeneous Fenton system

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Sulfonamides are synthetic antibiotics frequently used in veterinary medicine. After use they are being excreted in large portion directly to the sewage either in the native form as well as biotransformation products. Traditional sewage treatment systems are incompatible to eliminate many residual pharmaceuticals. A very significant rate of these chemicals entering environment constitutes of sulfonamides, which environmental concentrations are noticeable and moreover most of them are quite resistant to physical and biological degradation. Therefore, the elimination process of these chemicals from wastewater is a very important step preventing their emission into the environment. Advanced oxidation processes (AOP) present highly effective alternatives to traditional water treatment processes while degrading toxic and biorefactory organic pollutants such sulfonamides. These methods are based on the generation of highly reactive agents such as hydroxyl radicals (-OH), which are a very strong and non-selective oxidants able to mineralize some of organic compounds to CO2 and H2O. Among the various types of AOPs, Fenton process presents cheap and quite useful option, where radicals are produced by catalytic decomposition of hydrogen peroxide in reaction with ferrous ion. The main drawback of this system are ferrous sludge. This problem can be solved by using heterogeneous catalyst.
The present study focused on degradation of five sulfonamides: sulfathiazole, sulfadiazine, sulfamerazine, sulfamethazine and sulfadimethazine by heterogeneous Fenton system with Fe(II)/Al2O3 as catalyst. The influence of initial pH, amount of catalyst and initial concentration of sulfonamides were studied. Biodegradability and efficiency of degradation sulfonamides in wastewater is also presented.
Acknowledgment: Financial support was provided by Polish Ministry of Research and Higher Education under grant N N523 42 3737, BW 8270-5-0465-0, DS 8270-4-0093-10.

TH 198

Degradation of brominated flame retardants by the white-rot fungus Trametes versicolor

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Due to the environmental accumulation of polybrominated diphenyl ethers (PBDEs), the search of strategies for their elimination is of great interest. In the present work, *Trametes versicolor*, a white-rot fungus capable of degrading a wide spectrum of toxic contaminants, was tested for PBDE removal. Characterization of sludge from the El Prat de Llobregat WWTP (Barcelona, Spain) showed the presence of several PBDE congeners, although deca-BDE-209 was the most abundant. In vitro experiments with T. versicolor in liquid medium revealed elimination from 50% to 75% for deca-BDE-209. Similar experiments demonstrated removals of 85% and 67% (minimum degradation of 38% and 28%) for the penta-BDE and octa-BDE commercial mixtures. The fungus was then applied to sterile sludge obtained from two stages of the WWTP: the outlet of the anaerobic digestor and the thermally dehydrated sludge. For the former sludge, a slurry reactor with fungal pellets reduced the concentration of some congeners from 24% to 53%, while a solid-phase treatment of the dry sludge produced a remarkable degradation of 86% in the deca-BDE-209. The results suggest the potential of *T. versicolor* as a bioremediation agent for PBDEs removal.

TH 199

Uranium accumulation in aquatic plants: possibilities for phytofiltration

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The work presented here is a part the on going study on the uraniferous geochemical province of Central Portugal in which, the use of aquatic plants as indicators of uranium contamination is being probed using aquatic plants emphasizing their potential use in the emerging phytotechnologies. Several of the uraniferous deposits were exploited either by underground or surface mining methods. Many of the places were left in different stages of degradation. The samples were collected in running and in standing waters (lentic and lotic) in the places were it was possible to observe aquatic species. In these sites, samples of the waters and of the vegetable species were taken. The plants collected represented the free floating and the rooted emergent plants. In the ponds, only free floating plants were found growing. The methodology adopted for the determination of the U content in the water and plants was fluorometry.
Even though we have observed very low concentration of U in the fresh waters of the studied sites we found a set of vegetable species with the ability to accumulate U in concentrations which are orders of magnitude higher than the surrounding environment. We have observed that Apium nodiflorum, Callitriche stagnalis, Lemna minor and Fontinalis antipyretica accumulated significant amounts of uranium, whereas Oenanthe crocata excluded U. These results indicate substantial scope for proper radiophytoremediation and phytosociological investigation exploiting the native flora. These species show great potential for phytoremediation because they are endemic and easy to grow in their native conditions. A. nodiflorum and C. stagnalis have high bioproductivity and yield good biomass.

TH 200

Evaluation of the genotoxicity of textile effluents from the Region of Fez-Boulmane (Morocco) by the use of Saccharomyces cerevisiae mutation assay and plant micronucleus assay

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In order to investigate the genotoxicity effects of crude effluents from textile industries of Fez-Boulmane, Morocco, mutagenicity and phytotoxicity tests were carried out analysing different biological systems. Moreover the efficiency of a prototype of Sequencing Batch Reactor (SBR) working by activated sludges and obtained in Fez in a laboratory scale, was estimated by comparing the eco-toxicity results observed before and after wastewater purification in SBR system. Evaluation of the genotoxic potential was firstly investigated by means of mutagenicity tests on D7 strain of Saccharomyces cerevisiae in different growth phases. This strain, obtained from Zimmermann, allows the contemporary evaluation of three different genetic effects: mitotic crossing over (CO), genetic conversion (GC) and point reverse mutation (PM).
In addition phytotoxicity tests were performed on Allium cepa L. and Vicia faba L., considered plant model systems for eco-toxicological studies. The same tests were also applied on Lactuca sativa L. as an example of vegetable largely cultivated in riverside areas of Fez and normally irrigated with untreated river water. The considered parameters were the mitotic index (MI), the study of cytogenetic anomalies during anaphases and telophases and the analysis of micronuclei presence. The results obtained testing untreated wastewater demonstrated major mutagenic and phytotoxic effects in Saccharomyces and in the three analysed plant systems while after purification in SBR no more eco-toxicological consequences were observed.
The reuse of wastewater can be an obvious response to water shortage especially in African regions. But recycled wastewater can pose a danger to public health, environment, and agriculture if wastewater is not reused carefully. The data presented in this paper show the possibility of a good solution to tackle the problem of recycling wastewaters coming from textile industry pollution in Fez city, Morocco.

TH 201

Toxicity of biologicaly treated municipal and industrial effluents

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The environmental situation in the Baltic Sea has drastically changed over recent decades. Human activities both on the sea and throughout its catchment area are placing rapidly increasing pressure on marine ecosystems. Inputs of hazardous substances also affect the biodiversity of the Baltic Sea and the potential for its sustainable use.
Effluents from industrial or municipal sources may contain hundreds to thousands of chemicals, but only a few of them can be responsible for aquatic toxicity. The prediction of waste water toxicity usually does not take into account any possible interactions between the compounds in the wastewater sample. Moreover, some highly toxic chemicals may go undetected in a complex waste water mixture.
The aim of this study was to identify the most important sources of 11 hazardous substances of special concern and to evaluate potential toxicity of biologically treated sewage from Latvian municipal and industrial waste water treatment plants (WWTP) based on the Whole Effluent Assessment (WEA) approach.
Test results show that biologically treated municipal waste water is none or slightly toxic year-around, while toxicity of industrial waste water has a seasonal character showing higher toxicity in autumn and winter.
Study revealed that biological treatment is not sufficient for acquisition of good quality status of industrial waste waters. Results demonstrate the importance of using the 'whole-effluent' toxicity approach for a reliable assessment of wastewater quality.
This study was performed in the frame of INTERREG project COHIBA "Control of hazardous substances in the Baltic Sea region".

TH 202

Emerging pollutants in WWTPs effluents: ecotoxicological tests to assess the effects on specific organisms, representative of the aquatic environment

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A significant entry route of priority and emerging pollutants into the aquatic environment is the discharge from wastewater treatment plants (WWTPs). The increasing presence of these substances is of concern because there is a lack of information on their fate in aquatic systems.
The purpose of this study was to characterize the effects on specific organisms representative of the aquatic environment (i.e. algae, invertebrates, amphibians and fish cell lines) exposed to WWTPs effluents in order to assess their environmental risk. In addition, chemical characterization, including priority (7 metals) and emerging contaminants (22 pharmaceuticals, 12 fragrances, 5 preservatives and 5 endocrine disrupters) have been conducted.
Four WWTPs located along the Henares-Jarama-Tajo River Basin (central Spain) were considered; samples were taken monthly for a year. General quality parameters, metals and emerging pollutants were analyzed. Acute toxicity tests (Daphnia magna, Chlorella vulgaris, Xenopus laevis), subacute test (D. magna feeding), in vitro citotoxicity and subletal effects (RTG-2 fish cell line); a new test with embryos of X. laevis, considering different molecular biomarkers and a yeast test (S. cerevisiae), stably transfected with the rainbow trout estrogen receptor and the ß-galactosidase coding sequence (LacZ) as reporter gene, were used to estimate potential adverse effects.
This study was funded by the Spanish projects CONSOLIDER-INGENIO (TRAGUA) CSD2006-00044 and RTA2010-00004-C02

TH 203

Ecotoxicological evaluation of mixed domestic-industrial wastewater treatment plant effluent using Daphnia magna

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Organized industrial zone wastewater treatment plant receive mixed domestic and industrial influents and discharge into Gediz River with a capacity of 6,500 m3 / day in Manisa, Turkey. Samples from both influent and biological and chemical treated effluent wastewaters of treatmant plant were processed using bio-physico-chemical and toxicity tests. Toxicity of wastewaters were evaluated in an acute toxicity study using Daphnia magna as an aquatic experimental animal model. Both undiluted untreated (UWW) and treated wastewater (TWW) were severely toxic to D. magna and led to death of all exposed organisms. The 96-h ED50 value for UWW was 2.8 % and TWW was 20.4 %. The toxicity classification of effluent based on toxic unit (TU) showed that UWW was found high acutely toxic (35.7 TU) and TWW was acutely toxic (4.9 TU). Chemical analyses of UWW showed high concentrations of COD (1036.8 mg/l); TSS (58.1 mg/l); oil and grease (34.6 mg/l); total phosporus (5 mg/l), yet low concentrations of heavy metals according to effluent discharge standard regulations in Turkey. Chemical analyses of TWW showed that all parameters were lower than effluent discharge standards. In the Turkish Wastewater Control Regulations only toxicity dilution factor with fish is part of the toxicity monitoring

program of permissible wastewater discharge. There is no toxicity limitation in this regulations. In conclusion, the results confirmed that toxicity testing should be applied to effluent standard regulation. The results also clearly showed that D. magna assay was an excellent method for evaluation of aquatic toxicity of mixed domestic-industrial wastewater treatment plant effluents.

TH 204

Identification of potential toxicity caused by O3 and ClO2 treatment of pharmaceuticals in wastewater

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Chemical oxidation treatment is an effective innovative technology in wastewater treatment plants for removal of micro-pollutants in the effluent. In particular, ozonation (O3) and chlorine dioxide (ClO2) treatments are commonly used to degrade organic pollutants. By oxidation, the micro-pollutants are generally transformed to compounds that are easier to degrade biologically. There is, however, a risk that the transformation products will have structures similar to the parent compound and still be biologically active. It has been shown, for example, that transformation products generated from Carbamazepine by chemical oxidation were more harmful to aquatic organisms compared to the parent compound. The aim of our study is to evaluate the potential risk of oxidation treatment, using O3 and ClO2, due to formation of toxic transformation products. The ecotoxicological effects caused by the two oxidation treatments were evaluated for milli-Q water spiked with an API-mixture (containing 114 pharmaceuticals), wastewater (WW) and WW spiked with the API-mixture. Tests were conducted to evaluate the toxicity of the API-mixture itself and the spiked waters had an API concentration corresponding to the EC50/LC50 values obtained from these tests. The three test systems were subjected to different doses of the oxidants and used to evaluate acute toxicity for the cladoceran Daphnia magna (48h, OECD 202) and the alga Pseudokirchneriella subcapitata (72h, OECD 201). The survival end-points were complemented by oxidative stress biomarkers to understand effect mechanisms in different test systems and species. All tests were complemented with chemical analysis to confirm to what degree the pharmaceuticals have been mineralized and degraded by oxidation.
Early results with APIs in milli-Q water treated with ClO2 indicate an increased mortality rate of daphnids in higher concentrations oxidant while the growth rate of P.subcapitata was unaffected by the oxidation treatment.

NM02 - Fate and effects of nanoparticles

TH 208

C. elegans as an indicator of environmental and human health risk due to fine dust from wood firing

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Along with the growing importance of wood as a renewable low-cost fuel for residential heating a risk for human and environmental health is emerging due to high particle emission from wood combustion. This project aimed to adjust biological test systems for a practicable risk assessment of fine dusts from residential heating. Test systems are supposed to account for the effect mechanism of various particles. As relevant properties of the particles we anticipate size, shape and chemical adsorbents. At first trials are conducted in suspension with characterised model particles and a sample of fine dust from wood briquette firing.
The nematode C.elegans is an appropriate test organism for environmental as well as human medical science. In the nematodetest on reproduction (ISO 10872) different effects of particles were observed accounting for their size. Particles <1µm can be internalised while particles >1µm only affect the cuticle. The shape of particles suggested an even stronger effect. The nematodetest is to be amended for gene expression analysis considering subacute response potentially inducing chronic diseases in humans. Preliminary results indicate different expression patterns of gst-4 and sod-3 according to different particle size. Biomarkers have been selected according to expected reactions towards fine particles from wood combustion. Besides gst-4 and sod-3 for oxidative stress, tir-1 and nlp-29 for immune response, vit-6 for endocrine disruption and cyp-35C1 for xenobiotic detoxification were selected. A further assimilation of the test is planned for use of filter samples and a more direct exposure at the interface between atmosphere and liquids using a chamber (Cultex) designed for this purpose.

TH 209

Photo-induced toxicity of aged C60 in a marine invertebrate model

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While there is growing concern over the potential detrimental impact of engineered nanoparticles (ENPs) on the human and environmental health, little is known about their fate and behaviour. We have assessed the effect of photo-oxidative aging of C_{60} on its toxicity to the common mussel (Mytilus edulis). The DNA damage in the somatic cells (haemocytes) was assessed after 3 days of *in vivo* exposure to 1.0 mg l^{-1} fresh C_{60} (FC_{60}) and aged C_{60} (AC_{60}) using the Comet assay. Whilst both forms of C_{60} fullerenes caused significant increase in the level of the single strand breaks in the haemocytes, the aged form seems to be more genotoxic. In addition, significant bioaccumulation in all organs (i.e. adductor muscle, digestive gland and gills) was associated with clear signs of histopathological alterations. More subtle effects of AC_{60} and FC_{60} were explored using 2h pre-exposure of sperm followed by *in vitro* fertilisation and embryo development impairments were assessed. Aged C_{60} caused significant mortality among spermatozoa with subsequent decline of fertilization success. Severe developmental abnormalities were also observed in 48-hr embryos developed from AC_{60}-exposed sperm. Spermiotoxicity is associated to DNA damage caused by production of intracellular ROS during exposure to C_{60} (AC_{60} and FC_{60}).
This study underlines the putative photo-oxidative amplification of the ecotoxicological impact of a widely used ENP.

TH 210

Degradability of aged aquatic suspensions of C60 nanoparticles

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While studies of the potential human and environmental effects of C60 and its derivatives are emerging in the scientific literature, the environmental fate of C60 is still largely unknown. In this study, aged aqueous suspensions of C60 (nC60) were investigated in the respirometric OECD test for ready biodegradability. Two suspensions of nC60 were prepared by stirring and aged under indirect exposure to sunlight for 36 months, which resulted in relatively stable suspensions with a dark-brown colour. The suspended nC60 could not be extracted into toluene and indicating that the particles were no longer present as underivatised nC60 but had undergone a transformation. TEM images and particle tracking analysis showed that the suspension consisted of particle aggregates with a size of 156 nm (SD=54nm) and 139nm (Sd=49), respectively, but also contained smaller aggregates. Samples of the nC60 suspensions (approx. 20 mg/l) were inoculated with activated sludge (30 mgTSS/L) and incubated in a mineral medium under aerobic conditions for 28 days. After 28 days no mineralization of nC60 was observed. This was not due to inhibition of the biomass, since addition of 5 mg/L sodium acetate was completely mineralized within a few days. Even with this additional carbon source no mineralization of nC60 was observed after an additional 20 days. Based on these results, aged nC60 can be classified as not ready biodegradable according to the OECD test procedure.

TH 211

Bacterial test battery for risk assessment of fine particles originating from the combustion of wood

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Due to the increased use of renewable energy sources within the last decade, fine particle exhaust from the combustion of wood has become a relevant issue in human and environmental health risk assessment. The aim of this project was the development of bacterial screening tests that can be applied to fine particles as a whole. Hence these tests account for the bioavailability of contaminants adsorbed to the surface of particles. Three well-established bacterial tests were modified: The bacterial contact test (ISO 10871), the Umu test (ISO 13829) and the YES test. All tests are low in costs and can be performed within one day. The bacterial contact assay accounts for cytotoxicity of sediments by measuring the dehydrogenase activity in the aerobic bacterium Arthrobacter globiformis. By means of a genetically modified Salmonella strain genotoxicity of aquatic samples or extracts is determined in the Umu test. In our laboratories both tests have been successfully adapted for the evaluation of fine dust. In the YES test transgenic yeast reports estrogenicity of aquatic samples or extracts. Currently it is amended for the application with particles. To validate the adapted methods, fine dust in suspension, originating from wood briquettes, was examined in logarithmic concentrations from 0.001 to 10 mg/mL. The control was 1 mg/mL of Minusil 5, a crystalline quartz sand which has been proven to be non-toxic to both bacteria and yeast. 0.01- 10 mg/mL of the fine dust suspension triggered a concentration-dependent cytotoxicity in the bacterial contact assay compared to the control. No genotoxic effect was observed in the Umu test. Besides suspensions filters covered with fine particles were gained in the burning of beeches logs and were evaluated in the assays. They caused cytotoxicity and genotoxicity in the bacterial contact and the Umu test respectively compared to blank filters.

TH 212

Fullerene exposure to earthworms: from individual to population effects

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At present environmental risks of nanoparticles are still mostly unknown. Knowledge is especially lacking about possible effects on soil organisms and soil ecosystems. However, this knowledge is of importance because soil ecosystems may be exposed to nanoparticles and residence times of nanoparticles in soil may be long. We therefore conducted a study in which effects of nanoparticles on soil organisms were assessed. In this study, Lumbricus rubellus earthworms were exposed to fullerene C60 nanoparticles (nominal concentrations 0, 15.4 and 154 mg/kg soil), which were added to the soil. The particles, in soil extract, were visualized with TEM and showed aggregates of approximately 10 nm. Adult earthworms were exposed to C60 for 4 weeks and their offspring was exposed from cocoon to adulthood. Mortality, growth and reproduction were assessed at the level of the individual earthworm. These individual endpoints demonstrated that C60 exposure affected cocoon production, juvenile growth rate and mortality significantly. The results on individual parameters were used to model effects at the population level. The population model demonstrated reduced population growth rate with increasing C60 concentrations and a shift in stage structure for C60 exposed populations, with these populations moving towards a larger proportion of juveniles. This study indicates that C60 exposure may seriously affect earthworm populations. Furthermore, it was demonstrated that juveniles were more sensitive to C60 exposure than adults.

TH 213

Influence of the surfactant properties on the coagulation kinetics of functionalized engineered nanoparticles in water system

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As derivatives of engineered nanoparticles (ENPs), functionalized ENPs (FENPs) are widely used in biomedical, catalysis, and electronics fields.This proliferation induces a risk concerning the release of FENPs into the environment. To date, scientific effort has focused on evaluating the toxicity of ENPs but much less attention has been given to exposure assessment especially of FENPs in the environment. The objective of this study is to assess the fate of FENPs in aquatic environment. Functionalized gold nanoparticles (FGNPs) are employed as a proxy for FENPs. We investigated the effects of environmental parameters (pH, NaCl and natural organic matter (NOM) concentrations) on the coagulation kinetics of FGNPs by dynamic light scattering (DLS). In addition, we investigated the effect of size, surfactant type and FGNPs concentration by studying 30 and 100 nm citrate stabilized (CIT30 and CIT100) and 30 nm 11-mercaptoundecanoic acid stabilized (C1130) at concentrations of 5 ppm, 10 ppm and 20 ppm.
First, the coagulation rate of CIT30 and CIT100 showed the same linear relationship with FGNPs number concentration, but the relationship is different for C1130. Second, FGNPs showed high critical coagulation concentration (CCC) in the presence of the NaCl: C1130 (0.2 M) > CIT100 (0.1 M) > CIT30 (0.08 M). FGNPs are thus little influenced in their aggregation behavior by monovalent 1:1 salts. CCCs of CIT30 and CIT100 were similar; hence the particles size does not change the effect of NaCl. Conversely, C1130 showed a much higher CCC than CIT30 and stronger electrosteric repulsion was observed for longer surfactant chain such as 11-mercaptoundecanoic acid. Third, CIT30 and CIT100 aggregated only at pH < 3.0 whereas C1130 aggregated at pH < 5.7. FGNPs are thus stable on a wider range of pH that is not influ-

enced by the particle size. Nevertheless, the FGNPs show fast aggregation only at pH below the pKa of their surface functional acid group. Finally, addition of NOM decreased the coagulation rate of CIT30 and CIT100, but had surprisingly no effect on C1130 even at a NOM concentration of 100 mg/L. This might be attributed to the different bonding mechanisms between the core of the FGNPs and the different surfactants.
Those results indicate that the physico-chemical properties of the surfactant have more significant influence on the coagulation behaviour than the composition and size of the particles or some of the currently considered environmental parameters.

TH 214
Sedimentation and dissolution of nanomaterials in natural waters
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The behavior and fate of nanoparticles needs to be understood as part of their risk assessment. Sedimentation and dissolution are thought to be the main removal processes that are specific for nanomaterials. We studied the sedimentation behavior of silver, silica coated silver, ceria and fullerene nanoparticles. These particles were suspended at three concentrations, 0.5, 2.5 and 10 mg/L in 6 different natural water types. Water types and properties ranged from freshwater to seawater, from <0.1 to 25 mgC/L natural organic matter and from pH 4 to 8. Sedimentation was measured by quantifying the concentration of nanoparticles during a 15 day settling period by ICP-MS (metals) and UV_{vis} (fullerenes). Additionally, the particle size distribution and zeta potential were measured at different time points. In the same aquatic matrices after a 15 day equilibration period, the dissolution was measured by centrifugal filtration at 3 kDa. Results show that the silver nanoparticles capped with polyvinylpyrrolidone were extremely stable even in sea water. In comparison, the other three nanoparticles showed increased aggregation and sedimentation with increasing ionic strength. The relevance of these findings for developing and validating aquatic fate models for nanomaterials is discussed.

TH 215
Ecotoxicological characterization of carbon nanotubes according to standardized procedures used for the assessment of chemicals in the aquatic environment
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Carbon nanotubes (CNT), with an annual world production reaching several hundred tons, are a special category of nanomaterials with exceptional characteristic and numerous applications. Considering their current and future production, it is likely that some of them may contaminate the environment during each step of their life cycle (production, use and disposal), and especially the aquatic compartment which concentrates all kinds of pollutions. Thus, CNT should receive considerable attention as new, unknown and potentially hazardous nanomaterials. Nevertheless, little is known about their potential ecotoxicity, especially on aquatic organisms, with only few studies available.
Investigations of the environmental risk assessment related to the dispersion of Multi-walled carbon nanotubes (MWNT, Graphistrength C100) into the environment, synthesized by ARKEMA, were conducted in the framework of the Joint Research Laboratory NAUTILE (Nanotubes & Ecotoxicology).
In the regulatory scheme of ecotoxicological studies carried out to evaluate the ecotoxicity of a substance (REACH regulation), the approach taken by existing regulations is based on conventional and standardized bioassays and procedures (OECD guidelines). In this context, the potential impact of industrial raw MWNT was thus investigated using organisms belonging to different trophic levels: decomposers (bacteria), primary producers (photosynthetic green algae, Pseudokirchneriella subcapitata), primary consumers (invertebrates Daphnia magna and Crangon crangon) and secondary consumers (fish and amphibians, Xenopus laevis and Danio rerio), using classical ecotoxicological standardized procedures used to evaluate chemicals in the aquatic media.
The results indicated growth inhibition in amphibians exposed to 50 mg/L of MWNT. No toxicity in fish and daphnia up to 100 mg/L was observed. Toxicity was observed in the marine shrimp according the protocol of the "sea test"(but in the presence of an organic carrier). A notable effect has also been demonstrated at high concentrations with the growth inhibition test in algae (ErC50 = 120 mg/L, NOEC = 10 mg/L).

TH 216
Amorphous nanosilica induce ROS generation and DNA damage in keratinocyte
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Nanometer-size materials, such as amorphous silica particles (nSP), which are used as functional additives in cosmetics and foods, have quickly become essential to daily life. However, concerns over the potentially harmful effects of nanomaterials have been raised precisely because they possess novel properties that are different from those of microsized materials. A sufficient understanding of the relationship between the physicochemical characteristics of nanomaterials governing their toxicity and the identification of factors that influence their associated hazards are essential for the development of safer nanomaterials. Because the main focus of nanotoxicology research has been on hazard identification, however, linkage analysis between physicochemical properties and nanotoxicologies has been long on indicating a need for nanotoxicological study but short on developing the safer nanomaterials. In the light of this situation, the aim of this study was to investigate the relationship between particle size and in vitro hazard of nSP, especially focusing on DNA damage, using human keratinocyte cells (HaCaT). Specifically, we evaluated the relationship between particle size of nSP and the in vitro biological effects. Our results indicate that exposure to nSP of 70 nm diameter (nSP70) induced an elevated level of reactive oxygen species (ROS), leading to DNA damage. A markedly reduced response was observed using submicron-sized silica particles of 300 and 1000 nm diameter. In addition, cytochalasin D-treatment reduced nSP70-mediated ROS generation and DNA damage, suggesting that endocytosis is involved in nSP70-mediated cellular effects. Thus, particle size affects amorphous silica-induced ROS generation and DNA damage of HaCaT cells. Thus, particle size affects amorphous silica-induced ROS generation and DNA damage of HaCaT cells. We believe clarification of the endocytosis pathway of nSP will provide useful information for hazard assessment as well as the design of safer forms of nSPs.
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TH 217
Evaluation of immune modulating effect of orally administrated amorphous silica nanoparticles
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Summary:
Recently, the development of nanomaterials (NMs) with particle sizes below 100 nm is promoted extensively. These NMs have been already used in various applications such as cosmetics, medicines and foods, and become essential to our daily life. Thus, the exposure against NMs are unavoidable for us. Under this circumstance, there is increasing concern regarding the potential health risks by the unique mechanical properties of NMs. In most cases, however, the safety evaluation of NMs has been insufficient for ensuring their safety. In this regards, using amorphous nanosilica particles (nSPs), we are studying relationship between in vivo/in vitro distribution of nSPs and hazard. Because of nSPs has been frequently used as food additive, here, we examined in vivo distribution and biological influences of orally administered nSPs. We used 3 various sizes of nSPs with diameters of 30, 300 or 1000 nm (nSP30, nSP300 or mSP1000). Firstly, mice were orally exposed with nSPs for 28 days and we performed absorption test of nSPs by transmission electron microscopy (TEM) analysis. As a result, only nSP30 migrated to blood through gut and reached spleen. This fact suggested that nSP30 influence immune system. Next, to evaluate immune-modulating effect of nSPs, mice were orally exposed to ovalbumin (OVA) plus nSPs for 4 times at weekly interval, and the levels of OVA-specific response were determined. We found that oral exposure of OVA plus nSP30 tended to induce higher level of OVA-specific immunoglobulin (Ig) G and OVA-specific release of IFN-γ from splenocytes than OVA plus nSP300 or mSP1000. We also demonstrated that the levels of OVA-specific IgE levels causing type 1 allergy were enhanced in mice exposed to OVA plus nSP30. These results indicated that migration of nSPs below 100 nm to spleen could induce immune-modulating effect such as food allergy. Now, to create safer forms of nSPs, we investigate immune response related to physicochemical properties of nSPs. We believe that these data should provide basic information that can help risk analysis of NMs.
[Acknowledgement]: This study was supported in part by Grants-in-Aid for Scientific Research from JSPS; by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan; by a Global Environment Research Fund from Minister of the Environment; by Food Safety Commission; by The Cosmetology Research Foundation; by The Smoking Research Foundation; by The Takeda Science Foundation.

TH 218
Evaluation of biological response against various types of carbon nanotubes
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Summary:
Nanomaterials acquire revolutionary functions depending on nano size. Recently, Nanomaterials are applied to various fields as innovative materials. Particularly, carbon nanotubes (CNTs) begin to use in car parts and TV display, therefore, CNTs are close for our life. On the other hand, CNTs has filamentous shape like asbestos which induce mesothelioma. Because of this, it is thought that CNTs may have some problems. However, the knowledgement about safety of CNTs is still not enough.
Consequently, we evaluated the biological response, such as cytotoxity, DNA damage and inflammation by different-sized multiwalled CNTs (MWCNTs) and single-walled CNTs (SWCNTs). There is insufficient report about biological response against different-sized and shaped CNTs previously.
Macrophage cells, alveolar epithelial cells and mesothelial cells have possibility that exposure to CNTs, when human inhale it. At first, we examined cytotoxicity of CNTs on these cells. As a result, CNTs induced cytotoxicity in all cells. DNA damage caused by CNTs was evaluated by Comet assay. MWCNT caused stronger DNA damage than SWCNT, and the long and thick size/shape of MWCNT have significantly induced DNA damage. We then examined the CNTs-induced inflammatory responses. All of CNTs induced IL-1_ production in human macrophage cells. For further investigation of inflammation, C57BL/6 mice were intraperitoneally administered with various kinds of CNTs and counted total cell numbers in abdominal lavage fluid. As in the case of Comet assay, the long and thick size/shape of MWCNT-exposed mice exhibits increased total cell numbers. These results indicate that long and thick MWCNT are likely to cause severe biological effects and may lead to be the augmentation of carcinogenicity.
In order to management the risk of CNTs, we will investigate the kinetics in details and modify the CNTs to safety form at a future date.
[Acknowledgement]: This study was supported in part by Grants-in-Aid for Scientific Research from JSPS; by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan; by a Global Environment Research Fund from Minister of the Environment; by Food Safety Commission; by The Cosmetology Research Foundation; by The Smoking Research Foundation; by The Takeda Science Foundation.

TH 219
Genotoxic effects of nano-sized TiO2 particles in isolated leukocytes and fibroblasts. A comparison between human and dolphin (Tursiops truncatus) susceptibility.

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Many studies on NPs toxicity have been focused on their potential effects on human health, but scarce information are available on the susceptibility of endangered species, such as marine mammals. A possible accidental way of exposure to NPs during their industrial production is the skin; TiO2 NPs, present in solar creams are able to migrate to the corneal tissue and known to interfere with the immune system. This study is aimed to assess the profitability of isolated leukocytes for evaluating the potential genotoxic effects of NPs on toothed cetaceans and human lymphocytes and fibroblasts. The bottle-nose dolphin Tursiops truncatus was selected as study species being commonly reared in captivity. Previous studies had underlined a different susceptibility of human and dolphins leukocytes to pollutant exposure. In order to make a comparison human and dolphin blood, human (HuDE), murine (3T3) and dolphin fibroblast isolated from skin biopsies were used in this study. Blood samples from four males and a female specimens were achieved by the Adriatic SeaWorld "Oltremare"(Riccione, Italy). Human blood samples were taken from two healty male volunteers (24-30 years old), and processed as follows. Leukocytes were isolated by the lyses procedure and exposed in vitro to TiO2 (rutile and anatase) Nps.
Three experimental times of exposure (4, 24, 48h) and three doses (20, 50, 100 µg/ml) were tested. Genotoxicity was assessed by the Comet Assay, detecting DNA strand breaks. Cell viability was also assed by the Trypan Blue test. In order to assess aggregation, particles were observed with a Jeol 100 SX transmission electron microscope (TEM). Transmission electron micrograph showed that anatase and rutile particles coalesced in RPMI solution, forming variously sized aggregates after sonication, likely explaining a comparable behaviour of the two compounds both in dolphin and human cells. Results of the Comet assay showed that both the crystalline forms of TiO2 induced DNA fragmentation on a group basis. A marked variability among specimens was also detected. An higher susceptibility to TiO2 NPs exposure was assessed for dolphin leukocytes in comparison with human cells, speaking in favour of a particular attention to be paid to cetaceans endangered species .

TH 220
Biological effects of exposure to engineered nanoparticles in Saccharomyces cerevisiae
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Nanoparticles (NP) are particles of mater between 1-100 nanometers (nm). They exhibit different properties compared their bulk form due to the large surface to volume atoms ratio, and therefore have extensive application in many fields such as cosmetics and medicine. However, the small size of nanoparticles, comparable with the molecular machinery of cells, may affect normal physiological functions of cells and cause cyto- and genotoxicity and thus pose unknown risks to human health. The overall goal of our research is to better understand those potential adverse biological, and health effects that accumulation of nanoparticles may cause after chronic exposures. In this regard the biological effects of exposure of the yeast Saccharomyces cerevisiae to the nanoparticles TiO2, ZnO, CuO, single walled carbon nanotubes and Ag are assessed. The findings are then compared to the effects of exposure to the respective bulk form of the nanoparticles. The nanoparticles are characterized and their potential cyto- and genotoxicity are studied using a green fluorescent protein modified yeast-based screening system. The interaction between cells and nanoparticles are then analyzed more in detail using transmission electron microscopy (TEM) and finally the reasons for the potential toxicity (i.e. DNA breakage or oxidative stress) is studied by using comet assay and cell-free reactive oxygen production assay.
Our results show that TiO2-NP, ZnO and CuO-NP all show high cytotoxicity while Ag shows both cyto- and genotoxicity. At concentration of 31,25 mg/l, the nanoparticles do not produce any significant amount of reactive oxygen species. The TEM images confirm our preliminary data and show that the effects of the nanoparticles differ from each other. The main effect seems to be on the increase, and changes in size and shape of the yeast cell vacuoles. The cell walls are not broken due to the exposure, suggesting that the nanoparticless succeed in entering the cells despite the rigid cell wall and lack of endocytosis. Difference in cell wall size and presence of lipid droplets are also seen in the images. The TiO2 and ZnO bulk material are not cytotoxic, while CuO-bulk showed some genotoxicity. In conclusion, the nanoparticles affect yeast cells extensively, with more cytotoxicity than genotoxicity. More research is needed to understand the reasons for toxicity and the localization of the nanoparticles inside the cells.

TH 221
Cell-nanoparticle interactions and cytotoxic effects in human lung cells depend on particle properties and aggregation state
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The rapid increase and wide distribution of engineered nanomaterials, occurred in the last decade, suggest that human exposure to nanoparticles (NPs) is almost unavoidable. Thus the interactions with the biological systems and the potential adverse health effects must be better understood. Inhalation represents the main exposure route for humans, both in outdoor and indoor environment, but also the application of NP-containing consumer products has to be carefully evaluated. Metal oxides are a class of nanomaterials abundantly used in several industrial sectors and high concentrations of these NPs have been monitored in the sites surrounding factories. Previous in vitro studies confirmed metal nanoxides as powerful cytotoxic agents, thanks to their capacity to induce oxidative stress and DNA damage. Moreover, differences in particle size, aggregation and chemical properties may determine the extent of the cellular reactions to NP exposure and the mechanism by which NPs are taken up by epithelial cells.
This study aimed to associate the main cytotoxic effects induced on human lung epithelial cells (A549) with the physico-chemical properties and hydrodynamic behaviour of CuO and TiO2 NPs. After being characterised by TEM and DLS, NP suspensions were administered to cell cultures. Biochemical and morphological analyses were performed to evaluate stress responses and the cellular mechanisms involved in cell-particle interactions at different exposure times. CuO strongly diminished cell viability in a dose- and time-dependent manner. Metal ions dissolution contribute to the cytotoxicity but does not completely explain the high mortality observed. The effects of CuO were promoted by a rapid internalization of NPs, either surrounded by vesicles or free in the cytoplasm. Ultrastructural alterations were soon visible, mainly at cell membrane level, and oxidised lipids suggested that membrane systems may constitute a primary target.
In contrast TiO2 -treated cells resulted engulfed by particles internalised by endosomal vesicles but this phenomena failed to affect viability and cell ultrastructure. Besides the extremely high oxidative potential of Cu2+, these findings pointed out that the NP size and surface charge, coupled with the level and modality of particle aggregation, play a pivotal role in establishing the endocytic pathway, and thus the subcellular NP localization, which ultimately lead to different toxic potential.

TH 222
Effect of detonation nanodiamonds on fungi
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Nanodiamonds (NDs) are already widely used in various industries. But their bioactivity to different components of environment is still unknown. The goal of this work is to investigate the bioactivity of detonation NDs towards fungi by measuring growth of mycelia and spectral characteristics of micromycete culture fluids. Two samples of Ural NDs (size of free particles 15 and 100 nm) manufactured at the company "SKN"(Snezhinsk, Russia) were investigated. The effect of NDs (at 0.05 mg/ml) on fungi (Alternaria arternata, Cladosporium cladosporioides and Fusarium oxysporum) was assessed by comparing changes in biomass and spectral characteristics of metabolites released by fungi into growth medium treated with NDs and without NDs (control cultures).
Fluorescence emission spectra and quantum yield QY of filtered culture fluid were measured by luminescence spectrometer Solar CM 2203 under excitation at 270, 310 and 355 nm. Absorption spectra were measured using spectrophotometer Unico within spectral range 200[3DOTS]900 nm. It was found that the growth of fungi depends on size of nanoparticles. Maximum mycelia biomass observed at 15 nm NDs. More remarkable this effect was on the light-colored fungi. Dark-colored species of weakly respond to the virtues of nanodiamonds. Growth of mycelia biomass of A. alternata and Cl. cladosporioides correlated with the optical density of culture fluid. Addition of NDs to nutrient medium causes more rapid culture growth and hence higher concentration of metabolites. Shift of fluorescence emission maximum towards longer wavelengths, decrease of fluorescence QY may indicate the formation of larger aggregates of fluorescing metabolites.
The results of experimental investigation demonstrated that :1) NDs of 15 nm size are more effective on the growth of fungal biomass stimulation than NDs of 100 nm; 2) light-colored fungi are more sensitive to NDs than dark-colored species;3) the presence of NDs reduces the fluorescence quantum yield of fungal metabolites in culture fluid, which is associated with an increase in size of the macromolecules in the medium; 4) increase in the size of the macromolecules due either to the fact that the fungi in the presence of NDs emit exometabolites of higher molecular weight, or with the adhesion of metabolites on the surface of NDs (without depending on the size of NDs).
The research was supported by the Russian Education and Science Ministry (GK 02.740.11.06993)

NM03 - Risk assessment and risk management of nanomaterials

TH 226
Assessing the risks for aquatic organisms posed by waterborne copper and silver nanoparticles
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The increasing use of engineered nanomaterials in numerous industrial applications and consumer products are including the potential risk for human health and the ecological sustainability. The purpose of this study was to develop a risk-based probability model to predict the risks of nanoecotoxicity toward aquatic organisms posed by waterborne copper and silver nanoparticles (Cu/Ag NPs). Published experimental evidence based on Cu/Ag NP-zebrafish (Danio rerio) systems was adopted as the study data. Toxicodynamic based Hill model was used to reconstruct a concentration-mortality response profile. To estimate the exposure thresholds, the Weibull predictive model was employed. The derived probabilistic model can predict the risk of environmentally relevant Cu/Ag NPs for major Taiwanese rivers with predicted environmental concentrations. The results indicated that estimated thresholds of 95% confidence interval (95% CI) were 0.10~0.48 mg L^{-1} for Cu NPs and 2.69~2.73 mg L^{-1} for Ag NPs. The probabilities of a risk quotient (RQ) of > 1 ranged 17%~81% for zebrafish exposed to Cu NPs. This study found that Ag NPs exposure scenarios had no significant risks to zebrafish (RQ << 0.1). Results of this study imply that zebrafish can be used as a suitable bioindicator to monitor nanoecotoxicity of environmentally relevant Cu/Ag NPs that pose potential adverse effects toward aquatic organisms.

TH 227
Comparative toxicity of silver nitrate and silver nanoparticles to representative species of an aquatic trophic chain
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Nanotechnology field is growing with consequent release of manufactured nanomaterials and nanoparticles to the environment. Silver NP's are used as antibacterial agents in biotechnology and bioengineering as well as in textile industry and consumer products. In this study we compared the sensibility of thee different aquatic species to silver nitrate and silver nanoparticles and aimed to relate differences in toxicity to the differences in particle sizes and processes of uptake and distribution inside the organisms. Growth inhibition bioassay with the green algae Pseudokirchneriella subcapitata was performed according to the OECD 201 guideline with adapatations. Immobilisation assays with Daphnia magna was conducted under the OECD 202 guideline (OECD, 2004). Feeding inhibition assessment of Daphnia magna was conducted according to the methodology described in McWilliam and Baird (2001) and reproduction assay was conducted following the OECD 211 guideline (OECD, 1998). The zebrafish (Danio rerio) assay was in accordance with the guideline on fish embryo toxicity (FET) test (OECD, 2006). Zebrafish embryo were collected within 30 minutes after natural mating and selected under a stereomicroscope for the suitability of the eggs. The endpoints assessed were the number of coagulated eggs, irregularities in somite formation, tail detachment and the heart beat of larvae. We found no significant differences on the inhibition of growth of P. subcapitata exposed to silver nitrate and silver nanoparticle. Daphnia magna neonates were more sensitive to AgNO3 than to the AgNP, with a 48h-LC50 value ten times greater for AgNP. Fish embryo development was also studied under exposure to AgNO3 and AgNP and abnormalities were observed in embryo exposed to silver when compared to the control animals. The hatching rate was decreased in silver exposed embryo, and among injuries observed were abnormal notochord development, edema and weak heart beating rates. In conclusions, we addressed some of the effects of silver nanoparticles to three different aquatic species and it was possible to observe differences in toxicity between AgNO3 and AgNP. However, we believe that the toxicity profile of these compounds

would be better understood under the investigation of their uptake and distribution inside the organisms, which is an ongoing work.

TH 228

Long-term effects of iron-based nanoparticles at population level (Project Fe-NANOSIT)

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General aim of the project Fe-NANOSIT is the development of novel methods for the purification of contaminated ground- and industrial wastewaters based on nanoparticles (NPs) and nanocomposit structures. The project includes (i) the development and production of reactive NPs and nanocatalysts, (ii) detailed particle characterization, (iii) demonstration of the technological solutions at field scale, and (iv) detailed risk assessment following a multi-tiered approach from the cell to the population level.

For a risk assessment at population level, we apply the automated test system NANOCOSM, which analyses populations of Daphnia magna. The abundance and size structure of populations are quantified by image analysis. The NANOCOSM system allows the investigation of stressed population in the presence of intraspecific competition, which is a very important factor involved in the dynamics of natural populations. Hence, the NANOCOSM system enables a highly sensitive monitoring of long-term effects and recovery processes after exposure to NPs.

TH 229

Toxicity review on Al2O3, TiO2, ZrO2, SiO2, MWCNT and CeO2 nanoparticles

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The LCA & Ecodesign laboratory of ENEA is in charge of the assessment of nanofluid coolants impacts on the environment, health and safety (EHS), across their entire life cycle, for NanoHex (enhanced nano-fluid heat exchange) project (www.nanohex.eu). The NanoHex project, financed by Seventh Framework Programme for collaborative projects, is developing a high-performance coolant for adoption by industry in heat exchange systems using the enhanced thermal properties of nanofluids (NFs). The promising laboratory based nanotechnology results are translating into pilot-lines for the cooling of electronics primarily targeting Data Centres & Power Electronics.

The EHS assessment is being performed by the joint application of Risk Assessment (RA) and Life Cycle Assessment (LCA). The choice of the methods results from two main reasons: the recommendations in the Action Plan 'Nanosciences and nanotechnologies: An action plan for Europe 2005-2009' and the issues related to the sustainability of the emerging technologies (Sustainability Assessment of Technologies -SAT).

The article presents the results of a literature review on toxicity of the candidate nanoparticles (NPs) for NanoHex application in NFs, in order to consider the prevention and reduction of possible impacts of the new materials as well as technological performance.

Available studies of scientific community are not sufficient to characterize the effect of NPs and define a "hazard hierarchy"between different NPs because, at date, the difference among the procedures and results of toxicity tests are too broad. Nevertheless, some useful information about the toxicity can be drawn by the survey proposed in this work to consider impacts related to the toxicity besides the enhanced thermo-hydraulic properties of NFs based on NPs.

Further investigations are required to understand the behaviour of NanoHex selected NFs starting from the main NPs parameters (physical-chemical characteristics of NPs such as dimension, shape, crystalline form, z-potential and type of experimental tests such as in vivo or in vitro).

TH 230

Designing environmental friendly nanoparticles with Nano-QSAR

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New approaches that concern the study of materials having at least one dimension below 100 nm, called nanoscience and nanotechnology, are rapidly expanding. Particles in these size ranges, may exhibit properties drastically different from their larger counterparts having the same chemical composition. This enables novel, profitable applications of nanoparticles.

Together with an increasing role of nanotechnology in our every-day-life, we can also expect increasing emissions and increasing levels of novel nanoparticles in the environmental compartments. Thus, development of novel techniques that enable to design environmentally safe nanoparticles is of high interest.

Novel, fast and inexpensive procedures for designing nanoparticles with the expected properties as well as for performing a comprehensive human and environmental risk assessment can be developed without the need of extensive empirical and animal testing. Instead, a group of Quantitative Structure-Activity and Structure-Property Relationships methods (QSAR and QSPR, respectively) can be employed.

In this presentation we will demonstrate new contributions of our group to Nano-QSAR and Nano-QSPR methodology. The presentation will be focused on the four priority areas: (i) collecting and curating available experimental data; (ii) developing appropriate nano-structural descriptors; (iii) studying interactions between nanoparticles and biological systems; (iv) existing and newly developed Nano-QSARs and Nano-QSPRs.

TH 231

Iron-based nanoparticles and nanocomposit structures for removal of contaminants in ground- and industrial wastewaters (Project Fe-NANOSIT)

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General aim of the project Fe-NANOSIT is the development of novel methods for the purification of contaminated ground- and industrial wastewaters based on nanoparticles (NPs) and nanocomposit structures. The project includes (i) the development and production of reactive NPs and nanocatalysts, (ii) detailed particle characterization, (iii) ecotoxicological risk assessment and (iv) demonstration of technological solutions for the remediation of contaminated water recourses at field scale. Studies of possible environmental hazards caused by the particles play an important role since a sustainable technology development can only be warranted when risks for the environment can be excluded or fully controlled.

Groundwater treatment:

For the purification of contaminated groundwaters, a nano-structured composite material has been developed which combines the reactivity of zero-valent iron deposits with the sorption properties of the colloidal activated carbon carrier (Carbo-Iron). Carbo-Iron is applicable for injection into contaminated aquifers in order to form sorption/reaction barriers by controlled subsurface deposition directly within the flow passages of the contaminated water. Carbo-Iron's properties have been designed to target both: plume control and source remediation.

Waste water treatment:

Reactive nanocatalysts are studied for two target applications in the treatment of industrial wastewater: the Pd-catalyzed hydrodehalogenation as reductive treatment and particle-catalyzed Fenton-like oxidation of persistent non-halogenated pollutants as well as oxidation using magnetic photocatalysts. Highly-active Pd/magnetite and Fe-based magnetic nanocatalysts are designed, characterized and tested for their performance in the target reactions. All of the particles are supposed to be removed from the treated water streams and are subjected to recycling and regeneration processes.

Before implementing the particles into any technological development process, all of the particles under study are included into a strict risk assessment scheme which will be described in detail in this presentation.

TH 232

A proposed protocol for estimating the potential release of nanoparticles from nano-based coatings: the NANOHOUSE project

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Nanotechnologies utilizing engineered nanoparticles (ENPs) have been envisaged to become a flourishing industry with an expected annual turnover over 1.5 trillion euros by 2015 (Savolainen et al., 2010). "The Project on Emerging Nanotechnologies"has estimated that more than 1000 nano-products are marketed now, with a new entry every 3-4 week (http://www.nanotechproject.org). ENPs, since their unique features, are applied in many fields and products (e.g., textile, coatings, composites, medicine, etc.). An inevitable consequence of this constantly using of nano-based products is the potential human and environmental exposure to ENPs (Nowack and Bucheli, 2007; Kaegi et al., 2010). However, although the toxicity of some ENPs has been widely studied in both *in vitro* and *in vivo* assays, there still is a lack of information concerning ENPs release from nano-based products. Hence it is necessary to estimate their potential releases throughout the whole product life cycle.

Within the FP7 EU-funded NANOHOUSE project (*Life Cycle of Nanoparticle-based products used in house coating*; www-nanohouse.cea.fr), aiming at developing appropriate solutions for a safe and sustainable use of nano-based coating according to Life Cycle Thinking, the potential release of ENPs from selected nano-based paints by "wet route"is being evaluated. In detail, within the WP2 - Source identification, an experimental protocol has been proposed to estimate release from selected paints containing TiO_2, Ag and SiO_2 ENPs. The proposed protocol has been defined taking into account literature results, available standards (e.g.. ISO, ASTM) and input from project partners. In this work, the experimental protocol will be presented and discussed, highlighting: (i) type of panels coated with nano-based paints and their initial conditioning; (ii) pre-treatment of panels to simulate their weathering (i.e. accelerated weathering tests such as Taber test and UV exposure); (iii) leaching test applied on weathered and un-weathered panels to verify possible ENPs release; (iv) characterization methods and techniques needed to investigate ENPs in collected leaching liquid samples. Moreover, a special attention will be paid to available leaching standards able to estimate migration rate of ENPs, and characterization techniques/methods necessary to quantify ENPs release.

TH 233

The safety assessment of amorphous nanosilica on developmental and reproductive toxicity

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Recently, many nanomaterials (NMs) with innovative functions have been developed and already used in several fields. However, knowledge concerning the potential safety of NMs is still fragmentary. Especially, few studies have examined the effect of NMs on maintenance of pregnancy. Therefore it is essential to examine the effect of NMs on fetuses and pregnancies. In this study, we examined the reproductive toxicity of nanosilica particles with different size and surface modification for developing NMs with safety. Pregnant BALB/c mice were treated with silica particles with a diameter of 70, 300 or 1000 nm (nSP70, nSP300 or mSP1000, respectively) via intravenous injection. At first, we examined the influence of silica particles on fetuses. nSP70-treated pregnant mice showed the increase of fetal resorption rates and decrease of fetal weights compared to control mice, suggesting that nSP70 induced the miscarriage and fetal growth restriction. In contrast, nSP300 and mSP1000 did not induce these pregnant complications. Furthermore, we showed that nSP70 with functional COOH or NH2 surface modification group did not induce miscarriage and fetal growth restriction. These results suggest that appropriate surface modification suppresses these pregnancy complications. Next we attempted to clarify the mechanism of miscarriage and fetal growth restriction induced by nSP70. Sections of placentas were stained with hematoxylin and eosin. We observed variable structural abnormalities of placenta in nSP70-treated mice, although placentas of nSP300- and mSP1000-treated pregnant

mice did not show any significant abnormalities compared to control mice. These results suggest that nSP70 induce the structural abnormalities to placenta. Therefore we consider that the dysfunction of placenta is one mechanism of pregnant complications induced by nSP70. We believe that the knowledge obtained by these studies might provide basic and useful information for developing NMs with safety.
[Acknowledgement]: This study was supported in part by Grants-in-Aid for Scientific Research from JSPS; by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan; by a Global Environment Research Fund from Minister of the Environment; by Food Safety Commission; by The Cosmetology Research Foundation; by The Smoking Research Foundation; by The Takeda Science Foundation.

TH 234

Risk-oriented characterization of engineered nanoparticles

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It has been recognized that production and use of engineered nanoparticles (ENPs) are addressed by the EU REACH Regulation n. 1907/2006 that gives industries the responsibility of the Chemical
Safety Assessment (CSA) for any produced or imported industrial substance. The required CSA follows the traditional Risk Assessment (RA) framework for chemicals, including Hazard Assessment, Exposure Assessment and Risk Characterisation steps. Since the application of the traditional RA framework to ENPs is hampered by methodological concerns and data gaps, a novel approach for RA and prioritization of ENPs, including uncertainty evaluation, is being developed within the FP7-funded ENPRA project. The whole procedure will follow the Weight of Evidence (WoE) approach and will integrate experimental/estimated exposure and effect data provided by project partners or available in the literature that refer to a panel of 9 ENPs including titanium dioxide, zinc oxide, silver and carbon nanotubes. Among the experimental data required for RA purposes, the physical and chemical characterization of ENPs seems to be a critical point, on the basis of available literature data. Only a correct combination of adequate measurement techniques allows adequately characterizing a certain ENP and therefore avoiding the misinterpretation of the toxicological tests to be carried out for RA purposes. The set of techniques and needed information strongly depends on the investigated ENP, and only an expert evaluation allows their adequate selection and application. Additional critical issues are associated with the absence of reliable particle standards, the lack of reproducible administration protocols and the performance of a 'secondary' characterization of ENPs (i.e. behaviour under real conditions) in biological media during and after administration. Within the ENPRA project many efforts are addressed to investigate and solve these open issues within a RA perspective.

RA05 - Integrated science: Key to risk assessment

TH 238

To contract a pyrene-based physiologically based pharmacokinetic (PBPK) model for predicting urinary 1- hydroxypyrene (1-OHP) levels in human

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The objectives of this study are to characterize the concentration-time profiles in target organs and tissues by a pyrene-based physiologically based pharmacokinetic (PBPK) model, and to collect the published data of metabolism biomarkers (urinary 1- hydroxypyrene (1-OHP) levels) for model validation. We adopted the pyrene data from Tsai et al. (2004) for PAHs concentrations measured at highway to all station to account for the traffic pollution sources. The variation of urinary 1-OHP levels between post-shift and pre-shift exposure were measured. The input pyrene concentration and 1-OHP concentration of exposure groups were 105 ng m-3 and 2.12 μmol of 1-OHP/mol of creatinine, respectively. For simulating the inhalation pharmacokinetics of pyrene, we use a basic human compartment structure that has been previously used by Chiang and Liao (2006). The tissue compartments included in the model are: alveolus, lung, richly perfused tissues (brain, gut, kidney, spleen, and heart), fat, slowly perfused tissues (bone, muscle, and skin), and liver. Each tissue compartment is interconnected by arterial and venous blood. The PBPK model will be verified by comparing the predictions of urinary 1-OHP levels with the measured published data. The results shows the simulated highest time-course concentrations of pyrene were in fat (4.32[GREEKX]10^{-5} mg/l blood) at day 5, followed by rapidly perfused tissues (6.04[GREEKX]10^{-6} mg/l blood), slowly perfused tissues (3.56[GREEKX]10^{-6} mg/l blood), liver (1.34[GREEKX]10^{-6} mg/l blood), blood (3.83[GREEKX]10^{-7} mg/l blood), and lung (6.64[GREEKX]10^{-7} mg/l blood). Model validation by t-test statistical method shows that there is no significant difference between model prediction and experimental 1-OHP concentration in urinary.

TH 239

Assessing arsenic exposure and influenza A (H1N1) infection-associated lung function exacerbations risks

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Arsenic (As) is ubiquitous in the environment and known to lead to many human health problems related to pulmonary diseases. Recently, the animal experiment showed that As would significantly increase influenza viral titer. In this study, we are trying to link arsenic exposure and influenza A (H1N1) infection-associated lung function exacerbation and assess the risk based on mechanistic models. First, we apply the homogeneous Poisson process to obtain the relationship between viral load and percent predicted forced expiratory volume in the first second (FEV_1). Then, we construct the viral load-percent predicted FEV_1 and arsenic exposure-percent predicted FEV_1 relationships to link the As exposure and viral load. The result shows that 625 μg L^{-1} As increment leads to 1 viral load (log TCID50 mL^{-1}) increment. Second, we apply the Hill model to best fit the relationship of viral load and respiratory symptoms scores (RSS). Finally, a Hill-based model is also adapted to construct the response of RSS as the function of As exposure and influenza A (H1N1) infection. Given the amount of viral load in a specific day post infection (PI) and site-specific arsenic distribution, we could estimate the change of RSS and assess the risk under different levels of RSS. Our results show that West Bengal (India) has the most severe lung exacerbation risk due to the highest As concentration distribution (RSS: 0.11-0.51 (Day 0 PI); 0.28-0.76 (Day 1 PI); 0.60-0.91 (Day 2 PI)) followed by Taiwan, USA, Mexico, and Brazil, respectively.

TH 240

Lead human exposure to industrial emission and health risk assessment for selected area of Casablanca: a pilot phase in Morocco

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INERIS (French National institute for industrial environment and risks) methodology is used here to calculate the human health risk associated with lead emission exposure, from lead fondry and Battery Manufacturing Plants located in two communities namely: Sidi Bernoussi and Ain Sebaa in the region of Casablanca.
Inhalation and ingestion of lead-contaminated dust are identified as major exposure routes. Used Human Toxicity Values were chosen based on the first 3 Data bases (WHO, ATSDR, EPA). Data from sources and lead emission were obtained according to two approaches: actual direct measurement and modelling. Thus, four scenarios were built: Population of Sidi Bernoussi bordering the Battery Manufacturing Plants within a distance of 100 m, 450 m and 500 m and the general population located at 1,8 km of the sources of pollution. For the four scenarios, we calculated the Hazard Quotient (HQ) for the noncarcinogenicity effects and assessment of Unit Risk for Carcinogenic effects. Probabilistic risk assessment is also conducted to account for uncertainty and variability in the analysis.
For the noncancerogenic effects, ingestion of deposit lead was only considered for specific sub group of population (6 years of age and less).
In the absence of national blood lead surveillance program, Blood Lead Level (BLL) modelling Methods was performed to predict results in child.
Among the four scenarios considered, the calculated risk values were several times higher than the generally acceptable risk mainly in children from a shantytown. Effort must be taken to implement screening strategies to reduce childhood lead exposure.

TH 241

Integrating General knowledge and an understanding of basic science to evaluate environmental risk assessments predictions through compiled non-statistical relevant scientific data

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Developing countries normally have a problem in conducting risk assessment studies due to lack of an understanding of the importance of data collection. The strategy of data collection is often done in a half hazard manner, lacking proper sampling and understanding of the study as a whole. Correlations of relevant stresses is generally avoided and unattended in regulatory and policy issues, to give the risk assessment studies more meaning and relevance to possible solutions to be implemented. The case study of such ignorance and collection of unscientific data in issues related to the agriculture practiced in Punjab (INDIA).

TH 242

Substance flow analysis for micropollutants management in urban watersheds

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Micropollutants released by cities into water are of increasing concern as they are suspected of inducing long-term effects on both aquatic organisms and humans. Substances found in the urban water cycle have different sources in the urban area and different fates in this cycle. For example, the pollutants emitted from traffic, like copper or PAHs get to surface water during rain events often without any treatment. Pharmaceuticals resulting from human medical treatments get to surface water mainly through wastewater treatment plants, where they are only partly treated and eliminated. Once in the receiving waters (lakes, rivers, groundwater), these substances may re-enter the cycle through drinking water. It is therefore crucial to study the behaviour of micropollutants in the urban water cycle and to get flexible tools for urban water management. Substance flow analysis (SFA) was recently proposed as instrument for water pollution management in urban water systems. In this study, we propose to test the application of surface flow analysis for various classes of micropollutants to evaluate its application to urban water management. We chose the city of Lausanne as case study since the receiving water of this city (Lake Geneva) is an important source of drinking water for the surrounding population. Moreover a profound system-knowledge and many data were available, both on the sewer system and the water quality. We focused our study on some heavy metals, and some pharmaceuticals. For copper, the results show that around 1500 kg enter the aquatic compartment yearly. This amount contributes to sediment enrichment, which may pose a long-term risk for the benthic organisms. The major sources (total of 73%) of copper in receiving surface water are roofs and contact lines of trolleybuses. Thus technical solutions have to be found to manage this specific source of contamination. Application of SFA approach to pharmaceuticals reveals that combined sewer overflows represent an important source of contamination. These results will help in defining the best management strategy to limit Lake Geneva contamination. SFA is thus a promising tool for integrated urban water management.

TH 243

A novel risk assessment approach for the prioritisation of emerging substances and its application to 500 organic microcontaminants

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Given the huge number of chemicals in commerce, there is a need of prioritising chemicals for risk assessment and monitoring. This study is the first to assess the risk of 500 organic substances based on observations in the four European river basins of the Elbe, Scheldt, Danube and Llobregat. A decision tree is introduced that first classifies chemicals into six categories depending on the information available. The priority within each category is then evaluated based on two indicators, the Frequency of Exceedance and the Extent of Exceedance of Predicted No-Effect Concentrations (PNECs). These two indictors are based on maximum environmental concentrations (MEC), rather than commonly used statistically based averages (Predicted Effect Concentration, PEC), and compared to the lowest acute-based ($PNEC_{acute}$) or chronic-based thresholds ($PNEC_{chronic}$). For 56% of the compounds, PNECs were available from existing risk assessments, and the

majority of these PNECs were derived from chronic toxicity data or simulated ecosystem studies (mesocosm) with rather low assessment factors. The limitations of this concept for risk assessment purposes are discussed. For the remainder, provisional PNECs (P-PNECs) were established from QSAR models for acute toxicity to the standard test organisms *Daphnia magna, Pimephales promelas* and *Selenastrum capricornutum*. The prioritisation revealed that about three-quarter of the 44 substances with MEC / PNEC ratios above ten were pesticides.
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TH 244
Environmental assessment for pharmaceuticals under the National Environmental Policy Act
EA Mcvey
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The fate of pharmaceuticals in the environment has been studied for more than 50 years, with the presence and potential effects acknowledged shortly thereafter (Soulides et al. 1962; Voets et al. 1976; Peele et al. 1981; Lee & Arnold 1983; Grimes et al. 1984). It has gradually become apparent that risk assessments developed for the usual chemical contaminants cannot be applied carte blanche to pharmaceuticals, because they are developed to be highly active and specific in biological systems at low levels (Sybesma 1992; Stan & Heberer 1997; Halling-Sorensen et al. 1998; Daughton & Ternes 1999). Therefore, when applying risk assessment models to the environmental assessment of pharmaceuticals, regulators must take into account not only the complexity of the entity to be protected (the ecosystem at large), but also the complexity of the regulated article (pharmaceuticals).
Under the United States' National Environmental Policy Act (NEPA), before performing any action, a federal agency must assess the environmental impact of that action by preparing an Environmental Assessment (EA), followed by a Finding of No Significant Impact (FONSI), or an Environmental Impact Statement (EIS). An EA is a "concise"(i.e. 10-12 pages) document that assesses the potential environmental impact of an action. From the EA, regulators then determine whether a more in-depth EIS is required, or whether there can be a FONSI. If it is determined that an EIS is needed, a longer process begins to create a (in most cases) massive document which takes into account all possible environmental impacts of an action, including cultural and social impacts and those relating to environmental justice. The Act encourages multidisciplinary approach to risk assessment, by requiring this very broad and extensive view of environmental assessment, and by encouraging cooperation between various Federal entities to create an EIS.

TH 245
Using 'intelligent testing' ideas to design a targeted fish full life-cycle test: a case study
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For evaluating the potential ecotoxicity to fish of human pharmaceuticals, particularly potential 'endocrine disruptors', a fish full life-cycle test is deemed to be the "gold standard", as it covers all life-stages and measures long-term effects. However, by making the best use of mode of action, preclinical and clinical pharmacological and toxicological data (data generated during drug development from non-human mammals and humans), it may be possible to tailor the experimental design to focus on the most sensitive life-stages and biological endpoints. From an ethical perspective, such considerations have the potential to reduce the number of animals required for testing. In knowing the therapeutic dose and predicted environmental concentration, test concentrations could also be selected such that a range-finding study is not required.
This presentation provides a real example of how the existing data for an active pharmaceutical ingredient, bicalutamide, was used to design a targeted fish chronic study and to focus measurements where effects would be most likely to be seen. The results provided good quality data for risk assessment purposes and the study used approximately half the number of animals normally tested in a fish full life-cycle study and at a considerably reduced cost and time requirement.

TH 246
Non-traditional endpoints put to the test: a question of relevance and reliability
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The apical endpoints of survival, growth, and reproduction have long been the mainstay of environmental toxicology research as they integrate effects at the organism level. Yet, as science progresses the use of biomarker endpoints in early hazard assessment is becoming more common. The challenge is in relating the biomarker with an adverse effect that is relevant and reproducible. Bisphenol A (BPA), is an important high production volume compound used in the production of epoxy resins and polycarbonate plastics. It is also one of the most studied compounds, particularly for potential endocrine interactions. The database of ecotoxicological test results for BPA includes traditional guideline acute and chronic data addressing population level effects of survival, growth and reproduction, as well as a variety of studies with secondary and often unique sublethal or biomarker-type endpoints. For example, a number of highly replicated fish life-cycle and multi-generational tests in both freshwater and marine species have examined growth, development and reproductive success in combination with biomarker endpoints such as vitellogenin, gonadosomatic index, and gonad histology. In addition, analyses of sperm quality, gonad development, vitellogenin, genomic markers, and many other non-traditional endpoints have been reported in the literature, often evaluated in isolation of a link with more traditional endpoints. In determining the utility and relevance of these additional studies and their various endpoints in an environmental risk assessment framework, a clear understanding of the relationship between biomarker and sublethal findings, which may or may not be adverse, to population relevant assessment endpoints must be established and data quality and reliability must be determined. The evaluation of such existing information, including both traditional and non-traditional endpoints should be done in a weight of evidence approach in order to reach a scientifically defensible risk determination. In so doing, potential adverse impacts of the compound can be defined and subsequently used as measures of effects that are directly related to the assessment endpoints for a sound and robust risk assessment. A case study describing this approach for the evaluation and use of both traditional and non-traditional endpoints in a weight of evidence assessment will be presented using examples from the extensive database of fish studies available for BPA.

TH 247
How non-standard test methods and data analysis tools can benefit to ecological risk assessment of chemicals?
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Regulation mostly relies upon the results of standard toxicity tests that have been validated for a reduced set of species and endpoints. This pragmatic strategy ensures the reliability of toxicological data. Yet, current practices might not appropriately cover the broad range of toxicant mode of action (*e.g.,* endocrine disruptors - EDCs). In particular, the standard approach may fail to provide realistic risk forecasts when the test design is not sensitive enough to highlight toxicity and when the various standard tests provide conflicting results.
Through two case studies, we explain why the standard approach may not allow assessing properly the risk of reprotoxic pesticides in invertebrates. The first case study illustrates current issues in risk assessment of EDCs. In this context, we show how non-standard partial life-cycle (PLC) tests allow identifying sensitive development phases/endpoints to EDCs (*e.g.,* anti-androgens) along the life-cycle of *Lymnaea stagnalis*, a candidate species for the development of forthcoming OECD test guidelines. We also show how non-standard data analysis methods, such as mixed effect models, can be used to avoid underestimation of EDCs toxicity. The second case study illustrates how a non-standard approach can contribute to provide complementary data when standard approaches led to conflicting risk forecasts. In this context, we repeatedly exposed *L. stagnalis* to pulses of diquat and monitored life-cycle traits. We show how non-standard full life-cycle (FLC) tests allow assessing effective diquat concentrations more realistically and why non-standard models, such as TK-TD models, are relevant to characterise the time-course of biological responses to complex exposure patterns. For each case study, we show how to derive relevant effects criteria and include them in the regulatory risk assessment process. We then investigate whether including this additional data might modify current risk forecasts for the studied compounds.
This study illustrates how non-standard test procedures of different nature may contribute to ecological risk assessment of chemicals, when standard procedure are lacking or provide conflicting results. Most importantly, the use of some simple but relevant PLC and FLC tests methods with *L. stagnalis* should be promoted when standard approaches are suspected to provide unrealistic risk forecasts.

TH 248
The role of interspecific competition on the response of Daphnia magna to pesticides
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Most of the tests to address toxicity of pesticides are made on experimental organisms, typically Daphnia magna for fresh water, cultured individually. These tests are not realistic and many studies showed the inadequacy of this current practice in ecotoxicology. Animals potentially struggle in interspecific or intraspecific competition, interactions that are likely to be of crucial importance to obtain a response to toxicants' activity. Thus in ecotoxicology a subject for inquiry should be the role such biotic interactions play in determining the response to stressors.
The focus of this study is to understand the importance of competition on Daphnia magna after pesticide applications. Competitions between different species of cladocerans have been already investigated by several ecology studies. Daphnids respond with life history changes to the presence of competitors, which can influence their sensitivity to a chemical stress. However little is known about what the presence of a species of a complete different systematic group can cause. A laboratory mesocosm study on population of Daphnia magna competing with larvae of Culex pipiens showed a delay in recovery after multiple pesticide applications.
To understand the relative roles of the direct influence of competition on vulnerability to a pesticide, versus the influence of life history adaptation, a reproduction test (OECD guideline 211) was also conducted. We investigated mothers (F0) living singly or with three C.pipiens specimens in 50 ml of medium and their offspring (F1). Both were contaminated at the same levels of the pesticide pirimicarb used for the mesocosm experiment. Results showed a delay in growth and reproduction and an increased sensitivity to the contamination for the competition-experienced F0 daphnids, confirming the results obtained in the mesocosm study.
Animals were kept in semi-static test system with high amount of food, thus probably Daphnia magna might have perceived the presence of competitors and then adapted its life strategy at this new condition. What it is now needed to understand is how this change affects the sensibility to contaminants.
The interaction between toxicants and biological interaction is rarely investigated but we proved that interspecific interactions can negatively affect the sensitivity of one of the most common test organism in ecotoxicology. Therefore such interactions must be considered when conducting an ecological risk assessment.

TH 249
Competition delays the recovery from toxicant stress
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Recovery from toxicant stress is important to assess the field relevance of effects. Positive effects on recovery often occur after pesticide-induced mortality relieves competition pressure. We investigated how persistent competition pressure alters effects and the recovery of species.
Model communities of Daphnia (*Daphnia magna*) and Culex larvae (*Culex pipiens molestus*) were pulse exposed (48 h) to the pyrethroid Fenvalerate. Abundance and biomass of aquatic populations were monitored by non-invasive image analysis; adult mosquitoes were counted manually.
Short-term effects due to exposure were increased for both species by intraspecific competition. Long-term effects were exacerbated by interspecific competition: After pulse exposure close to the acute LC50 of 1 µg/L, the recovery of Daphnia was impaired by persistent competition with surviving Culex larvae for 31 days. Culex larvae profited from the slow recovery of Daphnia with an increased emergence until day 47 after pesticide pulse.
We conclude that in natural systems where competition is present, such competitive processes may prolong the recovery of the community structure. Hence, natural communities may be disturbed longer than predicted from mono species tests alone. To achieve a realistic pressure of

competition in test systems at population and community levels, we recommend initiating them with several generation times before contamination.

TH 250

Traits and stress - keys to identify community effects of low levels of toxicants in test systems

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Community effects of low toxicant concentrations are obscured by a multitude of environmental factors and biological interactions. To resolve this issue for community test systems, we propose a trait-based approach to detect toxic effects. A long-term experiment with outdoor stream mesocosms was conducted. It was established 2-year before contamination to allow the development of biotic interactions within the community. Following pulse contamination with the insecticide thiacloprid, communities were monitored for additional 2 years to observe long-term effects. Species were aggregated into trait-based groups that reflected stressor-specific vulnerability of populations to toxicant exposure. This reduced intrareplicate variation and increased the ability to detect toxicant related effects. Species with low intrinsic sensitivity showed only transient effects at the highest thiacloprid concentration of 100 µg/L. Sensitive multivoltine species showed transient effects at 3.3 µg/L. Sensitive univoltine species were affected at 0.1 µg/L and did not recover during the year after contamination. The new indicator SPEARmesocosm was calculated as the relative abundance of sensitive univoltine taxa. It detected long-term effects for five species at concentrations that were 1000 times below the concentration at which long-term effects of thiacloprid were detected by the PRC (Principal Response Curve) approach traditionally applied for mesocosms. We also found that species that were stressed, as indicated by a decline in abundance in control streams, were affected more strongly by thiacloprid than nonstressed species. We conclude that the grouping of species according to toxicant-related traits enables identification and prediction of community response to low levels of toxicants

TH 251

Ecological Risk Assessment of estuarine contaminated sediments: the Exposure-Dose-Response framework

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Sediment Ecological Risk Assessment has often been conducted integrating exposure and effects data using qualitative judgement to weight the different Lines of Evidence. To identify the Lines of Evidence more suitable in evaluating the impairment of sediment environments contaminated by metals, Lines of Evidence have been explored in the Exposure-Dose-Response framework. Data including sediment chemistry, bioaccumulation, toxicity, and biomarkers for molluscs, crustaceans and fish and benthic community assemblages were mined from the literature and from available databases. Data were gathered only for estuarine sediment environments contaminated by copper, cadmium, lead and zinc. The collected data were compared with sediment quality guidelines and explored for relationships within the Exposure-Dose-Response framework. The behaviour of the molar sum of the four metals was investigated for its possible use as a concise indicator of metal exposure and dose and for its relationships with Response. The Exposure-Dose-Response approach was also applied to the Lines of Evidence gathered from Lake Macquarie, an estuarine lagoon on the East Coast of Australia, historically contaminated by copper, cadmium, zinc, lead and selenium. The molar sum of the four metals showed to be a good indicator of contamination and bioaccumulation in relation to response. Dose-Response relationships were shown to be stronger than Exposure-Response relationships in the mined and Lake Macquarie data sets, by-passing the confounding factors due to bioavailability of metals in sediment. Molluscs and fish showed more consistent Exposure-Dose and Dose-Response relationships than crustaceans.

TH 252

Silkworm as a bio-indicator model in ecotoxicological studies on Cadmium (CD)

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Xenobiotic risk evaluation largely concerns the international scientific and social communities in order to assess sustainable threshold levels of pollution. Different available bioassays for ecotoxicological studies focused mainly on organisms such as earthworms, mussels and snails while only few insect species have been used up to date. This research aims at setting up protocols for testing some relevant biomarkers on Bombyx mori L. (Lepidoptera: Bombycidae) used as model. This insect species was chosen because it is easily reared under controlled environmental conditions by feeding on mulberry leaves or artificial diet.
Silkworm artificial diet was spiked with 0.01, 0.1, 1 and 10 ppm of Cd. Three microcosms were set up for each treatment and for control.
Twelve larvae of silkworm at the last instars were fed on artificial diet and exposed to pollutant for 6 days at 28°C with 65% relative humidity (RH) and a light:dark ratio of 15:9. After exposure, specimens of B. mori were opportunely treated to assess the metallothionein content, the DNA damage (comet assay), and the bioaccumulation of Cd.
The metallothionein content showed a significant difference between the treatment with the highest Cd dose and the control, while the comet assay showed a dose-response effect.
These first results seem to be promising to use silkworm as a starting model to promote other insects, more relevant from an environmental point of view, as bio-indicators. The application of other biomarkers and the collection of further data for confirming these preliminary information is foreseen in the next steps of this research.

TH 253

Using a soil-water flume on pesticide ecological risk assessment under Mediterranean exposure scenarios

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Integrated in a broader study on the fate and effects of plant protection products under Mediterranean conditions results on fate of three PPPs and their effects on aquatic and soil organisms are presented. Samples for both assessments were collected using a recently developed laboratory soil-water simulator allowing simulation of pesticide application, irrigation and collection of different matrix samples (soil, leachate and run-off). Model pesticides were chlorothalonil, ethoprophos and azoxystrobin, used in their commercial formulations and selected based on the result of acceptance in inclusion in Annex I of Directive 91/414/EEC and as being regularly used in Portuguese model crop systems (potato, maize, onion).
Different scenarios for each pesticide were created using reference soil from an agricultural area of Central Portugal. Simulator experiments ran under Mediterranean climatic conditions, and considered the specific crop type, mode of application and irrigation regime for each pesticide. Based on data from previous laboratory tests, soil samples for testing with soil organisms were collected only for ethoprophos, but leachate and run-off samples were collected for the three pesticides.
Data gathered from all matrices will be compared with data obtained from laboratory tests aiming to refine effect assessment data obtained in lower tiers. Furthermore run-off matrices will be used as contamination source on higher tier aquatic microcosms.
This study contributes to fulfil the need for integrated (water and soil) studies, especially mimicking more realistic exposure scenarios This will also lead to a refinement of methodologies to assess quality standards that will contribute to decision-making aiming at a sustainable use of pesticides towards water, soil and biodiversity protection, contributing to reduce soil degradation and water contamination at a national level.

Poster Corner abstracts

MOPC1 - Amphibians and reptiles in ERA (review)

MOPC1-1

A Review of the Inclusion of Amphibians and Reptiles in Ecological Risk Assessments

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To be realistic and robust, ecological risk assessments (ERA) should account for potential harm to all the relevant components of the ecosystems being evaluated. Amphibians and reptiles are important components of terrestrial and aquatic ecosystems; they drive important abiotic and biotic processes, which underpin the delivery of ecosystem services. In addition, some species of herpetofauna are legally protected both nationally and internationally. Amphibians and reptiles are excellent indicators of the health of the aquatic and terrestrial habitats in which they live.
In particular, amphibians are sensitive to environmental disturbance because of their biphasic life history, physiological characteristics, and specific microhabitat requirements; reptiles play a complex role in food webs where they can be predators or prey of invertebrates and vertebrates. In addition to contaminants, numerous other stressors affect the world's herpetofauna, including habitat degradation and loss, introduced species, global warming, and diseases. Publicly available literature was reviewed to determine the current status of including amphibians and reptiles in ERA. It was confirmed that seven amphibian ERA have been conducted on stressors ranging from ultraviolet radiation, acidic deposition, and organic, inorganic and nutrient contaminants. Fewer ERA included risks to reptiles; two ERA have been conducted for turtles where the risk of contaminants, as well as anthropogenic and physical stressors, was assessed. In conservation studies, four ecological assessments evaluated the impacts of non-native reptile introductions to native amphibians and reptiles. It was concluded that while amphibians and reptiles are key to providing many ecosystem services, the results of our review demonstrate that they are still rarely included in ecological risk assessments. However, with the establishment of the SETAC Ecotoxicology of Amphibians and Reptiles Advisory Group in 2010, it is hoped that amphibians and reptiles are included in all future applicable ERA.

MOPC1-2

Is it necessary to include reptiles in the risk assessment of plant protection products?

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In the proposal for data requirements under the new Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products (PPP) on the market it is proposed to include reptiles in the risk assessment for PPPs. This means that for each PPP it would be necessary to test a reptilian species, but from an animal welfare point of view this is undesirable. The purpose of this poster is to open a discussion on whether reptilian risk assessment should be included in the risk assessment process or whether we have evidence that this would not be necessary.
Fryday and Thompson (2009) reviewed information that might be suitable for assessing the possible exposure of reptiles to PPPs:
_ Many reptilian species can be found at the edges of fields and in hedgerows adjacent to fields. Therefore they could be exposed to PPPs via contaminated food in a similar fashion to birds and small mammals.
_ Exposure via contaminated food will be similar to that of birds except that a reptile is likely to consume more than ten times less of the same food type.. Therefore it can be predicted that exposure of reptiles through food will be less than for a similar-sized bird. The comparison may be valid for small species, like turtles and lizards, which may eat every day during periods of activity. In the case of snakes the comparison is more difficult due to the punctuated feeding pattern where they will eat a large prey item on one day and not feed again for several days or more. In these circumstances maximum daily exposure may therefore be far greater than that predicted for other species. More data on feeding patterns is needed to allow risk assessment for these species.
_ The review of toxicity data confirmed that very little acute toxicity data suitable for comparison with birds is available in the literature. For the five pesticides and one rodenticide for which acute toxicity data was available, reptiles were less sensitive than birds for four compounds and more sensitive to the remainder. Should sufficient data become available in future then consideration could be given to whether data from other species such as birds could be used to predict risk to reptiles.
Tentative hypothesis: Assuming reptiles have similar sensitivity to pesticides to that of birds, dietary risk for reptiles is covered in risk assessment for birds.

MOPC1-3

Variation in sensitivity of amphibians to toxic and non-toxic stressors

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Amphibian populations are declining around the world. Identifying the major causes of their decline is important to explain why amphibians are at risk and how to preserve them. Causes that may underlie amphibian declines include habitat loss, global warming, acidification, fertilizers, pesticides, toxic chemicals, invasive species, and infectious diseases. Loss of suitable habitat is considered to be the largest factor contributing to this decline. However, remaining habitats might be less suitable due to chemical pollution. In this study, the influence of acidification, nitrogen fertilizer (NH_4NO_3), heavy metals (Cd, Cu) and two insecticides on amphibians are evaluated to determine their sensitivity to these stressors. Data were derived from the database of the Dutch Institute for Public Health and the Environment, e-toxBase, as LC_{50} (mg/L) for Anura species. The fact that species differ in their sensitivity to the same stressor allows for the use of Species Sensitivity Distribution (SSD). SSDs are probabilistic models to analyze the variation of species sensitivity for one particular or a set of stressors. In log-logistic distributions, variation in sensitivity of species is described by the slope of SSD (β, parameter proportional to the standard deviation of distribution) and the average toxicity over species is described by the parameter ±/__sample mean of log-transformed values). To study the influence of different stressors on the variation within a population of amphibians, the difference between slopes was compared. The shallowest concentration-effect curve corresponds to β values of 0.65 for cadmium and 0.57 for copper (α is 9.3 and 4.3, respectively). The β for NH_4NO_3 equals to 0.41 (α=1.9), for insecticide camphechlor 0.36 (α=0.5) and for azinphos-methyl 0.39 (α=2.9). For acidification the curve is the steepest with the β value of 0.15 (α= pH 3.7), meaning that variation in sensitivity to acidity is the smallest. Previous studies on toxic stressors show that, in general, within a taxonomic order compounds with a specific mode of action have steeper slopes than that with a non-specific mode of action. Here, β values for insecticides are similar to the values characteristic to compounds with narcosis mode of action (β~0.4). This may imply that these insecticides have no specific mode of action on amphibians. The SSD analysis allows to rank the stressors according to the sensitivity of amphibians and identify the major threat causing their decline.

MOPC1-4

Evaluation of the embryotoxic potential of xenobiotics in Xenopus laevis using molecular approaches

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The aim of this work is to explore the validity of molecular endpoints as suitable biomarkers to evaluate the embryotoxic potential of contaminants and complex mixtures on a *Xenopus laevis* embryo assay. For this purpose, the effects of two contaminants of environmental concern (Bisphenol A and Chlorpyrifos), one emerging pollutant (Methylparaben) and several effluents from wastewater treatment plants around the city of Madrid (Spain) have been evaluated in a new frog embryo assay. The parameters considered in this test are lethality, embryo malformations, whole body length at 96 hours of exposure and mRNA expression of different genes involved in the embryonic development at 4, 24 and 96 hours of exposure. The response (inhibition or overexpression) of the studied genes will be compared with the morphological parameters in order to investigate the sensitivity of molecular endpoints for the early detection of sublethal effects. This work has been funded by Spanish projects CONSOLIDER-INGENIO (TRAGUA) CSD2006-00044 and RTA2010-00004-C02-00. L. San Segundo is a doctoral student supported by a FPI fellowship from Spanish INIA.

MOPC1-5

Assessment of ecotoxicological status of the Mediterranean population of Caretta caretta (Linneo, 1758) based on classes of age

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The loggerhead turtle (Caretta caretta) represents the most common sea turtle in the Mediterranean Sea. This species is a long-living (70-80 years) omnivore species that feeds on molluscs, crustaceans and fish potential subjected by threats deriving by contaminants. In addition to the accumulation of pollutants from food the threats for the Mediterranean population of this species are fishing, nesting beaches degradation and solid wastes. For all these reasons since 1996 the loggerhead turtle is listed as "Endangered"on the IUCN Red List of Threatened Species. Therefore, there is a need to develop sensitive tools to investigate the health status of the Mediterranean population of this species. The purpose of this study was to evaluate the ecotoxicological status of Caretta caretta through the responses of a set of biomarkers and the analysis of contaminants levels (PAHs, OCs and heavy metals) in blood, skin biopsies and carapax samples. Between 2008 and 2010 were collected 50 specimens of loggerhead turtles in several Italian rescue centers and during a survey in Spain (free-ranging specimens). Oxidative stress was evaluated on plasma samples by lipid peroxidation (LPO); potential genotoxic effects were investigated by Comet assay and ENA (erythrocytic nuclear abnormalities) assay in whole blood. Butyrylcholinesterase (BChE) activity was detected in plasma as biomarker of neurotoxicity. The presence and induction of CYP1A was detected by Western blot (WB) analysis in skin biopsies as biomarker of exposure to planar molecules. The results were processed by classes of age to evaluate a possible relationship between the specimens age and the different biomarkers responses and contaminants bioaccumulation. In accordance with Casale et al., (2009) the specimens were classified into three age categories (I: specimens from 0 to 5 years; II: from 5 to 10 years; III: more than 10 years) depending on the Curved Carapace Length (CCL) through the use of von Bertalanffy growth-model. The results of total PAHs, carcinogenic PAHs and OCs levels, lipid peroxidation, Hg levels and the frequency of micronuclei and total number of cells with nuclear abnormalities showed increasing values from I to III category to suggest a potential accumulation of contaminants with increasing of specimens age. The data obtained from this multi-trial approach represents a further sight that highlights the potential ecotoxicological risk of the Mediterranean population of Caretta caretta.

MOPC1-6

Effects of mosquito control measures on mortality and whole-body corticosterone levels in tadpoles of four Southeast Asian amphibian species

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Mosquito control methods include both chemical and biological approaches, and are commonly applied in wetlands and marshes that also serve as amphibian habitats. In Hong Kong, mosquito control measures are applied by local authorities in urban and rural areas to control mosquito-borne diseases. In this study, the toxicological and ecological effects of three mosquito control methods used in Hong Kong, larvicidal oil, the organophosphate insecticide temephos, and the bacterium *Bacillus thuringiensis*, on tadpoles of four native wetland-breeding Southeast Asian amphibian species (the ornate pigmy frog [*Microhyla ornata*], paddy frog [*Fejervarya limnocharis*], Asian common toad [*Bufo melanostictus*], and brown tree frog [*Polypedates megacephalus*]) were determined in a mesocosm exposure. Eighty 84-L plastic containers (55 L water/container) were set up and allowed to be colonized by local insect fauna for 30 days prior to commencing the exposure. The mesocosms were randomized by species, treatment, and replicate (n=5; 40 tadpoles per container). Tadpoles were exposed to one of four treatments: freshwater control, 4.70 ml larvicidal oil, 10.70 g temephos, or 1.60 ml B. thuringiensis per container for 28 days. Larvicidal oil and *B. thuringiensis* were re-applied every seven days, while temephos was applied only once at the beginning of the exposure. Effects on tadpole survival and whole-body corticosterone levels were investigated, as well as impacts on the aquatic insect community in the mesocosms.
Tadpole survival in the mesocosms was highly dependent on the presence of Odonate predators. Odonate abundance was reduced by the temephos treatment, which had a positive effect on tadpole survival. The larvicidal oil was found to be toxic to the tested species. Whole-body corticosterone levels were determined by enzyme-linked immunosorbent assay following tadpole homogenization and extraction. The effects of the three mosquito control methods on whole-body cortiscosterone levels will be discussed.

MOPC1-7

Sensitivity of embryos and tadpoles of the green frog Pelophylax perezi (Seoane, 1885) to lethal levels of copper

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Metals are well described as responsible for survival reduction and development disturbance on several stages of aquatic organisms when present at high concentrations. This work aimed to compare the sensitivity of embryos and tadpoles of green frog *Pelophylax perezi* (Seoane,1885) to lethal levels of the essential metal copper. Eggs and tadpoles were collected at a coastal freshwater pond in Northern Portugal. Eggs were kept in the artificial medium FETAX until reaching the Gosner stages 9-11. Afterwards, jelly-coat was removed and they were exposed to a gradient of copper concentrations (0 to 3.2 mg Cu L-1) until the 96 h. Survival and malformations rate, time to eclosion and larvae body length after the eclosion were monitored during the assay. Tadpoles were kept for at least 24 hrs in FETAX and, at the Gosner stage 25-28, were exposed to a copper gradient (0 to 2.4 mg Cu L-1), for 96 hrs. At the end of the assay survival was checked.
For embryos, the 96h-LC50 and 96h-LC20 were > 3.2 and 1.4 mg Cu L-1, respectively. The 96h-EC50 and 96h-EC20 for malformations were > 3.2 and 1.2 mg Cu L-1, respectively. Significant differences were observed on the body length in the three highest copper concentrations but no differences were found in time to eclosion. For tadpoles the LC50,96h was 0.9 mg Cu L-1, revealing that this life stage of *P. perezi* is more sensitive to copper than embryos, and, thus, suggesting that though assays with larval stages should be abolish from risk assessment, an exhaustive evaluation of embryo relatively to tadpoles sensitivities should be carried out, to allow the use of scientifically sound safety factors, aiming at avoiding risk of under- or overestimation.

MOPC1-8

Lethal and sublethal effects of nitrogen pollution on anuran larvae

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Human activities dramatically increased the amount of inorganic nitrogen released in ecosystems through the application of fertilizers in agriculture, the generation of human and livestock waste, and the combustion of fossil fuels. This nitrogen eventually reaches water bodies where it can, in the form of nitrate, nitrite and ammonium, be toxic to aquatic organisms. Our objective was to test in the laboratory if prolonged exposure of anuran larvae to inorganic nitrogen at low and environmentally relevant concentrations could affect survival, growth, development and behavior. Tadpoles of *Hypsiboas faber* (Anura, Hylidae) were exposed to 0, 2.5 and 10 mgN/L of nitrate, nitrite and ammonium during 21 days in a static renewal test. We demonstrated that if there is extended exposure even relatively low concentrations of inorganic nitrogen can cause lethal and sublethal effects on anuran larvae. Nitrate, the most abundant N form in nature, has low toxicity when compared to nitrite and ammonium, causing no effects even in the largest manipulated concentration. Tadpoles exposed to 10 mgN-nitrite/L had survivorship 20% and mass 12% lower than controls. However, it is unlikely that the concentrations of nitrite we manipulated in the laboratory are common in nature, especially under aerobic conditions. On the other hand, 10 mgN-ammonium/L reduced survivorship in 10% and activity rate in 22% relative to controls. This concentration is within the upper range of concentrations observed in nature. This study contributes to the development of ecotoxicology in Brazil by establishing the basis for the employment of native amphibians as model experimental system. Future studies that aim to assess the environmental risk of nitrogen contamination should monitor concentrations in natural habitats and evaluate the effects of synergistic interactions between inorganic nitrogen and other physical, chemical or biological stressors to amphibian larvae.

MOPC2 - New developments and perspectives in Life Cycle Sustainability Assessment

MOPC2-1

Life cycle based indicators for quantifying and monitoring the environmental impact of European Union

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Sustainable development is an underlying objective of the European Union treaties. It is mainstreamed to the European Union policies, along with the approach chosen to facilitate meeting this objective"'life cycle thinking. So far, the tools necessary to measure the progress towards this ambitious goal have been missing. Therefore, the JRC/ESTAT in consultation with DG ENV and the EEA developed a new type of life cycle based indicators for quantifying and monitoring the environmental impact of the European Union. This paper presents the framework for the life cycle based indicators.
These indicators serve the implementation of modern life-cycle based environmental policies, like the Sustainable Consumption and Production Action Plan (COM(2008) 397), the Thematic Strategy on the Sustainable Use of Natural Resources(COM(2005) 670), and the Thematic Strategy on Prevention and Recycling of Waste (COM(2005) 666).
Life cycle based indicators are essential to monitor progress and objectives towards sustainable consumption and production, with particular focus on the separation of environmental impacts from economic growth. The indicators take into account the impacts and resources consumed, both inside and outside the EU, that are associated with goods and services consumed and the wastes produced.
The life cycle based monitoring indicators include:
- Decoupling Indicators (resource efficiency indicators) which measure the total environmental impact of European Union in relation to the resources used.
- Basket-of-Product Indicators which informs about the environmental impact indicators for products, covering a representative selection of goods and services consumed in the EU.
- Waste Management Indicators cover the entire waste management chain for most relevant waste types generated and treated in the EU.
The indicators will be used to monitor progress towards sustainable development in the policy areas of resources, consumption and production, and waste, allowing the detailed analysis of the European production and consumption. They will support the sustainable use of natural resources without shifting of burdens in a globalised economy as well as foster material and energy resource efficiency and reduced environmental pressure of waste management.

MOPC2-2

Feasibility of current SLCA methodology for technology assessment

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Life cycle thinking within sustainability assessment of products so far mainly addresses environmental aspects (Life Cycle Assessment). However, assessing economic and social aspects gain increasing attention; the latter especially since the publication of the UNEP-SETAC Guidelines for Social Life Cycle assessment (SLCA) of products in 2009.
Based on two case studies on sustainability assessment of technologies for water respectively fuel provision in developing countries, social aspects along the life cycle of those technologies were identified. Special focus was laid on the "use-phase"of technologies respectively the production phase of the services provided.
It is discussed if the assessment of technologies is actually within the scope of SLCA, which focuses on products. Since it consequently was considered to be, current SLCA-methodologies - summarized in the UNEP/SETAC Guidelines - were analyzed with regard to suitable impact categories and indicators for describing the identified social sustainability aspects.
The paper thus examines the basic applicability of SLCA guidelines to address social aspects and indicators which are considered as relevant to evaluate the "sustainability performance"of technologies. Finally, possible additional social indicators for technology assessment within management systems are proposed, giving an outlook on how further development of SLCA methodologies could look like.

MOPC2-3

New guideline for sustainable chemicals management

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Today's chemicals management has the responsibility to avoid long-term and irre-versible damage to human health and environment. Existing data of a chemical pro-vides important information for the producer and the applicant to decide whether a chemical is able to perform distinct functionalities. Additionally, existing concepts dealing with the assessment of chemicals impact on health and environment are mostly restricted to special applications. However, it is necessary to develop a new approach for industrial operators to address all aspects of sustainability in production and usage of chemicals.
Therefore, based on the analysis of three existing concepts, the guideline "Criteria for Sustainable Chemicals"for producers and operators was developed. In this guideline, substance characteristics were accounted for by distinct substance-orientated assessment criteria in terms of hazardous properties, exposure assess-ment and risk characterisation. Additionally, application-orientated assessment crite-ria were established to refer to methods of processing and use.
In this new guideline not only inherent and intrinsic properties and potential sub-stance exposure were addressed. Also aspects of sustainability of chemicals along their whole life-span were accounted for: emission and transport, resource and en-ergy demand as well as social implications. Overall, the guideline was designed as a level-adapted risk reduction concept with 15 substance- and application-orientated assessment criteria with focus on relevance, applicability and underlying information.
For the first time, a guideline concentrates on essentials for a holistic sustainable chemicals management and therefore helps producers and operators to green their chemical portfolio as well as their internal processes.

MOPC2-4

Development of inventory database aimed at sustainability assessment considering environmental, economic and social aspects

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As sustainable and prosperous global society is pursued, LCA aiming at environmental aspect is expected to expand considering sustainability such as social and economic aspects. Although several studies of life cycle social impact assessment have been proposed, the existing inventory databases consider mainly environmental aspect.
In this study, we developed inventory database focused on triple bottom line, which contains environmental, economic and social aspects, using input-output analysis. On the environmental aspect, natural resources, water resources and biodiversity as well as climate change is considered. On the economic aspects, a framework considering the effect of production inducement is designed. Third, on social aspects, labor inducement effect is evaluated. In addition, we have a plan to include labor accident in our database.

MOPC2-5

Methodological development of regional sustainability analysis

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Recently the methodological aspects of the sustainability LCA induce increasingly bigger interest among LCA researchers. Many assessments connect to sustainable product systems, renewable resource system or regional and local sustainable models. This paper describes an applied method of a regional sustainability assessment. It is based partly on LCA and partly on sectors mutual relationship.
The basic concept of our analysis is built on the LCA standards ISO 14040 and ISO 14044 but it is supplemented by economic and social elements, because according to our concept all (three) pillar of sustainability should be integrated into the assessment. In the development of the concept we used the following models as a starting point (EIOA, MFA, REEIO, CALCAS, GEM-E3, EVR) selecting their advantage to build a measurable, relatively simplified, transparent, and coherent model specified to our region (at NUTS 2 level) that represents an underdeveloped, a former heavy-industrial centre.
In this model LCA is built on a matrix structure, the environmental impacts have been aggregated as an environmental performance index, while we measure the economic and social impacts by scenario and trend analysis. For system border we chose the region's geographical border, the function unit is the unit of GDP produced in the region. The data analysed in the model generated from regional material flow and sector specific production data statistics.
The model does not integrate the whole economical activity; it focuses for those sectors, which have a more considerable role in the development of the region and can determinate its sustain-

able path. It involves:
- sectors which cover the 80 percent of the environmental load focusing on the largest polluter producers at company level - environmental aspect;
- sectors where the added value is the highest - economic aspect,
- sectors which employs the most- social aspect.
The selection of the dominant sectors is based on long term statistical data analysis provided by the Hungarian Statistical Office.
Conclusion
Recently we are working at the elaboration of a demonstrative project, which can represent and verify the model's viability and its adaptability through an example of a selected sector's evaluation. This can help us characterise the further steps and directions to improve the model and sustainability assessment.

MOPC2-6
Selection of LCA-based environmental criteria for multi-criteria decision support in a biorefinery context
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Triticale is a non-food crop that can grow on marginal lands. It may therefore be an interesting candidate for biorefinery feedstock. To help identify sustainable pathways for three different products made from triticale: ethanol, polylactic acid (PLA) and thermoplastic starch (TPS), a multi-criteria decision-making (MCDM) panel is conducted which will consider four aspects: product portfolio competitiveness, technico-economics, biomass procurement and environmental impacts. Given the number of aspects considered, the number of criteria per aspect should be as few as possible while remaining representative, comprehensive and easy to understand. Consequential Life cycle assessment (C-LCA) has been chosen to calculate the environmental impacts of each scenario, taking into account affected processes and indirect land use change (iLUC). Because the study is limited to the context of Alberta, the triticale scenario will be compared to wheat and corn scenarios, which are the most common local feedstock for biorefineries.
The C-LCA midpoint categories impacts and interpretation will be presented to a preliminary panel along with a productivity-weighted measure of land use that represents the potential competition with food. In this preliminary panel, the weighting of the different impacts will be assessed, enabling to calculate a smaller set of criteria adapted to the biorefinery context in Alberta for the environment aspect. This presentation will present the LCA-based criteria definition for the environmental aspect and its integration in the overall MCDM panel.

MOPC3 - Data-driven, knowledge-based, and QSAR modelling in ecotoxicological assessment

MOPC3-1
An uncertainty-based framework for the integration of data from different sources in ecotoxicological risk assessment
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Estimation of risks caused by environmental stressors involves uncertainties. These uncertainties might have impact on the results of the risk assessment and, thus, to the decision making if risk assessment is supporting a decision making process. Therefore it is important to recognize and quantify uncertainties during the risk assessment process. The identification and characterization of uncertainty sources are two capital issues to develop a transparent and objective decision support system to both integrate different data and perform any risk assessment analysis.
BCF has been used as a benchmark endpoint to develop a decision support framework based on uncertainty values obtained from the differences between experimental and predicted BCF values for a training set of chemicals. BCF data has been treated as a continuous endpoint and the uncertainty of each predictive test method has been obtained following the same methodology. As a result of such analysis it is possible to obtain a point estimate for the mean error of the method and, what is more relevant, a confidence interval for the mean error which gives an idea of the uncertainty of each method tested on a new substance.
The uncertainty values relative to the alternative methods are compared next. This gives a realistic expectation of the possibility to use a certain value for a specific endpoint. The relevance of a method in the final risk characterization may be expressed in terms of its uncertainty. The advantage of this approach is that different methods can easily be compared and a clear objective procedure is defined and applied unambiguously.

MOPC3-2
SMILES-based QSAR model for bioconcentration factor
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The Simplified Molecular Input Line Entry System (SMILES) has been used as a representation of the molecular structure to build up quantitative structure - property relationships (QSPR) for the prediction of the logarithm of bioconcentration factor (logBCF) of a large set of organic compounds (n=473). Quite good predictions of logBCF for external test sets (n=105) have been obtained for three random splits into subtraining, calibration, and test sets, in spite of lower statistical quality of the models for the subtraining set. An attempt to explain this phenomenon from a probabilistic point of view is discussed. This is also useful to evaluate principles for the definition of the applicability domain for these models. A new global SMILES invariant ATOM-PAIR (calculated with CORAL software, http:/www.insilico.eu/CORAL) has been examined as a potential tool for improvement of the statistical quality of the logBCF prediction.

MOPC3-3
QSAR for toxicity of aromatic aldehydes to Tetrahymena pyriformis using correlation weights of physicochemical situations
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A physicochemical situation in the molecular structure can be represented by means of a (0,1)-descriptors, where 0 is the indicator of absence and 1 is the indicator of presence. For each physicochemical situations and each molecular fragment that can be calculated from the simplified molecular input line entry system (SMILES), correlation weights, producing a maximum correlation coefficient between the endpoint and the optimal descriptor, can be obtained by the Monte Carlo technique. This approach has been used for the QSAR model of the toxicity of aromatic aldehydes to Tetrahymena pyriformis (-logC, C is the 50% growth inhibitory concentration). The model is characterized by the n=35, R2=0.63, Q2=0.58, RMSE=0.287, F=56 (sub-training set); n=23, R2=0.88, R2pred=0.87, RMSE=0.290, F=166 (calibration set); and n=19, R2=0.89, R2pred=0.86, RMSE=0.210, F=138 (test set). It has shown that the model is both robust and convenient for the physicochemical interpretations. The described calculation can be carried out with CORAL software available on the Internet (http:/www.insilico.eu/CORAL).

MOPC3-4
QSAR models for toxicity of organic substances to Daphnia magna built up by using the CORAL freeware
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CORAL (CORrelations And Logic) is a freeware available on the Internet. This freeware is designed to build up quantitative structure - property/activity relationships (QSPRs/QSARs). The molecular structure for CORAL should be represented by the simplified molecular input line entry system (SMILES). Optimal descriptors calculated with SMILES are a mathematical function of the presence or absence of SMILES elements. The element is one or two symbols which cannot be examined separately (e.g., 'Cl', 'Br', etc.). The basic idea of our approach is to calculate correlation weights for each element or combination of the elements by the Monte Carlo method. These coefficients serve to calculate the descriptor correlated with the endpoint for the training set, hoping that this correlation will also hold for the external test set. These descriptors can be improved by taking into account global physicochemical situations in molecules. An example of the physicochemical situation is the presence of oxygen, nitrogen, and sulphur. Another illustration of the mentioned situations is the presence of oxygen and nitrogen accompanied but no sulphur. One can calculate these situations with SMILES and represent them by descriptors combine 0 (absence) and 1 (presence). Using this software we developed a model for the prediction of the toxicity to Daphnia magna (LD50). The statistical characteristics of this model are the following: n=114, r2=0.7082, q2=0.6969, s=1.04, F=272 (subtraining set); n=108, r2=0.7366, r2pred=0.7238, s=0.877, F=296 (calibration set); and n= 75, r2=0.7897, r2pred=0.7790, s=0.847, F=274 (test set).

MOPC3-5
Classification scheme for screening metabolic half-lives of organic chemicals in fish
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For the regulation of chemical substances under REACH, the bioconcentration factor (BCF) is an animal-intensive and high-cost endpoint. So far, in silico alternatives to experimental testing focus on the octanol/water partition coefficient as predictor for the passive-diffusive uptake and storage in the organism. Recently, a fragment-based scheme for predicting metabolic half-lives from molecular structure has been introduced, enabling the possible extent of BCF reduction due to biotransformation to less hydrophobic metabolites. Estimation of metabolic half-lives is also needed for multimedia fate modelling.
In the present communication, a new classification scheme is introduced that enables the discrimination between fast-, intermediate- and slow-metabolizing compounds based on their structural features and associated property profiles.
Normalized estimated values for whole-body in vivo metabolic half-lives (t1/2) for fish of about 600 organic compounds for scheme developing were taken from the work of Arnot et al. 2009. Rules for a classification scheme where developed using physical-chemical and molecular properties and structure fragments, all of which were generated with our in-house software ChemProp (Schüürmann et al. 1996).
The proposed classification scheme allows a screening-level assessment of metabolic biotransformation half-lives for organic chemicals in fish, and can be integrated into existent schemes for predicting BCF as additional correcting factor.

MOPC3-6
Reactive toxicity of michal acceptors: quantitative prediction based on quantum chemical reaction barriers
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One important property to predict reactive toxicity of Michael acceptors is their electrophilic reactivity towards thiols. For a set of 35 α,β-unsaturated aldehydes, ketones and esters, quantum chemical transition-state calculations including consideration of different conformers have been performed for their model reaction with CH_3SH as surrogate of glutathione (GSH), focusing on an addition pathway with initial protonation of the carbonyl oxygen. The GSH-thiol reactivity in terms of logarithmic second-order rate constants, log k_{GSH}, can be predicted with the calculated reaction barriers, $\Delta E^{\ddagger 0}$, with r^2 (squared correlation coefficient) = 0.96 if combined with an indicator variable $I\alpha$ to discriminate between α-H and α-substituted derivatives. For an augmented set of 46 α,β-unsaturated carbonyls aquatic toxicity quantified as logarithmic 50% growth inhibition after 48-h exposure, log EC_{50}, of the ciliates Tetrahymena pyriformis were taken from literature. Class-specific regression models for predicting log EC_{50} were developed using a combination of log K_{ow} (logarithmic octanol/water partition coefficient) and predicted log k_{GSH} or calculated $\Delta E^{\ddagger 0}$ and $I\alpha$ yielding r^2 values up to 0.96 (aldehydes), 0.92 (ketones), and 0.83 (esters), respectively. Our results confirm the recently reported difference in hydrophobicity and reactivity contributions to the aquatic toxicity of Michael acceptors between aldehydes, ketones and esters. The derived regression models appear useful as in silico tools for screening the electrophilic reactivity and toxicity of α,β-unsaturated carbonyls in the context of integrated testing strategies for REACH, enabling an early discrimination between narcosis-level and reactive toxicants.

MOPC4 - Innovative approaches of toxicant identification and structure elucidation

MOPC4-1
Effect-directed analysis (EDA) of transformation products of triclosan formed during (photo)oxidative processes
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Transformation processes in the aquatic environment and in water treatment systems may signifi-

cantly change the toxicity in comparison to the parent compound. For an overall risk assessment, the toxicity of the transformation products should be investigated. The direct identification of the transformation products, their synthesis if they are not readily available, and evaluation of their toxicity, is a rather tedious and expensive procedure. As an alternative, an effect-driven approach allows the priority setting for further identification and evaluation of toxicity. Here, the toxicity of the mixture of transformation products is tested and specific transformation products are identified only if the toxicity does not follow the degradation kinetics of the parent compound, which indicates that the transformation product is more toxic than parent, presumably because a new toxicophore is formed.
This screening-level approach will be illustrated on the example of the antimicrobial agent triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol). The toxicity of transformation products was investigated during four (photo)oxidative processes (direct phototransformation, triplet-induced photosensitized oxidation, oxidation by hydroxyl radicals and ozone). In a first step, we followed the evolution of the specific toxicity of the parent compound (growth inhibition of E. coli) during its degradation. The specific toxicity decreased proportionally with the decrease of triclosan concentration. We concluded that the antibacterial activity of the degradation products was insignificant in comparison to the toxicity of triclosan. In addition, the induction of cytochrome CYP1A, measured as EROD activity in fish liver cells, was analysed in order to detect dioxin-like compounds. Our work confirmed formation of 2,8-dichlorodibenzodioxin (2,8-DCDD) during direct photodegradation of triclosan. The dioxin-like activity measured by the EROD assay corresponded to the concentration of 2,8-DCDD measured by HPLC analysis. Moreover, we revealed the formation of CYP1A-inducers during ozonation which could not be explained by 2,8-DCDD. Therefore, this degradation mixture was fractionated according to hydrophobicity by preparative HPLC and the active fraction localized by application of the bioassay, whereupon the toxic compound is identified.
Our approach can be used as a first step in toxicity assessment of transformation products and facilitates identification of toxic products formed.

MOPC4-2
What are the constituents of heavy fuel oil that cause toxicity to fish?
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Heavy fuel oil (HFO or Bunker C) is a thick, viscous residue of refining that is used in large heating plants, industrial processes, and ships as an inexpensive source of energy. Compared to crude oil, it contains much smaller proportions of low molecular weight alkanes and mono- and diaromatics, and higher proportions of high molecular weight PAH, waxes, resins, and asphaltenes. Because it is transported by truck and rail in relatively small quantities, there is a high frequency of shipments, and a high risk of spillage. In aquatic ecosystems, Bunker C tends to sink because it has a specific gravity close to 1.0, and brief weathering or contact with sediments accelerates sinking, usually as large blobs or tar balls. Little is known about toxicity, although a spill in Wabamum Lake, Alberta contaminated whitefish spawning shoals and impaired embryo survival. Our lab tests have demonstrated that HFO is more toxic to fish embryos than crude oil, in proportion to its higher concentrations of PAH. We have used embryo toxicity as a tool for an effects driven fractionation of HFO to test the hypothesis that it is PAH in HFO that are responsible for toxicity. This paper will present our progress in fractionation, chemical analyses, and toxicity testing.

MOPC4-3
Identification and compartmentalization study of emerging EDCs in an impacted river system by using an EDA approach
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The last decade dealt with the increasing occurrence of emerging pollutants (e.g. hormones, pharmaceuticals) that trigger adverse effects in river systems. Thus, strategies for their identification are needed. Effect directed analysis (EDA) approach aims at identifying such adverse chemicals. Its current use is the most often restricted to dioxin (AhR), estrogen (ER) or androgen (AR) receptors pathways while recent evidences suggested that others signalling pathways (e.g. PXR) involved in endocrine regulation can be disrupted. Hence identifying environmental active pollutants on these other pathways will enhance risk knowledge on endocrine disruption in situ.
We report here the use of a battery of in vitro reporter gene bioassays including classical and emerging pathways to characterize the contamination of both sediment and surface water (polar organic integrative sampler (POCIS) and semi permeable membrane device (SPMD)). This allowed to integrate a wide range of active chemicals and to assess their distribution between river compartments. To isolate and identify these chemicals, a two-step fractionation was then used. This overall approach was applied to a river site under mixed anthropogenic pressure where fish are impacted.
The bioassay profiling revealed estrogenic, anti-androgenic, dioxin-like and PXR-like activities in sediment while very strong estrogenic and PXR-like activities were measured in POCIS as regard to what reported scientific litterature. Bioanalysis of SPMD extracts is under progress. A first fractionation of sediment extract led to 4 fractions. F3 (polar) exerted only strong estrogenic and PXR-like activity while dioxin-like was mainly detected in F1 and F2. This demonstrated the occurrence of semi-polar to polar ligands of ER and PXR in sediments. Then F3 was hyperfractionated, estrogenic activity was mainly detected at the same elution time as BPA and 17β-E2 while PXR-like activity was mainly detected in less polar fractions.
In summary, this study demonstrates the usefullness of an EDA approach based on a multi-receptors/muti-compartment approach to assess and identify a diversity of EDCs and to study their distribution between water compartment The active fractions will be investigated using accurate mass spectrometric techniques while further fractionation strategies will be adressed on POCIS extracts. Purification on purified nuclear receptor column will be also tested on active fractionation for an optimized identification.

MOPC4-4
Automated strategies to identify compounds based on mass spectrometry and calculated properties
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The identification of active compounds in complex environmental samples using integrated chemical and biological approaches often relies on chromatographic separation followed by mass spectrometric detection due to sample amount and purity. Effects often cannot be fully explained by compounds identified using mass spectral databases alone and it follows that many compounds (e.g. transformation products or emerging pollutants) not within the databases may be responsible for the unexplained activity. An alternative to database searching is to use structure generation approaches to generate all possible compounds matching the analytical data and then reduce these candidates by progressive exclusion based on calculated properties. The identification of compounds based on gas chromatography-electron impact-mass spectrometry (GC-EI-MS) and structure generation techniques has been improved by combining a number of strategies into a programmed sequence. This builds on the mass spectral interpretation and structure generation procedures in the program MOLGEN-MS by the calculation of additional data following structure generation, such as the boiling point, octanol-water partitioning coefficient and steric energy. Several exclusion criteria were applied progressively to eliminate candidates that did not match the analytical information available. The effectiveness of the proposed sequence was tested on a case-study of 29 structures of formula $C_{12}H_{10}O_2$, which showed that the combination of mass spectral interpretation and the additional exclusion criteria reduced the number of possible candidates down to the correct set of substitutional isomers in most cases. The method was then tested on unknown spectra isolated during mutagenicity-based effect-directed analysis (EDA) on river water sampled using blue rayon as a passive sampler in the Elbe River in the Czech Republic. Two compounds were identified in an active fraction using structure generation techniques based on GC-EI-MS spectra, confirmed analytically using both GC and LC-based techniques. These compounds were not responsible for the sample mutagenicity, but no additional spectra of interest were found using GC-based analysis. LC-Orbitrap-MS/MS analysis of the same samples revealed tens to hundreds of peaks of interest following subtraction of the blanks, highlighting the need to incorporate LC-based methods into EDA studies and develop similar database-independent identification strategies for MS(^{n}) spectra.

MOPC4-5
Contaminants identified in sewage Effluent using an LC/ ToF fast screening approach
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In recent times the use of pesticides, herbicides and fungicides has steadily increased, in an effort to meet global food demands. A percentage of such chemicals applied to crops will, inevitably, end up leaching into the surrounding soil and waterways. Strict environmental monitoring is mandated, which endeavours to safeguard the environment and protect plants and wildlife from harm as a result of exposure to these types of chemicals.
A novel QTof instrument coupled with ACQUITY UPLC, along with the Waters ToF Screening Pesticide Database, and POSI±IVE software data processing, was used to rapidly screen treated sewage effluent that had been extracted using SPE Waters' OASIS HLB cartridges. A generic screening UPLC gradient was used, with a total run time of 2 minutes. The mobile phases used were 10 mM ammonium acetate solution in water and 10 mM ammonium acetate in methanol. Extracted sewage effluent was successfully screened for pesticide contaminants at ultra-trace level. Use of the MSE functionality of the QTof instrument enabled the acquisition of exact mass data for both precursor and fragment ions in one screening run, with a high level of reproducibility. The RMS variation in acquired exact mass across a peak for thiabendazole was 1.5 ppm. This provides increased confidence in the detection and identification of compounds present, as result of the accurate and precise exact mass data acquired, and also allowed the use of very narrow chromatographic extraction windows, as low as 0.5 mDa in some instances.
In addition, the use of ChromaLynx XS software for non-targeted spectral deconvolution enabled further discovery and identification of a non-targeted pharmaceutical compound in natural river water during a pesticide screening run. This was confirmed by examination of the high energy MSE fragment ion data, which exhibited characteristic fragments at the retention time for that compound.

MOPC4-6
Utilizing novel software algorithms to streamline LC-MS analysis for targeted and non-targeted screening in environmental applications
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The availability of full scan ultra high resolution LC-MS instruments allows for rapid analyses of samples without the need to develop specific MS methods for target components. At the same time, it is possible to re-interrogate the same dataset for the identity of unknown or unexpected components since full scan data is acquired over the entire chromatographic run. Historically, significant time has been devoted to manual data mining required when searching for unexpected components. We describe the use of software for environmental screening applications to improve processing throughput of ultra-high-resolution, accurate-mass LC-MS data. The software incorporates several novel algorithms for data processing, including advanced component detection, parameter-less peak integration, and intelligent elemental composition determination.
In addition to these algorithms, automated library and database searching can be enabled to facilitate identification and throughput. The software can be used to screen and report targeted components in an automated fashion and at the same time obtain information on potential unexpected or unknown contaminants in samples

TUPC1 - Integrated testing strategies (ITS) for bioaccumulation

TUPC1-1
Integrated Testing Strategy (ITS) to optimise the assessment of bioconcentration under REACH framework
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Aim of the REACH legislation is human and environmental protection. To achieve this goal the use of available information results achieved by alternative methods are preferred, not only to save animals but also to increase the information robustness. To optimize the choice of the methods to satisfy all REACH requirements, an ITS (Integrated Testing Strategy) is recommended. It combines the available knowledge about the target chemical (identity, exposure, effects) with alternative information: non-optimal in-vivo data (e.g. BMF or BAF data), values from testing (in-vitro) and non-testing (e.g. QSAR and read-across) methods.
In this work an ITS for bioconcentration assessment has been developed beyond the REACH requirements and the official guidelines. The ITS for bioconcentration assessment takes into consideration: 1) annual production tonnage, 2) experimental information (including in-vivo and in-

vitro data), 3) physicochemical properties, and 4) data from non-testing methods. As one component, this ITS contains a waiving scheme to identify the compounds that can be safely considered non bioaccumulative (nB). This waiving scheme is made up of two logP-based worst-case QSARs and a decision tree based on physicochemical properties. The performance of the waiving scheme was tested using a large dataset. More than 50% of the nB compounds can be classified this way. The ITS allows to further reduce the number of compounds needing an in-vivo test using alternative methods. The ITS presented here is implemented in a web tool to help users in the decision-making process.
Financial support by the OSIRIS project (GOCE-CT-2007-037017) is gratefully acknowledged.

TUPC1-2
Screening for low aquatic bioaccumulation: physico-chemical constraints
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Physico-chemical properties related to the bioavailability of xenobiotics in aquatic environments have been tested for their ability to identify chemicals with low bioconcentration potential. Cut-offs in lipophilicity (log KOW < 3 or > 10), solubility and volatility (log Henry constant < -11 [atm/(mol/L)]), degradability (ready biodegradability, hydrolysis) and ionisation (> 5% ionisation at pH 7) have been adopted and combined into a decision tree based on 382 industrial chemicals [1]. The 5-parameter classification scheme was externally validated with 49 pesticides and successfully confirmed with 83 bioaccumulative compounds. The applicability domain of the model has been described in terms of chemical classes (excluding polybrominated compounds (> 4 Br), organometallics, compounds with perfluorinated fragments, substances with an acyclic alkyl moiety (chain length > C7) and thiols) and ranges of physico-chemical properties. The present tool [2] allows to securely de-prioritise more than 50% chemicals of low concern with regard to the B criterion (BCF < 2000). Bioassays with compounds with these physico-chemical constraints may be waived because testing may be technically not possible and does not appear scientifically necessary in PBT and risk assessments.
[1] EURAS. 2007. CEFIC LRI Goldstandard Database. http://ambit.acad.bg/ambit/php/euras.php, accessed 16.12.2009.
[2] Nendza M.; Herbst, T.; Screening for low aquatic bioaccumulation (2): Physico-chemical constraints, SAR QSAR Environ. Res., (in press).
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TUPC1-3
Use of conditional inference trees in support of B and non-B classification for aaiving of experimental BCF testing
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Under the European REACH regulation substances of very high concern, including persistent, bioaccumulative and toxic (PBT) chemicals, require authorization and their use may be restricted. According to Annex XIII of REACH, the bioconcentration factor (BCF) may be used as a surrogate to characterize bioaccumulation potential. To improve animal welfare and reduce costs of experimental testing of chemicals, QSARs are accepted by the legislation to identify bioaccumulation potential. Here we present a scheme for identifying chemicals for potential waiving of experimental BCF studies that extends an initial decision scheme based on physicochemical properties (1), that uses 382 chemicals from the CEFIC database (2) as training set and classifies the chemicals into "non-B"and "undecidable"using 5 descriptors. Conditional inference trees (3) (*c*Tree) separate the variable selection from the splitting procedure, reduce overfitting and avoid selection bias towards explanatory variables with many possible splits. After analysis of multiple collinearities and variable importance 10 variables were selected to construct the *c*Tree. This tree classifies the remaining 179 undecidable chemicals from (1) into B, non-B, and still undecidable. Ctrees show also how a classification was obtained: the paths from the root to a particular leaf detail the rules to classify substances. From the 179 chemicals, 37% (45/122) of the non-B chemicals and 19% (11/57) of the B chemicals could be correctly classified, the remaining 123 chemicals are still undeciable. In combination with the initial decision scheme based on physicochemical properties, 60-70% of chemicals could be proposed for waiving their experimental BCF tests and therefore the work presented here has the potential to contribute to monetary savings as well as saving of animal lives in the framework of REACH.
[1] Nendza M.; Herbst, T.; Screening for low aquatic bioaccumulation (2): Physico-chemical constraints, SAR QSAR Environ. Res., (in press).
[2] EURAS. 2007. CEFIC LRI Goldstandard Database. http://ambit.sourceforge.net/euras/, accessed 16.12.2009.
[3] Hothorn T.; Hornik K.; Zeileis A., Unbiased Recursive Partitioning: A Conditional Inference Framework, Journal of Computation and Graphical Statistics, Volume 15, Number 3, Pages 651-674, 2006.
Acknowledgement - This work was supported by the EU 6th Framework Integrated Project OSIRIS (contract no. GOCE-ET-2007-037017).

TUPC1-4
A cost-based classifier for bioaccumulation assay waiving
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The development of robust and reliable models for bioassay waiving requires the development of robust classifiers able to distinguish between active and non-active chemicals with high sensitivity. Different types of information such as physicochemical properties or molecular descriptors have been reported in the literature as the basis to develop QSAR models to predict the bioaccumulation potential of chemicals (Zhao et al., 2008)**.**This work explores the feasibility of using a cost-based learning scheme to develop high sensitivity classifiers suitable to be used as the basis for waiving bioassays.The data set used in this work corresponds to an experimental data obtained according to official guidelines (Dimitrov et al., 2005) that contains logBCF information for 511 chemicals. This dataset was filtered and cleaned and final database containing 473 chemicals was obtained. The dataset covers a wide range of logBCF (-1.0 to 4.85) where chemicals have been identified as either B or nonB according to Annex XIII of REACH (i.e., chemicals with BCF>2000 are considered as having highly bioaccumulation potential). The resulting dataset after applying the logBCF threshold is highly imbalanced (i.e., 391 nonB chemicals (82.6%) and 82 B substances (17.4%)). The best classifier was obtained from the combination of an alternating decision tree (ADTree) with a cost-sensitive wrapper (Metacost). To avoid false negatives (i.e., 100% sensitivity) the required false positive to false negative cost ratio was 1:25. Similar results were obtained using Atom Center Fragments (ACF). The current work shows that the use of cost-sensitive learning schemes facilitates the tuning of the performance of classifiers based on the cost assigned to false negative classifications. This is especially useful when dealing with unbalanced data sets as well as for the development of assay waiving models that require the development of high sensitivity classifiers.

TUPC1-5
Models to estimate the BCF from octanol/water partition coefficient - validation and applicability domain
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The software system ChemProp provides several models to estimate the bioconcentration factor in fish from the octanol/water partition coefficient. In turn, the octanol/water partition coefficient can either be taken from experimental values, or calculated from the chemical structure. For models with known training sets, the applicability domain is checked. A data set with experimental data independent from the model training sets is used to estimate the uncertainty of the predictions. A refinement is provided by a result correction derived from the estimation errors for chemicals of this data set that are similar to the test compound. A separate external test set is applied to validate the performance of the implemented models and their uncertainty estimation. This study is financially supported by the EU Integrated Project OSIRIS (contract No. 037017).

TUPC1-7
An assessment of the bioaccumulation model in EUSES
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Assessment of environmental exposure is an essential element of chemical risk assessment. The decision support instrument European Union System for the Evaluation of Substances (EUSES) is currently recommended for government authorities, research institutes and chemical companies within the EU to carry out quantitative model based exposure assessments when appropriate monitoring data are lacking. However, the algorithms employed in the bioaccumulation module of this model are more than 10 years old and our scientific understanding of bioaccumulation has progressed considerably since then. This study presents a comparison between estimates of human daily intake by EUSES and a new steady state food chain model (OSIRIS) which employs more recently published process descriptions. Calculations were made for a wide range of chemicals defined by their octanol-air (K_{OA}) and octanol-water (K_{OW}) partitioning properties. This model comparison showed that the greatest differences between the models were for chemicals with high K_{OW} and high K_{OA} due to differences in estimation of bioaccumulation in fish and root crops. Predicted concentrations in fish were up to four orders of magnitude lower in EUSES. One reason is that dietary uptake is not considered in this model, but also due to a generally lower predicted bioconcentration factor for high K_{OW} compounds. EUSES predicted much higher concentrations of hydrophobic compounds in root crops because it assumes chemical equilibrium between the roots and the soil compartment, whereas the OSIRIS model treats root accumulation as a mass-transfer limited process.

TUPC1-8
Integrated testing strategies (ITS) for bioaccumulation: 3R-directed optimization of in vivo BCF testing
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Under REACH, assessment of the bioconcentration potential is required for chemicals with a log Kow greater than 3 if it is produced or imported in quantities greater than 100 t/year. The gold standard for the determination of the bioconcentration factor (BCF) is the OECD Test Guideline 305 with fish, which is demanding in terms of resources, costs and number of animals. Integrated? Testing Strategies (ITS) for bioaccumulation assessment take advantage of a variety of information sources in order to estimate whether a chemical is bioaccumulative (B, BCF > 2000) or very bioaccumulative (vB, BCF > 5000). Although the ITS aims to come to a conclusive statement on the BCF of a test substance solely on the basis of non-animal (in silico, in vitro) data, for a number of substances still the in vivo test may have to be performed. Here, we discuss potential approaches to further develop the existing BCF test guidelines by refining the test and by reducing the animal needs for the test. Possibilities to optimize the OECD TG 305 with respect to the 3R principles include reduction of sampling frequency for determination of the kinetic parameters as well as the reduction of test concentrations (only one instead of two concentrations). Another option is to explore the utility of fish embryos for bioaccumulation testing. Embryos, due to their yolk content, readily absorb xenobiotics, and they posses already a functional metabolic capacity. Thus, the Fish Embryo Test (FET), hwich is currently under valdiation as alternative to the acute fish lethality test, may serve at the same time as a BCF test, or at least a BCF screen. A similar double use of a test to determine both toxicty and bioaccumulation may also be possible for fish early life stage tests. However, the potential of these approaches for 3R-directed optimization of in vivo BCF testing remains largely un-exploited to date.
Financial support by the OSIRIS project (GOCE-CT-2007-037017) is gratefully acknowledged

TUPC2 - Life cycle thinking in the waste management sector: one solution fits all?

TUPC2-1
LCA software for evaluating the environmental performance of municipal solid waste management systems: a comparison
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Within the last years, a number of LCA software has been developed to evaluate the environmental behavior of municipal solid waste (MSW) management systems. These tools can be applied as life cycle management tools by technical managers responsible for incorporating environmental criteria into the decision making process when selecting a waste management system for a municipality. However, these software present very different functionalities and characteristics, and therefore may provide different results. In this paper, a comprehensive comparative analysis of six LCA tools specifically applied to waste management systems has been developed: IWM-2 (Integrated Waste Management), IWMMM (Integrated Waste Management Model for Municipali-

ties), WARM (WAste Reduction Model), LCA-IWM (LCA-Integrated Waste Management), WISARD (Waste Integrated Systems for Assessment of Recovery and Disposal) and EASE-WASTE (Environmental Assessment of Solid Waste Systems and Technologies). This analysis was conducted at three levels: 1) modeling and assessment of a complex MSW management system in each software in order to examine whether there are differences in the obtained results in terms of overall impact of the MSW management system; 2) modeling and assessment of alternative MSW management systems in each software in order to analyze whether the preference order is maintained; and 3) analysis of the life cycle inventory applied in each software for each waste management treatment in order to justify the differences obtained in the previous comparisons.

TUPC2-2

LCA as a decision support tool for waste management planning in Lombardia Region

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Waste management plans play a key role in achieving sustainable development by saving resources and recovering materials and energy from waste. Their aim is to provide a planning framework for the following: compliance with waste policy and target achievement; outline of waste characteristics and sufficient treatment capacity for managing waste; control of technological measures; outline of economy and investment requirements.

Life cycle assessment (LCA) has been chosen by Regione Lombardia as a strategic support decision tool in the preparation of its new waste management plan, which will address 2015 as the target year for the implementation. The goal is to use the life cycle thinking approach to assess the current regional situation and thus to give useful strategic indications for the future waste management.

Regione Lombardia is among the most advanced in Italy, as well as in Europe, for municipal waste management. In the year 2009 the total production of about 5 millions ton (500 kg per capita per year) was split between 48% of source separation for material recovery, 32% of energy recovery (mainly via incineration), 16% of mechanical-biological pre-treatment (MBT) and only a negligible amount to landfill.

The project started in November 2010 and it will be finished by the end of 2011. By bearing in mind that a "status part"(i.e. assessment of the state-of-the-art of waste management) and a "planning part"(i.e. definition of future developments in waste management) are the key elements of a plan, the first phase of the study consists in the analysis of the present management of municipal waste in Lombardia (baseline scenario). This implies the identification of all the fluxes of materials (both the source-separated fractions and the unsorted residual waste - URW) in terms of quantity, characteristics and destination, together with the characterisation of the most important treatment plants, in terms of their capacity, energy and materials consumption, emissions in the environment, energy and materials recovery.

The critical analysis of the results of the baseline scenario will allow to design some possible scenarios for the year 2015, with the final goal of a further improvement of the environmental performances of the regional system.

In parallel with the regional assessment, three more detailed LCAs will be carried out for the evaluation of three provinces within Lombardia, characterised by a different approach on waste management.

TUPC2-3

Screening environmental and human health impacts of municipal solid waste management in Asian cities

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Solid waste management has become a major environmental problem for the fast growing towns and cities of developing Asian countries. Asia generates annually roughly 4.4 billion tons of solid waste (SW) that consists for 0.8 billion tons of municipal solid waste (MSW). Present solid waste management in most Asian countries is disastrous. There is an urgent need in Asia for municipal solid waste management (MSWM) systems that effectively separate and process hazardous and non-hazardous waste in a healthy and environmentally sound way.

Municipal waste composition in Asia is broadly similar, though slightly influenced by climatic and cultural variations, and clearly differing between high income countries versus middle and low income countries. This enables a screening environmental and human health impact assessment and comparison based in quantitative and semi-quantitative information that matches enough across Asian countries at the same time contrasts enough between MSWM services and technologies and involved environmental and human health impacts. The screening assessment combines life cycle assessment with elements from risk assessment and environmental impact assessment. Quantitative and semi-quantitative information needed for the screening assessment is abstracted from the fast amount of relative certain information stored in existing databases and full (life cycle) assessments.

Asian MSWM stakeholders will be greatly helped with a screening assessment of existing and available MSWM services and technologies and their potential environmental and human health impacts. This facilitates them to evaluate and compare MSWM services and technologies on a screening level, before going into more details for the relevant ones in their specific MSWM context. The presentation will present and underpin the screening assessment approach illustrated with examples.

TUPC2-4

Life cycle inventory of a new technology for the inertization of MSWI fly ashes

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The quantity of fly ashes produced by municipal solid waste incineration (MSWI) is a significant percentage of municipal solid waste incinerated. Fly ashes are an hazardous waste, since they contain large quantities of soluble salts (NaCl, KCl, calcium compounds) and significant amounts of toxic heavy metals (Pb, Zn, Cr, Cu, Ni, Cd) in forms that may easily leach out.

Fly ashes inertization can facilitate their recycling as a secondary material instead of landfilling. The best available techniques for the inertization of fly ashes can be grouped into three categories: separation processes, solidification/stabilization processes and thermal processes.

Among the solidification/stabilization processes is the COSMOS process, recently developed at the Chem4Thech laboratory of the Università degli Studi di Brescia (northern Italy). This process combines the stabilization of the metals in a solid matrix with a successive washing treatment.

Recently, the European Commission has funded, with the Life+ program, a project called "COSMOS project"(LIFE08 ENV/IT/000434), that has the objective to demonstrate the industrial applicability of the COSMOS process, including the assessment of the possible environmental advantages. More information about the COSMOS project can be found here: http://www.cosmos.csmt.it.

Within the COSMOS project, with the aim to compare the environmental performance of the COSMOS process with viable alternatives, a comparative life cycle assessment (LCA) is being conducted.

In this work, the methodology and the main results of the life cycle inventory phase of the LCA of the COSMOS process will be presented. Of great interest for the life cycle inventory phase is the availability as a source of primary data of a pilot plant for the COSMOS inertization process funded through the COSMOS project.

TUPC2-5

Estrategies for improving the life cycle management of construction and demolition waste in Spain

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Spain is one of the major producers of construction and demolition (C&D) waste in the European Union, however, presents a low level of C&D waste recovery (lower than 40%) which is well below the target level established by the Waste Framework Directive -Directive 2008/98/CE- by 2020 (minimum of 70% by weight of non-hazardous C&D waste).

In order to correct the current situation and meet the requirements of the Directive 2008/98/CE is necessary to lay down a model of sustainable construction. This requires the application of ecological principles able to maximize resource efficiency and minimize waste in construction processes, use and end of life of buildings and infrastructure. One of the main challenges for the construction industry is the production and use of construction materials based on the recycling of these same materials, in order to closing the loop of materials and to minimize waste generation. Those countries with a shortage of raw materials and/or space to build landfills are the most advantaged in terms of prevention, reuse and recycling of C&D waste (e.g. Holland, Belgium or Denmark, all of them reach C&D waste recovery rates above 80%).

This work presents the drawbacks of C&D waste management in Spain and expounds some strategies to improve the current situation, some of which are being promoted in other countries with satisfactory results. We propose strategies to deal effectively with waste management throughout the entire life cycle of construction, from design, planning and execution of construction projects until the end of life of buildings and infrastructure, in order to minimize waste generation and to establish a closed cycle construction.

Firstly, we present some waste minimization strategies to incorporate at the early stages of project (design and planning considering waste minimization). Secondly, we detail some measures to reduce waste generation during project implementation and to improve the efficiency of subsequent treatments (material control, separate collection on-site and selective demolition). Thirdly, we describe new high-efficient recovery technologies (integrated treatment plants). Finally, we discuss some measures to boost the competitiveness of the market for recycled products (establishing quality standards, defining applications for recycled products, etc.), emphasizing the role of Government to promote the development of the measures raised (taxes for landfilling, incentives for recycling, etc.).

TUPC2-6

Reduction of environmental impacts in the production process of Portland cement by using Refuse Derived Fuel (RDF)

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Waste management is one of the biggest environmental issues and still a key topic for a sustainable society. Then, a possible alternative is the use of wastes as fuels for high energy consuming industries, then allowing to lower the overall emissions.

This study highlights the environmental advantages deriving from the use of Refuse Derived Fuel (RDF), partially substituting the use of conventional fossil fuels in the production of a Portland cement clinker. It is known that, due to the high production volumes and consumption, especially by fast growing countries, such as China and India, cement is one of the most impacting materials on the environment. For such a purpose the production process of a Portlan cement (CEM II/A-LL 242.5R) was studied by LCA, by following the ISO 14040 and 14044 regulations. The study followed "from cradle-to-gate"perspective and considered three different functional units in order to exploit the main outputs of the system: i) 1 ton of clinker, ii) 1 ton of cement, iii) 40 bags (25 kg each) of cement. The indicator used were GWP100 (IPCC 2007) and Eco-Indicator 99 (2001). The system was coincident to everything was connected to the production process; in the study only human resources and impacts linked to the costruction and end-of-life of machinery and transportations were neglected.

RDF is a dry grinded solid fuel obtained from the treatment of urban solid wastes. In the Robilante plant, where the cement is produced, two different factories making RDF are present: I.D.E.A. Granda that produces high-quality RDF and Ecodeco that produces normal RDF. The first one has a heating value of about 5000 kcal/kg, while the second of 4500 kcal/kg.

Both GWP100 and Eco-Indicator 99 agree that the use of RDF allows a considerable reduction of the impacts on the environment. In particular Eco-Indicator 99 states that the overall reduction was about 25%, while GWP100 detected a lowering of the CO2-equivalent emissions of about 8%. The stage involving the higher impacts is the production of clinker, since during the production of the cement, the addition of other raw materials such as gypsum and limestone allows a reduction of the overall impacts. The use of paper bag considered in the study gave credits in terms of CO2 equivalent.

TUPC3 - Carbon-based nanomaterials in the environment: tools for detection, quantification, and characterization

TUPC3-2

Towards the isolation, separation and quantification of condensed carbonaceous nanoparticles in soil and sediments

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Due to their unique chemical and physical properties, the use and mass production of condensed carbonaceous nanoparticles (CCNs), such as carbon nanotubes (CNTs) and fullerenes (C60) is on the rise. Correspondingly, concerns regarding the potential environmental and human health effects of CCNs are also increasing. However, the lack of methodologies to quantify and differentiate between various forms of CCNs in complex matrices has prevented us from assessing the potential impacts of their release into the environment. Here we present the on-going devel-

opment of a method to isolate, separate and quantify different forms of naturally occurring and man-made CCNs in soils and sediments. Our approach consists of: 1) extracting from the natural matrix the CCN isolated during a chemo-thermal oxidation treatment (CTO-375); 2) fractionating the extract into different CCN (CNTs, C60s, and soot) forms by size exclusion chromatography (SEC); and 3) quantifying and characterizing the isolated fractions via optical techniques, spectroscopy, and/or elemental analysis. This far we have adapted the CTO-375 method for large sample sizes (recoveries of spiked CCN between 97 and 155%) and have tested the CTO-375 for isolating mixtures of CNTs, C60s and soot in soil and sediment matrices. We have also developed a method to solvent extract native and spiked CCNs from CTO-375 treated soil and sediments with extraction efficiencies up to 57 ± 15% for individual solvents and 121 ± 32% when multiple solvents were used successively. Finally, we have also tested diverse identification/characterization methods to differentiate between the CCN types found in the extracts. Most of them are not selective enough to identify different CCN types in our batch extracts, but it is likely that after fractionation the characterization/identification of cleaner and more concentrated CCN subfractions will be easier. Our next targets are: a) to evaluate size exclusion chromatography (SEC) to separate and fractionate the extracted material; b) to couple and test the whole system together; c) to test the methodology on real samples.

TUPC3-3

Testing the resistance of fullerenes to chemothermal oxidation used to isolate soots from environmental samples

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Due to potential breakthroughs in a broad range of applications for manufactured carbonaceous nanoparticles (MCNPs) over the past two decades, the use and mass production of materials such as carbon nanotubes (CNTs) and fullerenes is on the rise. However, research regarding the fate and transport of these materials in the environment has been limited due to the lack of a method to quantify MCNPs in complex environmental matrices. Here we tested the resistance of five different fullerenes to chemothermal oxidation at 375 °C, a method that has been widely used and tested for quantify black carbon (BC) in soils and sediments, and was recently tested for isolating CNTs in spiked soils and sediments that contained naturally occurring BC. Of the fullerenes tested, C60 was the one that survived CTO-375 the most (50%) while C70 was the one with the lowest survival rate (< 1%). Standard additions of C60s to soil and sediment yielded recoveries between 18 and 45%. Although lower than some of the recoveries that have been observed for soot and CNTs, these results demonstrate the capability of CTO-375 to isolate C60s from solid environmental matrices. Contrary to what would be expected, standard additions of C70, C76/78, and C84 yielded higher survival rates when added to soil and sediment samples than in their pure form. These results indicate that the mineral matrices from the soil and sediment may have a catalytic effect towards C60s and a protective effect towards C70, C76/78, and C84.

TUPC3-4

Occurrence of fullerenes in aerosols from the Mediterranean Sea atmosphere

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Carbon-based nanoparticles are present in the environment due to different causes including natural events, incidental sources, as industrial and combustion processes, and during the recent years due to the use and production of carbon based nanomaterials for nanotechnological applications. Therefore, occurrence assessment of carbon-based nanoparticles is required for determining their environmental cycling and impacts.

This work describes the development, optimization and validation of a liquid chromatography coupled to mass spectrometry (LC-MS) method for the determination of a selection of fullerenes in environmental samples. The presented method has been applied to the study of the occurrence of natural and synthetic fullerenes (C60, C70, N-methylfulleropyrrolidine, C60 pyrrolidine tris-acid ethyl ester, [6,6]-Phenyl-C61 butyric acid butyl ester and [6,6]-Thienyl C61 butyric acid methyl ester) in airborne samples from the Mediterranean Sea. This new method reached sensitivities in the pg/m3 range, with repeatabilities between 1,8% and 13,2%.

While the presence of fullerenes in wastewater [1] and its occurrence in the atmospheric particulate (mainly associated with coal combustion processess [2] and domestic kitchen stoves burning natural gas/air and propane gas/air mixtures [3]) have already been reported, to our knowledge, this work is the first study to be about the occurrence and quantification of fullerenes in sea airborne.The results can be reasonably related to incidental emission and posterior atmospheric transport and deposition, underpinning the need of studying the possible risks associated to the presence of carbon nanoparticles in the environment and the need of evaluating the possible consequences of their ubiquitous distribution, which is facilitated by long range atmospheric transport.

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[2] S. Utsunomiya et al. "Uraninite and Fullerene in Atmospheric Particulates", Environmental of Science and Technology, 2002, 36 (23): 4943-4947
[3] L.E. Murr et al. "Carbon nanotubes, nanocrystal forms, and complex nanoparticle in common fuel-gas combustion sources and the ambient air", Journal of Nanoparticle Research, 2004, 6(2): 241-251

TUPC3-5

Characterization and toxicity tests of multiwalled carbon nanotubes and cyclodextrin polymers on selected test organisms

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The escalating use of engineered nanomaterials has since led to the need to study their possible adverse effects to the environment. The key in carrying this out is in their full characterization and testing on living organisms of different trophic levels. In our laboratories, selected carbon-based nanomaterials in the likes of pristine multi-walled carbon nanotubes (MWCNTs), oxidised multi-walled carbon nanotubes (oxMWCNTs), cyclodetrin polymers pCDs, and cyclodextrin-multiawalled carbon nanotube polymers (CD-oxMWCNTs) were exposed to Daphnia pulex (water flea), Vibrio fisheri (luminescent bacteria), Selenastrum capricornutum (algae) and Poecilia reticulatae (fish). The nanomaterials were characterized and their toxicities were observed on these test organisms. The toxicities were explained in the light of the nanomaterials' characterization. The particle size distribution was found to be the same for both pristine and oxidised MWCNTs (14.5-93.7 nm). The FTIR showed that the acid oxidation used to produce oxMWCNTs introduced new functional groups that made these nanomaterials more toxic than their pristine counterparts. The EDX showed some residual Fe-catalyst impurities in the pristine MWCNTs and TEM showed amorphous carbon which was not observed in the oxMWCNTs. The toxicities observed also showed oxMWCNTs were more toxic than pristine MWCNTs. The surface charges of pristine MWCNTs were 0 mV and oxMWCNTs was 3.4 mV, thus pristine MWCNTs agglomerated while oxMWCNTs dispersed well in water. The SEM images of these materials showed a fibrous appearance. The polymers were polymerized using hexamethylene diisocyanate (HMDI) as a linker showed that their particle sizes were comparable yet the toxicity of CD-oxMWCNT was higher than that of pCDs through the tested organisms. Their charges were 0 and 0.7 mV, respectively. Their particle size distribution was 10-79.2 nm. The SEM images of the nanomaterials showed a somewhat razor sharp, flaky appearance of CD-oxMWCNTs while that of pCD was only flaky. The conclusions drawn were that the primary factor of nanomaterials' toxicity is their size. The surface charge or absence of it thereof, is also important in the materials's toxicity since it determines its mode of penetration into the organism. Full characterization is therefore, key into the understanding of interaction of nanomaterials and the environment.

TUPC3-6

Sorption of hydrophobic and very hydrophobic PAHs onto carbon nanotubes in the low concentration range

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Production of CNTs is expected to increase rapidly in the future and CNT release into the environment has been shown. Understanding the interactions between organic contaminants and CNTs is therefore essential for evaluating the potential environmental impact. Although a great deal of work has been published in the past years, data is still limited in terms of compounds, concentrations and conditions investigated. This can be mainly explained by some limitations associated with classical batch setups, traditionally used to measure sorption. First, the high sorption capacity of CNTs results in extremely low concentration of sorbates remaining in the aqueous phase. Second, classical separation techniques (e.g. centrifugation or filtration) are often not adequate to efficiently separate the CNTs and liquid phase under conditions where CNTs may be partially dispersed (e.g. presence of humic acids). Questions thus remain open on the possible succession of sorption mechanisms occurring over a range of concentration and environmental conditions.

In the present study, we applied a passive sampling method (POM-SPE) previously validated for sorbents with similar characteristics as CNTs to investigate aspects of sorption that have not been studied to date. The first objective was to gain a better understanding of the interactions between polycyclic aromatic hydrocarbons (PAHs) and CNTs over a range of concentrations. Sorption isotherms of phenanthrene and pyrene were measured over six to seven orders of magnitude. In the low concentration range (pg/L), sorption of both PAHs was linear on a non-logarithmic scale. Sorption can thus be described using a single sorption coefficient over several orders of magnitude. Isotherm fitting over the whole concentration range showed that the CNT sorption capacity was directly related to their surface area. Sorption coefficients for 13 PAHs (11 of which were never reported to date) were also measured using the passive sampling method. Conversely to results previously reported at higher concentrations, no competition seemed to occur in the low concentration range. Sorption affinity also appeared to be directly related to the hydrophobicity of the compounds (Kow) which is extremely valuable for the development of predictive equations. Further experiments are currently undergoing to study sorption under conditions in which CNTs are dispersed and to analyse how sorption characteristics are affected.

TUPC4 - Hazard assessment for the marine environment and regulation

TUPC4-2

Marine ecotoxicology testing: towards a standardised suite of chronic methodologies for chemical risk assessment

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Several European regulatory regimes include requirements for the hazard assessment of chemicals in marine (water and sediment) systems, but few specify the ecotoxicology test methods that should be used. In particular there is a lack of consensus on the methods that should be used to assess chronic marine toxicity. The absence of a standardised suite of chronic marine tests has led to a reliance on those (usually few) academic studies available in the open literature, or on the use of "read-across"from freshwater testing data. Chronic marine chemical hazard assessment can therefore be hampered by the low quantity/ quality of appropriate ecotoxicology data, or by differences in the fate and behaviour of chemicals and the physiology of receptors in freshwater and marine systems. Effects specific to marine systems and organisms can be missed, and the uncertainties inherent in "read-across"from freshwater data to marine receptors require the application of large safety factors to any toxicity thresholds derived in this way. Chronic marine ecotoxicology testing methodologies have been developed for use in marine monitoring programmes (e.g. OSPAR JAMP/CEMP) and for assessing the hazards posed by industrial discharges (e.g. OSPAR Whole Effluent Assessment) but these methods have not been widely applied in single chemical hazard assessments. This may be because, in validation terms, the techniques are considered to be relatively immature compared to the freshwater methods, or because of the potential challenges in adapting the methods for use with more rigorous single-chemical testing systems. This poster evaluates those chronic marine ecotoxicology methods currently prescribed for use in European marine monitoring programmes and assesses their potential for adaptation for use in regulatory chemical hazard assessments. The available methods are compared using the criteria set out in the comprehensive validation assessment framework developed by the European Commission's FP6 NORMAN Network (with a focus on single-chemical testing). Based on the outcomes of these evaluations a standardised suite of chronic marine ecotoxicology tests is proposed, along with suggestions for optimizing their performance for use in chemical hazard assessments for marine water and sediment.

TUPC4-3

Integrated assessment of bioeffects monitoring data in the Baltic Sea - a case study with flounder, eelpout and herring

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In the light of the EU Marine Strategy, integrated monitoring and assessment receive increasing attention. Data for integrated assessments are converted to a traffic light system, enabling com-

parison of multiple bioeffects on a common scale. Threshold values are adjusted for fish species as well as for bioeffect methods. If different species are combined in one approach, they should be comparably sensitive to the parameter measured in general. In a case study with data on PAH metabolites in bile, fish diseases, liver histopathology, and vitellogenin measured in flounder (*Platichthys flesus*), eelpout (*Zoarces viviparus*), and herring (*Clupea harengus*) from the Baltic Sea this approach was tested. The data were collected during sampling campaigns of the pan-Baltic BONUS+ project BEAST which is targeted at developing integrated measures of chemical pollution and tools to detect and understand human-induced pressures on the Baltic Sea ecosystem. The results will contribute to science-based recommendations for the implementation of an integrated chemical-biological effects monitoring strategy for the assessment of ecosystem health.

TUPC4-4

The derivation of a marine predicted no effect concentration for bisphenol A: the case for a species sensitivity distribution approach under REACH

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REACH guidance allows for the derivation of predicted no-effect concentrations (PNEC) based on (1) the traditional "assessment factor"approach that divides no observed effect concentrations (NOEC) of sensitive key species by a factor of typically 10 to 1000 and, (2) species sensitivity distribution (SSD) approaches when data exists for at least 8 taxonomic groups. Statistically based approaches, such as SSD, make use of the full distribution of available no-effect values, taking into account the variability that might be observed between taxonomic groups. Current European Chemicals Agency guidance developed for REACH provides specific procedures on the use of the SSD approach for the assessment of freshwater, while not so for marine water. Marine environments are considered to be more diverse and thus greater uncertainty may exist in the extrapolation between species responses. Therefore, within the guidance, calculations of PNEC for the marine environment are typically addressed using assessment factors of 10 to 10,000, depending on the number of acceptable toxicity tests available. Since freshwater and marine data can be combined in the assessment factor approach to derive a PNEC for marine water, this same practice should be appropriate for developing a marine PNEC using the SSD approach. This is especially true when high quality chronic freshwater and marine data exist for multiple taxonomic groups. Bisphenol A (BPA), is an important high production volume compound used in the production of epoxy resins and polycarbonate plastics. With the addition of new chronic marine aquatic toxicity studies using the sheepshead minnow (Cyprinodon variegatus) and the mysid shrimp (Americamysis bahia), BPA now has an extensive toxicity database of both freshwater and marine organisms from diverse taxonomic groups. Freshwater and marine PNEC for BPA were derived using both the traditional assessment factor and an SSD-based approach. PNEC derived using assessment factor and SSD-based approaches are compared and will be used to revise the risk characterization for BPA in freshwater and marine environments for submission of the "technical dossier"under the REACH regulation.

TUPC4-5

Deriving a safe level for copper in the marine environment

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The last five years have seen significant developments in the approaches used to define safe levels for metals. In particular, the better understanding of bioavailability has provided new tools such as the Biotic Ligand Model which have allowed the derivation of 'Predicted No Effect Concentrations' (PNECs) based upon improved use of available data. The approach described shows how these tools can be used to derive a PNEC for copper in the marine environment, and provides information on how the safe level was tested experimentally.

TUPC4-6

Deriving a safe level for copper in marine sediment

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Predicting the effects of metals in the sediment compartment has historically been a challenging task, because of the ameliorating processes which occur in the sediment compartment. The use and applicability of the Equilibrium Partitioning Model to derive safe levels for copper in the marine sediment environment is assessed, and conclusions drawn based upon experimental data available.

TUPC4-7

Marine risk assessment of molybdenum under REACH: generating data, developing of a species sensitivity distribution and PNEC derivation

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In an effort to generate a robust $PNEC_{marine}$ for risk assessment purposes which is based on the SSD approach, a marine research program was initiated by the REACH Molybdenum-Consortium as part of the REACH dossier preparation. As a first step, the International Molybdenum Association (IMOA) commissioned a thorough evaluation of all existing chronic toxicity data for molybdate in the marine environment. Based on the outcome of this review a testing program was conducted aimed at generating the data necessary to 1) develop a species sensitivity distribution for molybdate, and 2) derive a $HC_{5,50\%}$/PNEC for the marine aquatic compartment.
One reliable chronic data point was identified in open literature for the mussel *Mytilus edulis*. Consequently, nine different tests were conducted at four different testing laboratories, representing eight taxonomic groups that are relevant and typical for the marine environment: the copepod *Acartia tonsa*, the diatom *Phaeodactylum tricornutum*, the oyster *Crassostrea gigas*, the mysid *Americamysis bahia*, the micro-alga *Dunaliella tertiolecta*, the macro-alga *Ceramium tenuicorne*, the sheepshead minnow *Cyprinodon variegatus*, the sea urchin *Strongylocentrotus purpuratus* and the sand dollar *Dendraster excentricus*. Where possible, the chronic toxicity experiments were conducted according to internationally accepted standard testing protocols (e.g., EPA, ASTM, ISO). Dissolved molybdate levels were measured and reported effect levels (NOECs, EC_{10}-levels) were based on these measurements.
No-effect levels for dissolved molybdenum were situated between 4.4 mg Mo/L and 1,174 mg Mo/L, i.e., a difference of a factor of 267 between the most and least sensitive species. The mussel *M. edulis* and the copepod *A. tonsa* were the most sensitive species. The least sensitive species were the oyster *C. gigas* and two algal species (*D. tertiolecta*, *C. tenuicorne*). The $HC_{5,50\%}$ (± 95%CL) that was associated with the Log-Normal Distribution that was plotted through the chronic data was 5.74 mg Mo/L (95%CL: 0.58 - 21 mg Mo/L). Application of an assessment factor of 3 on this HC5,50% resulted in a $PNEC_{marine}$ for molybdenum 1.192 mg Mo/L. This value is in line with the Final Chronic Value (FCV) of 1.16 mg Mo/L that was determined according to the USEPA methods, i.e., a method based on the four most sensitive genera - taking into account the size of the toxicity database (n=10).

WEPC1 - Assessment of the effects of veterinary drugs on dung and soil organisms

WEPC1-1

Higher tier test strategy for dung fauna organisms - from regulatory perspective

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According to European legislation, an environmental risk assessment of veterinary pharmaceuticals for dung fauna is required if the substance acts as a parasiticide for the treatment of pasture animals. However, the demonstration of the environmental safety of those substances for dung fauna was strongly hampered by the fact that no standardized tests were available until recently. Therefore, starting with recommendations from the SETAC advisory group DOTTS (Dung Organism Toxicity Test Standardization) test systems for phase II tier A tests on the mortality of dung fly and dung beetle larvae were developed. The dung fly test guideline No. 228 was adopted by OECD on October 2008, while an OECD guidance document for standard laboratory tests with dung beetles is available since 2009. If a risk is identified for dung organisms in phase II tier A of the risk assessment process, a higher tier test strategy is required for tier B - but currently no advice is given for those studies in the existent EMEA and VICH guidelines. No validated higher tier laboratory or semi-field methods are available to assess structural and functional effects of pesticides in dung. Therefore, three workshops took place in 2007, 2008, and 2009 with international dung fauna experts in order to find an appropriate test strategy for higher tier dung fauna (Aveiro group). Possible strategies were discussed and adequate endpoints were defined for higher tier tests. Further on, strategies and endpoints were analysed for their practicability in laboratories considering different dung species, test compounds and endpoints.
The aims of the presentation are:
-To present details of higher tier testing requirements.
-To highlight the current state of knowledge regarding methods for dung organisms and to discuss methods to assess the 'impact' of parasiticides on dung community structure and function.
-To give an overview on higher tier laboratory and field methods which may be appropriate for Tier B dung organism effects studies.
-To discuss modeling approaches for higher tier dung testing.

WEPC1-2

Veterinary drug ivermectin and behaviour of earthworms

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Since 1990s behaviour responses of earthworms to contaminated soils have been well documented and two systems for avoidance test were developed (two-chamber and six-chamber system). The avoidance test has been described in ISO 17512-1 since 2008.
Although the effects of many substances on behaviour of earthworms have already been tested the information on influences of veterinary drugs (e.g. avermectins) are still lacking. Avermectins are broad spectrum parasiticides belonging to macrolide endectocides. Nearly all avermectins administered to animals are excreted into cattle manure (98 %). High concentrations of avermectins in soil and feces of treated animals can have negative effects on invertebrates, including earthworms, which play an important role in decomposition of dung on pastures. Studies were already performed to measure effects of avermectins on mortality, reproduction (cocoon production, hatching of juvenile earthworms), bioaccumulation and growth.
We studied the influence of ivermectin (belonging to avermectins) on behaviour of two earthworm species, Eisenia fetida (epigeic species) and Lumbricus terrestris (anecic species). We measured the effect of ivermectin on two earthworm species with avoidance test. The avoidance test was performed with two different soils, Lufa 2.3 natural soil and soil provided by Cinkarna Celje (Slovenia). The same soil was used on both sides of the test vessel. Earthworms Eisenia fetida were exposed to both types of soil, Lumbricus terrestris only to soil provided by Cinkarna Celje. Both soils were spiked with following ivermectin concentrations: 8, 32, 64 and 256 mg kg-1.The results of the avoidance test were presented as the mean number (±SD) of living earthworms in the test soil and the percentage of remaining worms in the test soil. Test soil is considered to be toxic if more than 80 % of earthworms migrate to control soil.
Our study showed that ivermectin, under experimental conditions stipulated by ISO 17512-1, does not have a deterrent effect on earthworms up to the highest concentration tested (256 mg kg-1). The avoidance test with Lumbricus terrestris exposed to soil from Cinkarna Celje showed that in all concentrations more than 20 % of the earthworms stayed in the test soil. We observed the same distribution of worms in the avoidance test with Eisenia fetida exposed to soil from Cinkarna Celje. Eisenia fetida in Lufa 2.3 soil was attracted to soil spiked with 32, 64 and 256 mg ivermectin kg-1 soil.

WEPC1-3

Sublethal effects of two parasiticides on the dung beetle Aphodius constans

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Currently, no advice is available how to proceed in case a risk is identified when testing the effects of a parasiticides on dung organisms in Phase II, Tier A laboratory tests. In this contribution we want to present (A) two test ideas using the beetle species Aphodius constans, (B) compare these

results with data gained in the larval Emergence Test with the same species published recently as an OECD Guidance paper and, [C] discuss these ideas in the light of the development of a higher-tier test strategy with dung organisms. The main properties of the existing dung beetle test as well as in the elongated larvae test and the adult reproduction test differ primarily in the end-point measured, the life-stage of the test organisms and the duration of the test. As a general rule, the new tests try to cover more than one life stage and a sub-lethal endpoint, aiming to increase the sensitivity of the test. These methods have already been set-up at two laboratories (ECT GmbH (Germany) and of the University of Montpellier (France)), indicating that the test performance is possible without considerable problems. All tests performed so far were valid according to the (preliminary) criteria proposed so far. Test concentrations were spiked into cattle dung using acetone as a solvent. All concentrations are given as nominal values in mg a.i./ kg dung dry weight (DW). As an example the results obtained in the Adult Reproduction Test, performed with Ivermectin in parallel in the laboratories of ECT GmbH and the University of Montpellier, are as follows: Despite the fact that different concentrations were used in the two tests, the resulting EC50 values were almost the same: 0.29 (0.21 - 0.40) and 0.32 (0.24 - 0.43) mg a.i./ kg dung dry weight (DW) were determined at ECT GmbH and the University of Montpellier, respectively. The corresponding EC50 value obtained in the Elongated Larvae Test at ECT GmbH was 0.26 mg a.i./ kg dung dry weight (DW). Thus, with both new methods a higher sensitivity compared to the established OECD Larvae Test was found. The results obtained in the two laboratories show that the new test methods are practical and sensitive. However, so far only two parasiticides could be tested. In the long run, and assuming standardisation as an OECD guideline is completed, these tests could be used for Phase II, Tier B in case a risk has been identified in lethal laboratory tests in Phase II, Tier A.

WEPC1-4

Lessons learned during the selection of a dung beetle species as a bioassay for insecticidal residues in cattle dung

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Pastured animals treated with parasiticides may faecally excrete residues harmful to non-target organisms. European legislation requires parasiticides to be tested for such effects using species of dung-breeding flies and dung-breeding beetles (Scarabaeidae). The latter include species that may be 'dwellers', 'tunnelers', or 'rollers'. Dwellers develop in dung on the soil surface. Tunnelers and rollers develop in dung which is deposit by the parent beetles into the soil. Adult tunnelers bury dung into the soil immediately below the pat, whereas adult rollers bury dung some distance from the pat. The beetle, Aphodius constans, is a dweller species that recently has been approved as a test species for insecticidal residues in dung (OECD 2009). However, results of toxicity tests using A. constans may not extend to tunnelers and (or) rollers. Thus, the tunneler species Onthophagus nuchicornis, recently was evaluated as a second species by members of the SETAC advisory group DOTTS (Dung Organism Toxicity Test Standardization). Preliminary results using adults field-collected in spring, showed O. nuchicornis to have high susceptibility when developing from egg-to-adult in cattle dung containing residues of the parasiticide, ivermectin. These results could not be replicated in a subsequent ring test (8 organizations in five countries), because adults field-collected in autumn failed to exhibit reproductive behaviour. This observation indicates that adult O. nuchicornis require an obligate diapause period prior to mating. A second international ring test is planned for spring 2011 using overwintered beetles.

WEPC1-5

Effect of oral ivermectin application to pony's on the dung colonising insect fauna of a semi-natural pastureland

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The use of large herbivores for the management of vegetation in nature conservation areas in The Netherlands creates opportunities for dung insect fauna and wildlife that feeds on these invertebrates. However, the routine application of toxic anthelmintics such as ivermectin to these herbivores potentially affects dung fauna populations. The negative impact of ivermectin and other macrocyclic lactones used in animal husbandry on dung fauna are well documented in the scientific literature. However, it is largely unknown to what extent these effects do also occur in conservation areas. Moreover, past research on the ecological effects of anthelmintics like ivermectin has tended to focus mostly on the insect fauna in cattle dung and less on the possible negative effects in dung of other large herbivores used for conservation purposes such as sheep and particularly horses. We conducted a combined field and laboratory experiment in order to investigate the effects of ivermectin treatment of pony's on the dung colonising insect fauna in The Netherlands. A second goal of the study was to test a method to evaluate the effects of parasiticides on dung insects in the field. The field experiment was conducted in a semi-natural grassland situated in a connective corridor of the Dutch Ecological Main Structure (EHS). The pasture is managed in a non-intensive, organic way and grazed all year round by a small herd of Dartmoor pony's. Dung pats of ivermectin-treated and untreated pony's were left in the field for colonisation and taken to the laboratory to capture emergent insects over the following 3 months. The study demonstrated that oral ivermectin treatment of pony' s at the recommended medical dose has a negative effect on the emergence of some of the most important families of flies that colonise the dung of these animals: Sphaeroceridae, Muscidae and Stratiomyiidae. Although dung beetles are abundant in the study area, very few of them emerged from the pony dung incubated in the laboratory. The exact cause for this remains to be investigated. In general, the study design is suitable to evaluate the effects of parasiticides on dung insects under field conditions such as in testing for higher-tier risk assessment.

WEPC1-6

Effects of antibiotics on microorganisms and the nitrogen cycle in soil

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The fertility of soil largely depends on the availability of nutrients, particularly on inorganic nitrogen, and thus, on the continuous formation of ammonium and nitrate by soil microorganisms. Consequently, the balanced functioning of all pathways of the nitrogen cycle has to be guarantied in soil.

Veterinary drugs, e.g. antibiotics, enter the environment via manure application and influence the soil organisms and nutrient cycles. The present study gives information on the effects of manure from pigs treated with sulfadiazine (SDZ) and difloxacine (DIF), respectively, on soil microorganisms and the nitrogen pathways (nitrogen mineralization, nitrification, denitrification) in a mesocosm experiment under controlled moisture, temperature and light conditions. SDZ and DIF containing manures were added to an agricultural soil (Orthic Luvisol), respectively. Then, pre-grown maize seedlings were planted and cultivated on the soil. Microbial analyses with focus on the rhizosphere were performed at the beginning and in distinct intervals up to 62 days after manure addition.

The microbial biomass amount and all processes of the nitrogen cycle were stimulated by manure addition. However, the presence of SDZ restricted the enhancing effect of manure significantly. The nitrogen mineralization was influenced temporarily. The potential nitrification and denitrification showed decreased activities in both soil compartments during the whole incubation time. In contrast, DIF applications caused minor effects. This can be attributed to the fast adsorption of the antibiotic.

WEPC1-7

Effects of monensin and lasalocid on heavy metal accumulation in woodlice

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Monensin and lasalocid are polyether ionophore antibiotics used in prevention of coccidiosis in the poultry industry and as growth promoters in cattle. They enter the environment via the faeces of treated animals. As ionophores, they affect the passage of ions across cellular membranes and could therefore change the patterns of heavy metal accumulation in soil organisms. These effects were studied on woodlice Porcellio scaber. The animals were exposed to cadmium and lead in the presence of monensin or lasalocid and their Cd and Pb contents were measured. The results imply that ionophore antibiotics could have a marked effect on the accumulation of heavy metals in soil organisms on metal-contaminated sites.

WEPC1-8

Ecological vulnerability of wildlife species for ivermectin

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The ecological vulnerability method is developed as an additional tool in risk assessment. The method uses ecological traits for individual species of wildlife to assess (1) their exposure to soil contaminants, (2) internal regulation and toxicological sensitivity to toxicants, and (3) potential for population recovery from harmful effects. Vulnerability of wildlife species was studied for different model compounds, including the persistent veterinary pharmaceutical ivermectin, with low to medium toxicity. The species did not include dung insects. The field distribution of ivermectin is more patchy than other persistent compounds, and can be very different between plots. Vulnerability of wildlife species for ivermectin was mostly determined by exposure through habitat: a preference for soil as the main habitat increases vulnerability. The life history strategy characteristics of species vulnerable to ivermectin are intermediate in the spectrum from r-strategy (opportunistic) to K-strategy (long-lived). In the category population recovery, most characteristic factors correlated with a low vulnerability. The most vulnerable species included Slow worm, Viviparous lizard, European mole, and Sand lizard. The method assesses direct effects on species. Indirect effects such as food depletion of higher animals caused by ivermectin effects on invertebrate food species cannot yet be properly predicted by our method for lack of generic data. This should be further studied.

WEPC2 - New developments in aquatic macrophyte testing, higher tier risk assessment and ecotoxicology

WEPC2-1

Rooting for Lemna: the toxicological and statistical sensitivity of an under-utilized endpoint

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Duckweeds (*Lemna* spp.) are floating macrophytes used extensively in ecotoxicology for regulatory testing, although their utility as a standard test species has been scrutinized due to their lack of an extensive rooting system from which toxicity can be assessed. Despite criticism, root length is a valuable endpoint to use in the development of risk assessment models such as the Biotic Ligand Model (BLM) for plants, where metal accumulation on the root is empirically related to toxicity. The purpose of this study was to assess the toxicological and statistical sensitivity of root length as an endpoint compared to the standard endpoints of plant dry weight and frond number. To achieve this, the toxicity of copper, nickel and lead to *Lemna minor* was assessed using a standard bioassay. Testing was performed with surface waters (5 to 7 waters per metal collected from various American locations) that had a range of water quality characteristics (e.g., DOC, pH, and $CaCO_3$/L hardness). To assess the toxicological sensitivity of each endpoint, IC_x values (10%, 20% and 50% plus CIs) were calculated by using non-linear regression analysis to describe the relationship between the measured concentration of total dissolved Cu, Ni or Pb in solution and new growth only in the endpoints. To assess statistical sensitivity of each endpoint, the coefficient of variation, the minimum detectable difference, and the replication required to detect a 20% changed were calculated. Root length was found to be intermediate of frond number and dry mass, in terms of statistical sensitivity, with dry mass being the most sensitive endpoint evaluated. In terms of toxicological sensitivity, all IC_xs assessed for comparable testing scenarios (i.e., same metal and surface water) were within 10-fold of each other, with Cu being the most toxic metal, followed by Ni, then Pb. Still, no one endpoint was consistently the most responsive under tested exposure conditions. Overall, root length was found to be as statistically sensitive as the standard endpoints and at times significantly more toxicologically responsive. In contrast to the critique of duckweed lacking of a testable rooting system, this study shows that root elongation can be a valuable complement to currently recommended endpoints. These results allow for enhanced ERAs for aquatic macrophytes, since the endpoint is well suited to *in-situ* field measurements and consistent with the development of the BLM for plants.

WEPC2-2

Lemna minor vs. Myriophyllum aquaticum: sensitivity and recovery potential after short and long term exposure to atrazine

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Two concepts have been highlighted as tools for refining ecotoxicological risk assessment and adding more ecological realism: the trait-based approach that has been built upon the hypothesis that sensitivity is a function of biological characteristics, and vulnerability analysis that combines susceptibility to exposure, sensitivity to a stressor and recovery potential. In recent years the risk assessment for aquatic macrophytes has triggered increasingly scientific attention, because in the past they had been barely taken into account in ecotoxicological assessment even though they are a significant component of aquatic ecosystems. Despite the great variety of species and important differences in morphology, physiology and life form, aquatic plants are only represented by duckweed species in risk assessment. Water and water-sediment tests with *Myriophyllum acquaticum* were recently developed in order to demonstrate more realistic exposure scenarios in refined risk assessment.
The poster will show the results of non-simultaneously conducted single - species laboratory toxicity test with two macrophyte species *Lemna minor* and *Myriophyllum aquaticum* exposed (short and long term) to atrazine - systemic herbicide with well known mode of action (inhibition of photosynthesis) still widely used throughout the world in agricultural applications. The recovery potential of both species after short and long exposure was estimated using growth rate, biomass and concentration of chlorophyll a as endpoints. *M. aquaticum* proved to be more sensitive to atrazine than *L. minor* after short as well as long term exposure. Also, its recovery potential is by far lower: *L. minor* recovered completely after short (3 days) and long (7 days) exposure to all test concentrations (max being 1280 _g/l) after only 6 and 5 days respectively, while the recovery of *M. aquaticum* was significantly slower and less efficient even after short term exposure (3 days): after 12 days long recovery period, it turned out that the concentration of 320 _g atrazine/l had caused irreversible changes and the recovery was impossible. The comparison of the results gained in water and water-sediment toxicity tests of atrazine on *M. aquaticum* will also be presented in a poster. The different experimental setup, different endpoints, the relative species-sensitivity and the applicability of vulnerability analysis (focusing on recovery potential) in ecotoxicological risk assessment will be discussed.

WEPC2-3
Myriophyllum spicatum as a test organism for eco toxicity testing and the impact of sucrose in the test medium on the photosynthesis activity and sensitivity of the test species
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The use of plant protection products is a common practice in modern agriculture and forestry. Their use, however, may result in risks for the environment because even correct application can not always prevent a possible contamination of the off-field environment. A contamination of surface waters may occur through driftage, precipitation, run-off and drainage from treated surfaces.
Dicotyledonous macrophytes are not part of the initial risk assessment of plant protection products in the aquatic environment although they are an important part of the ecosystem. In this context alternative test methods with Myriophyllum sp. are under development.
The Ecotoxicological Laboratory of the German Federal Environment Agency has conducted a standardised single-phase test system with the dicotyledonous water milfoil Myriophyllum spicatum following the ASTM Designation E 1913-04 (in Andrew's medium with 3% sucrose). In order to obtain substance-specific ecotoxicity data, a single-phase test system without sediment was chosen, to get results that are independent of the distribution of the test substances between water and sediment. The exposure of the test-plants only in the water phase reduces time and effort for analytics and facilitates modelling of the results.
Also, a direct comparison with results from investigations with the monocotyledonous duckweed Lemna spec. (OECD Guideline 221) is possible.
The used Andrew's medium and the presence of sucrose in the test system could impair photosynthesis activity and thus the sensitivity of the test species. To check and to quantify these unknown effects further tests with photosynthesis inhibitors were conducted. The results of tests with Lemna minor in a sucrose-free medium and Myriophyllum spicatum in Andrew's medium with 3% sucrose will be compared directly.

WEPC2-4
Effects of linuron on a sediment-rooted aquatic plant in sediment-dosed test system
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The availability of standardized methodologies to assess the environmental risks of pesticides to non-target aquatic plants is currently limited. Aquatic plant test guidelines are only available for algae and Lemna as representatives of primary producers. These studies may not be sufficient especially in the case when contamination via sediment is an important exposure pathway. In that case information on toxicity to sediment-rooted aquatic macrophytes may be required. Therefore a standardized test method protocol for the rooted aquatic macrophyte, Myriophyllum sp. has currently been proposed. This test method enables the study of exposure to a substance either via the water phase or via the sediment. Spiked sediment of the chosen concentration has to be prepared by addition of a solution of the test substance directly to the sediment. Test vessels have to be filled with spiked sediment. Three plants with known weight and shoot length are assigned to each replicate.
A study was initiated to investigate the effects of exposure via the sediment on a sediment-rooted aquatic plant, i.e. Myriophyllum spicatum. The objectives of the experiment were to quantify the exposure in the aquatic plant test and to assess the development of linuron concentrations in the compartments of the test system. The sediment was spiked with a concentration range of the herbicide linuron. Plant pots with sediment and three unbranched top shoots of Myriophyllum spicatum were placed into test vessels with standardized artificial sediment and Barko and Smart medium, and cultivated for 21 d under optimized conditions in a climate room. During the test period herbicide concentrations in sediment, water medium and plants were monitored. Although the artificial sediment was covered with a thin layer of sand and 1cm layer of non-contaminated sediment in order to minimize the exchange between sediment and the overlying water, linuron was already measured in the water layer in toxic concentrations at day 7. It is evident that linuron has not only effects on the plant through sediment exposure but also through exposure in the water medium. Overall effects of linuron on Myriophyllum spicatum are presented and discussed.

WEPC2-5
A single species test with the filamentous green algae Oedogonium sp. for higher tier risk assessment
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Metaphyton, which is defined as filamentous, free-floating and scum-forming algae, is often the largest contributor to total algal productivity, especially in nutrient-rich shallow ponds and ditches. Many epiphytic algae and invertebrates associated with scums of metaphyton differ from those present in the plankton and on the sediments. Hence, metaphyton scums are unique and important communities within pond ecosystems.
When assessing environmental risks of chemicals and pesticides, aquatic primary producers are often represented by phytoplankton, macrophytes, and periphyton. However, it has been recently shown that also the metaphyton might be important to consider in environmental risk assessment due to the partly high sensitivity of its species to pesticides: In aquatic mesocosm studies, herbicides and fungicides were often found to cause pronounced direct effects on the metaphyton biomass (e.g. *Oedogonium*) and, as a consequence, indirect effects on water chemistry, species composition of plankton and invertebrates, and development of macrophytes. Despite its high ecological relevance, guidance for biotests with metaphyton is lacking until now.
Oedogonium is one of the most common members of the metaphyton as well as the genus *Cladophora*, which may also frequently detach from substrates to form floating scums. We developed a method for ecotoxicological testing of these filamentous green algae in the laboratory in vessels with and without sediment. The poster will show the experimental setup and exemplary results for one pesticide.

WEPC2-6
Bioaccumulation of pharmaceutical active compounds and their metabolites in Ceratophyllum demersum and their resulting effects on the chlorophyll content
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The occurrence of pharmaceuticals in surface water is an emerging problem worldwide. They are present in surface water at concentrations in the µg L-1 range. If pharmaceutical active compounds (PhACs) are transferred to drinking water, the resulting involuntary low-dose medication of large population groups may compromise public health. In addition, aquatic life may be affected. The physiological effects of 5 PhACs such as Bezafibrate (BZF), Carbamazepine (CBZ), Ibuprofen (IBU), Levonorgestrel (LNG) and Metoprolol (MET) were tested on a submerged macrophyte *Ceratophyllum demersum* in the laboratory over 1, 4, and 7 days. Concentration of PhACs in exposure medium and tissue was determined with LC/MS-MS. Bioaccumulation is important in the interactions between living organisms and PhACs in aquatic systems. Hence, to create a broader basis for the evaluation of the ecotoxicological relevance of PhACs, bioaccumulation tests were performed with different concentration of the five selected PhACs. As an additional ecotoxicological endpoint chlorophyll content was assessed.
Uptake of the PhACs in *C. demersum* showed a two step-process. In the first 24 h the PhACs were taken up more rapidly than in the second phase, which lasted until the end of the experiment. Total Chlorophyll and chlorophyll b were significantly decreased in exposed plants. LC/MS-MS measurement revealed not only the bioaccumulation of the PhACs, but also the presence of metabolites, which yields to the conclusion that the PhACs are partly biotransformed in the macrophyte.
The data of this study clearly revealed that PhACs can be accumulated by the submerged plant *C. demersum*, having also an effect on the total chlorophyll and chlorophyll b content.

WEPC2-7
Impact of the herbicide Indaziflam in macrophyte-dominated microcosms including macrophyte-bioassays
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Aquatic toxicity tests with the herbicide Indaziflam indicate that primary producers, particularly Lemna gibba, comprise the most sensitive standard test species. As a higher-tier test, an outdoor microcosm experiment was conducted characterized by naturally established macrophyte-vegetation and in situ macrophyte-bioassays. The bioassays were introduced to achieve concentration - response relationships for a wider array of macrophyte species. Eighteen outdoor microcosms were used to study herbicide concentration - response relationships during a 70 day post-treatment period (4 controls and 2 test systems each once received 0.01 - 0.032 - 0.10 - 0.32 - 1.00 - 3.20 and 10.0 µg a.s./L). Effects on macrophytes (biomass, growth), phytoplankton and zooplankton (species composition, abundance) and water quality parameters (e.g. pH, Dissolved Oxygen) were evaluated using univariate and multivariate statistical techniques.
In the microcosm study, several macrophyte species were far more sensitive than phytoplankton and zooplankton taxa. The lemnids Lemna gibba (in situ bioassay) and Spirodela polyrhiza (volunteer species) were most sensitive (NOEC of 0.01 µg a.s./L). Several non-lemnid bioassay plants started to show effects at (Myriophyllum spicatum, Elodea canadensis) or above (Salvinia natans, Potamogeton natans, Sagittaria sagittifolia) a treatment level of 0.32 µg a.s./L. The sensitivity of "free-growing"Myriophyllum spicatum was similar to that in the in situ bioassay and recovery of both "free-living"and bioassay Myriophyllum plants was not observed at a treatment of 1.0 µg a.s./L and higher. For macrophytes that showed a relatively minor growth in controls (Ceratophyllum demersum, Ranunculus circinatus, Lemna trisulca) hardly no treatment-related effects could be demonstrated.

WEPC2-8
Effects of aquatic macrophytes on concentrations of contaminants in mesocosms
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Aquatic macrophytes play important structural and functional roles in aquatic ecosystems. For example, they change their chemical environment by increasing pH and the diurnal variation thereof, cause fluctuations in dissolved O_2 levels and produce organic matter, both solid as well as dissolved organic carbon (DOC). Such changes in the chemical environment affect both concentration and speciation of contaminants in aquatic ecosystems. To determine the magnitude of such changes, experiments were conducted to monitor the impact of macrophyte growth form and macrophyte biomass on concentration and speciation of contaminants in various ecosystem compartments, i.e. sediment, plants and surface water. Effects of growth form were studied in a potted-plants experiment performed in mesocosms. The effects of biomass were studied in four flow-through experimental ditches. Concentrations of pesticides (dimethoate, λ-cyhalothrin, metribuzin and imidacloprid), pharmaceuticals (ibuprofen) and heavy metals zinc (Zn) and copper (Cu) were monitored over time. Concentrations of Cu and Zn diminished rapidly, the decrease

in the controls including sediment and lacking macrophytes being rapid as well. This implies an important influence of sediment and a minor additional impact of macrophytes for these heavy metals. For strongly absorbing contaminants macrophytes and sediment both act as an important sink. Macrophytes have an enormous influence on pH values and diurnal pH fluctuations. Different growth forms seem to act differently in this respect. As a consequence submerged, floating end emergent plants show different effects on contaminants which are subject to hydrolysis, like dimethoate. Initial results show that aquatic macrophytes also change their chemical environment by producing DOC. As absorbing contaminants also bind to DOC, DOC thus acts as a carrier for these compounds. Because part of the DOC fractions can be highly mobile, the interaction between DOC and hydrophobic organic contaminants can result in potentially high concentrations in surface water. Further research is needed on the role of DOC in the absorption and dissipation of contaminants.

WEPC3 - Tropical ecotoxicology

WEPC3-1

Assesment of physico-chemical and ecotoxicological characteristics of Limeira stream, the Paraiba Valley, São Paulo - Brazil

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The Paraíba Valley is located in southeastern Brazil, an important center of economic and industrial development of Brazil and need special attention in relation to water resources. The Limeira stream, a tributary of the left bank of the river Paraíba do Sul, in this basin there are military and abrasives industries, eucalyptus plantations and pasture areas. To evaluate water quality in this environment were analyzed physico-chemical and ecotoxicological properties of the stream between September 2007 and July 2008. The limnological parameters analyzed in water were: turbidity, conductivity, DO, pH, COD, BOD, nitrate, nitrite, total phosphorus, total dissolved solids, hardness, metals and fecal coliform. The acute and chronic toxicity were evaluated using the cladocerans Daphnia similis and Ceriodaphnia silvestrii, the algae Pseudokirchneriella subcapitata. The indexes of Aquatic Life Protection and Trophic State were applied. Cluster analysis (CA) and Principal Component Analysis (PCA) were applied for the information obtained in this environment. The results showed that seasonality influenced the dynamics of the environment and the analyzed variables, particularly in rainy periods. The increasing of particulate materials into the environment aquatic reflected the values of conductivity, hardness and STD. Spatial variation was suggested due anthropogenic influences in river, especially at the point P1, area that received discharges of solid waste from a military industry. Acute effect was observed in Sept/07, in points P1, P2, P4 and P6 to D. similis. This results may be related to the presence of Fe and Pb in water with values above the allowed (0.3 mg.L-1 and 0.01 mg.L-1, respectively) according to CONAMA Resolution 357/05.Chronic effect was observed in the water to the microcrustacean C. silvestrii in Feb/08, Mar/08 (P2) and the alga P. subcapitata in Mar/08 (P1) and May/08 (P1, P2). The presence of Fe and Pb in Feb/08 and Mar/08, may have influenced the results of toxicity assays, causing chronic effects to organisms. The Limeira stream showed regular water quality according the index of Aquatic Protection Life and was regarded as mesotrophic in all sampling points, conform the Trophic State index. The analysis of CA and PCA indicated that this stream can be divided into three main groups according to degree of pollution: group 1 (P0, P3), group 2 (P1, P2) and group 3 (P4, P5, P6) had low pollution, high pollution and moderate pollution, respectively.

WEPC3-2

Methodologies for aquatic model ecosystem studies in tropical climate zones: lessons learned from Thailand and way forward

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Little research has been done so far into the environmental fate and side effects of pesticides in the tropical freshwaters. In addition, those studies conducted in tropical regions have focussed almost exclusively on single species laboratory tests. Hence, fate and effects of pesticides on higher-tier levels have barely been studied under tropical conditions. To address this lack of knowledge, four outdoor aquatic model ecosystem experiments using two different test systems were conducted in Thailand evaluating the insecticide chlorpyrifos, the herbicide linuron and the fungicide carbendazim. Results of these experiments and comparisons of recorded fate and effects with temperate studies will be presented. In addition, the pros and cons of the methodologies applied will be discussed as well as indications for i) possible improvements; ii) important aspects that should be considered when performing model ecosystem experiments in the tropics; and iii) future research.

WEPC3-3

The herbicide Atrazine impairs biotransformation, inhibits antioxidant defenses and increases DNA damage on a Neotropical fish species

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Atrazine, an herbicide widely used throughout the word, is considered a potential contaminant to the aquatic environments. The presence of atrazine in water has been detected above the limits determined by the official guidelines in many countries; even so, the effects of this herbicide on fish are not fully understood. At present, more studies are necessary to evaluate genetics and biochemical biomarkers in the freshwater fish *Prochilodus lineatus* exposed to atrazine to understand the mechanisms of toxicity of this herbicide. In this context, the goal of this work was to evaluate atrazine effects on biotransformation enzymes, antioxidant defenses and the occurrence of DNA damage on juveniles of *P. lineatus* after acute exposure to sublethal concentrations of the herbicide. This was done through the determination of the hepatic activity of CYP1A and glutathione-S-transferase (GST), antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), the amount of reduced glutathione (GSH) in the liver and the occurrence of DNA lesion on blood, gills and liver cells by the comet assay. The results showed that exposure to 10 μL^{-1} of atrazine for 96 h reduced the liver activity of GST, which is the main route for the metabolization of the herbicide. Besides, fish exposed to both atrazine concentration, for 48 and 96 h, showed an inhibition of EROD activity, indicating that this herbicide might act as a potential endocrine disruptor. Both atrazine concentrations produced a general reduction on reduced antioxidant defenses, with the exception of GR activity, that was stimulated. The occurrence of DNA damage, in comparison to control groups, was significantly higher in blood cells of fish exposed to 10 μL^{-1} of atrazine for 48 and 96 h, in liver cells of fish after 48h exposure to 10 μL^{-1} of the herbicide and in the gills cells of fish exposed to 2 μL^{-1} of atrazine for 48 h. These results clearly show that atrazine interferes on biotransformation and antioxidants defenses and promotes DNA damage on different tissues of *P. lineatus*.The limit for atrazine in water (2 $\mu g.L^{-1}$) set by the Brazilian guidelines (CONAMA, 2005) is not safe to protect this freshwater fish species.

WEPC3-4

Mercury contamination chronologic trends in a tropical ecosystem (Ceará, Brazil)

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Mercury (Hg) is a highly toxic persistent and global pollutant. Estuaries are transition ecosystems linking the terrestrial and ocean environments and are composed of an interdependent mosaic of subtidal, intertidal and surrounding terrestrial habitats. A mosaic of mangrove forests and associated plant communities defines the most tropical habitats. In this work, the study area is located in the State of Ceará, (Brazil) using a low contaminated area (Ceará river estuary) and a reference site (Pacoti river estuary). Hg was quantified by pyrolyse atomic absorption spectrometry in surface sediment cores and tree annular rings cores to obtain profiles of Hg contamination. Sediment cores in the reference site Pacoti estuary shows levels ranging from 0.01 to 0.02 µg g-1 whereas the Ceará estuary shows levels ten times higher in the top sediment layers but decreasing to in depth (around 30cm) to levels similar to the reference site. Similarity trends in both sediment and tree rings cores are presented and discussed. Data on historical trends of Hg contamination could provide a useful tool understanding past patterns, evaluating present levels and predicting future evolution.

WEPC3-5

PBDEs, PCBs, and DDE in eggs of aplomado falcons (Falco femoralis) from Chihuahua and Veracruz, Mexico

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The northern aplomado falcon (Falco femoralis septentrionalis) is a species threatened by habitat loss and agriculture in grasslands and tropical habitats in eastern Mexico. Currently there is little information about the potential impacts of environmental contaminants on raptor populations in eastern Mexico or in tropical regions. From 2004 to 2007 we collected 23 addled eggs from different nests of aplomado falcons in 4 regions of Chihuahua and Veracruz, Mexico. The eggs were analyzed for persistent organic pollutants including organochlorine pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). Of all the organochlorine pesticides, only DDE, a metabolite of DDT, was found in all egg samples at concentrations ranging from 129-7850 ng/g wet weight. Twenty PCB and six PBDE congeners were detected in over 50% of the eggs analyzed. Total PCB concentrations ranged from 44-2802 ng/g wet weight and total PBDEs from 62-798 ng/g lipid weight. There were no significant differences in DDE concentrations in eggs from the 4 regions in Chihuahua and Veracruz; however, total PCBs were significantly greater (P=0.015) in Coyame than in Ahumada in Chihuaha, but were similar with the other two regions in Veracruz. Total PBDEs were significantly higher in eggs from Veracruz than in those from Chihuahua. The most prominent congener was BDE-153, followed by BDE-99, -154, -100, and -47. This pattern of BDE-153 dominance is consistent with that reported for peregrine falcons in various parts of the world. Overall, concentrations of PCBs, PBDEs, and DDE were below those that could be associated with negative impacts on the aplomado falcon population in northern and eastern Mexico.

WEPC4 - Experimental approaches for determining bioavailability, sorption and NER formation of chemicals in soils and sediments

WEPC4-1

Toxicity and bioavailability of geogenic polycyclic aromatic compounds from coal

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Coals contain native polycyclic aromatic compounds (PAC) that are generated during the diagenetic process of coal formation from biological precursor materials [1, 2]. The amount of PAC depends on the various properties of coal, e.g. origin, coal rank or parent material. In abundant areas of the world soils and sediments are highly contaminated with unburned coal particles as a result of longtime mining activities, transport and usage of coal and overburden material [3]. A large part of PAC in coal-rich soils could be ascribed to coal particles [4]. It is assumed that the coal-derived PAC are of low environmental concern because coal acts as a very strong geosorbent for hydrophobic substances resulting in very low bioaccessibility indicating low environmental risk and reduced remediation costs.

In this investigation, eight coals of varying origin, coal rank and chemical properties were used in bioassays to study the toxicity and in particular bioavailability of coal-bound PAC. Apart from polycyclic aromatic hydrocarbons (PAH) heterocyclic aromatic compounds (NSO-PAH) which have not yet received much attention have been taken into account. NSO-PAH often show high toxicity and a higher mobility compared to PAH.

Sediment contact assays using ground coal as a substrate were performed (fish embryo assay with *Danio rerio*, nematode contact assay with *Caenorhabditis elegans* and a bioaccumulation test with *Lumbriculus variegatus*). The results are compared to the results of the same test systems performed with the solvent extracts in liquid media and additional tests (Neutral red retention assay, EROD assay, Ames fluctuation assay) to assess the toxic potential of the compounds present in the extracts. PAC were analyzed using one- and two-dimensional gas chromatography-mass spectrometry.

The results show that the coals used in the fish embryo contact assay did not cause any effects, while the extracts led to embryo mortality at very low concentrations and also caused strong effects in the other test systems. We conclude that the toxic compounds are not bioavailable to fish embryos when exposed in a contact assay whereas solvent extracts of the coals provide a very high toxic potential.

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WEPC4-2

Variability of Phenanthrene and Lindane extractability and bioavailability between ten artificial soils

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In standard soil bioassays, the artificial soil defined in OECD and ISO guidelines has been used routinely since 1980's. The use of standard soil helps to comparability and interpretation of measured data because natural soils are very variable. However, it is not clear how variable are artificial soils prepared in different laboratories. In our study, ten artificial soils were collected from different countries and differences in 14C-phenanthrene and 14C-lindane extractability and bioavailability were investigated in aging experiment. At each sampling point (1, 14, 28, 56 days after soil contamination), three extraction methods were employed:. extraction with DCM (dichloromethane) or MeOH (methanol) which represent exhaustive techniques and extraction with HPCD (hydroxypropyl-β-cyclodextrin) which is used as a mild extraction able to predict microbial degradation. The bioavailability of 14C-phenanthrene to microbes (Pseudomonas sp.) was measured by 15-day-respirometry assay. After 28 d contact time, comparable amounts of 14C-phenanthrene associated residues were extracted from all soils by DCM, not significantly different, the day 56 showed significant decrease in all artificial soils, except artificial soil 8 Significantly different amounts were extracted by HPCD (1d 49-83%, 14d 44-79%, 28d 20-72%, 56d 11-48%) and the aging effect was clearly apparent. Extraction of 14C-lindane by DCM and MeOH was not significantly different between soils at any sampling time. Significantly different amounts were extracted by HPCD (1d 68-92%, 14d 66-86%, 28d 61-79%, 56d 58-74%). The degradation of 14C-phenanthrene showed typical kinetics with rapid mineralization in the first 24h, slow-down in following 2-4 days and coming to plateau in next days. Total amount of phenanthrene mineralized was significantly different between soils (1 day 40-67%, 14 day 64-100%, 28 day 30-76%, 56 day 17-73%).
The observed variability was not correlated at any of soil properties, this means that there is no available predictor of these differences and it must be futher studied.
To summarize, variability of phenanthrene and lindane extractability and bioavailability was observed in artificial soils from different laboratories. The question remains if these differences might affect the results of ecotoxicity tests.

WEPC4-3

Fate and bioavailability of pyrene and lindane in sterile artificial and real soils

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Artificial soil is considerably different from real soil despite it was developed to serve as "model soil"in soil toxicity tests. From our earlier studies, it is apparent that organic carbon is not only determinant of pollutants fate, bioavailability and toxicity. Despite of this fact, some methodologies still use organic carbon for direct recalculation of toxicity or risk between different soils, i.e. also between artificial and real soils. This assumption might lead to the final risk under- or overestimation. Differences between artificial and natural soils are caused not only by components proportion (i.e. relatively high total organic carbon content) but also by their character - peat and kaolin clay are the most critical components affecting the fate of contaminants. This study is posing claim to investigate the differences in bioavailability of selected POPs between artificial and natural soils and its changes in time. Three natural soils were used with different total organic carbon content (TOC): arable soil (1.2%), grassland soil (3.5%) and forest soil (10.2%), and three artificial soils were prepared to have the same TOC as these real soils. Soils were sterilized to avoid the influence of microbes, and contaminated by radiolabelled 14C-pyrene and 14C-lindane. Total activity, DCM and HPCD extraction and bioaccumulation in earthworms *Eisenia fetida* was measured after 1, 14, 28 and 56 days. According to results it is clear that i) decline of bioavailability of selected compounds in time was less evident in sterile soils than in non-sterile soils studied in the past, ii) bioaccumulation and extractability showed different trends for pyrene and lindane, and iii) differences between artificial and natural soils were observed however they had the same TOC. These conclusions are in accordance with previous studies which also reported than not only organic carbon content (TOC) but also its character and other soils properties must be seriously considered in artificial to real soil extrapolation.

WEPC4-4

Fate and behaviour of Azoxystrobin, Isoproturon, Cypermethin and Diazinon in four UK soils under laboratory conditions.

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Soil is a highly complex matrix in which pesticides are often applied on purpose directly or indirectly. This study aimed to investigate the fate and behaviour of azoxystrobin, isoproturon, diazinon and cypermethrin in four natural UK soils under laboratory aerobic conditions and for a period of 100 days. Fate and behaviour in this study were described in terms of losses due to biodegradation and extractability into three extractions solutions and the respective formation of non extractable residues. Losses of the pesticides related ^{14}C-activity increased with time of incubation mainly due to biodegradation. Losses increased significantly ($p>0.05$) with incubation time in almost all the microcosms. Greater losses were observed in the case of diazinon and cypermethrin in Redesdale. Incubation time had also significant ($p>0.05$) negative effect on the extractability of all the pestcides in the three extraction schemes employed here ($CaCl_2$, HP-β-CD and acetonitrile). The effect of the incubation was shown to have a respective impact in the formation of nonextractable pesticide related ^{14}C-residues. This study highlights how different extraction schemes, as these used in this study affect the availability of azoxystrobin, isoproturon, diazinon and cypermethrin in the four soils. It is also evident from this study that the HP-β-CD was able enhance the solubility of the pesticide molecule into the aqueous solution . Minerilisation extent for diazinon and cypermenthin was relatively high indicating increased availability of these pesticides to the soil microorganisms. This study highlights that degradation and sorption-desorption are processes unique to each soil and pesticide system and thus special consideration is required when interpreting data from laboratory based fate studies for purpose of prediction or the pesticide fate of pesticides in environmental media.

WEPC4-5

Sorption-desorption behaviour of 14C-isoproturon and 14C-azoxystrobin in soil.

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Sorption-desorption isotherms are useful tools for the investigation of the fate and behaviour of pesticides in the soil. Isoproturon, a widely used herbicide, and azoxystrobin, a novel fungicide were investigated in this study in two organically managed soils. A standard batch equilibrium method was employed followed by serial desorption method. A linear partition isotherm model and Freundlich isotherm model were used for the data interpretation together with a simple desorption-release model. Pesticide polarity and soil organic carbon were shown to be the major factors determining pesticide's partitioning within the soils. Effects like sorption and desorption isotherm nonlinearity were shown to be more pronounced in the case of azoxystrobin; this was attributed to its stronger association to the soil. Observed hysteretic phenomena were attributed to specific interactions (H-bonding) among the pesticides and the soil. Azoxystrobin soption desorption data derived from this study are unique and offer an approximation of the fate and behaviour of azoxystrobin in agricultural soils. Previously published data were mainly gained through evaluation of the pesticide main characteristics (structure, charge, side chains). In the case of azoxystrobin, for the first time, in this study sorption-desorption profile of this pesticide in agricultural soils were reported.

WEPC4-6

Long-term persistence of various 14C-labeled pesticides in soils

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The environmental fate of the ring-14C-labeled herbicides methabenthiazuron (MBT), ethidimuron (ETD), and the fungicide anilazine (ANI) in soils was evaluated after long-term aging in lysimeters under outdoor conditions. Pesticide applications were performed at customary rates in 1988/1992/1994 for MBT (totalling 8.79 kg had'1) and 1997 for ETD (1.23 kg had'1). ANI was applied on two different lysimeters in five consecutive years from 1985-1989, with a total of 9.68, and 19.78 kg had'1, respectively. All pesticide treated soils were subject to environmental aging and crop-rotation until sampling in April 2006. Analyses of residual 14C-activity in the upper soil layer revealed 19% (ETD; 0-10 cm depth), 35% (MBT; 0-30 cm depth), 49% and 43% (ANI; 0-30 cm depth), respectively, of initially total applied 14C-activity. Soil sample extraction using accelerated solvent extraction with acetone-water mixture as a solvent yielded 90% (ETD-soil), 26% (MBT-soil), and 28% and 37% (ANI-soils), respectively, in total of the residual 14C-activity in the sample, after 8 consecutive extraction steps. Analyses of the extracts by means of LC-MS/MS and radio-HPLC revealed the parent compounds ETD and MBT in considerable amounts (24.5 µg ETD kg-1 and 45.9 µg MBT kg-1 soil, accounting for 3% and 2% of total active ingredient applied, respectively, calculated per layer 0-10 cm ETD-soil / 0-30 cm MBT-soil, and m-2), as well as dihydroxy-anilazine as the main ANI metabolite. Additionally, radio-HPLC analyses of the concentrated extracts revealed two metabolites for ETD. The results demonstrate the long-term persistence and extractability of various pesticides in soils even after prolonged environmental aging. Detailed knowledge about the mechanisms of molecule binding, entrapment, persistence and degradability in the soil matrix is therefore of continued importance. A reassessment of the long-term fate of chemicals in terms of environmental risk assessment and sustainable resource protection should be encouraged.

THPC1 - Atmospheric chemistry, transport and deposition

THPC1-1

Passive atmospheric sampling of Polychlorinated Biphenyl (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs) in urban and industrial sites in the coastal area of Milazzo, Sicily

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Passive air samplers consisting of polyurethane foam disks were deployed concurrently at 18 locations and exposed for 60-90 days from February to September 2010 at Milazzo (coastal area) of Sicily to screen for levels of PCBs and PAHs in the atmosphere. The sampling sites were located on the vicinity of an industrial Settlements. Derived air concentrations (pg/m3) ranged as follows: the sum of 12 PCB congeners, BDL-182 for Period 1 (February-May), from 64-3721 for Period 2 (May-July), and from 94-4260 for Period 3 (July-September). The PCBs with 6-cl were the most predominant congeners accounting for the 40, 52 and 39 % for the periods 1, 2 and 3, respectively. Following for the 5-cl (37%) during the first period, and for the 4-cl (24% and 31%) during the second and third periods respectively. For PAHs air concentrations ranged from 1114-4513 for Period 1, from 776-6131 for Period 2, and from 1475-20249 for Period 3. PAH with 3-6 benzene rings were analysed, the most abundant was 3 ring-congeners accounting for 63 ± 8 % (period 1), 77 ± 8 % (period 2) and 71 ± 8 % (period 3) of the total PAHs composition. Phenantrene alone accounted for the 48 ± 3 % of the total PAHs composition during the first period and for the 68 ± 8 % and 51 ± 5 % during the second and third period respectively. The percentage for 4 rings were 36 ± 8 % (period 1), 23 ± 8 % (period 2I) and 28 ± 7% (period 3). These results shows a clear seasonal variations for the targets compounds. Period 3 showed the highest concentrations for PCBs and PAHs. This information is useful to identify hot spots of PCBs and PAHs on local and regional scale.

THPC1-2

Concentrations of organochlorine pesticides (OCPs) current use pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) using passive air sampling in Tuscany region, Italy

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Passive air samplers consisting of polyurethane foam disk were deployed over a 3-month period from April to July 2008 at 15 locations throughout the Tuscany Region in central Italy. Sampling sites were selected based on different criteria including urban, rural and agricultural sites. The purpose of the study was to assess the gas-phase concentration of selected historic use organochlorine pesticides (OCPs) and current use pesticides (CUPs) on a local and regional

scale. Five of the historic-use OCPs and seven CUPs were frequently detected. These include α- and γ-HCH, HCB, α-endosulfan, and DDT for OCPs. Trifluralin, diazinon, chlorpyripos methyl, chlorpyripos ethyl, malathion, chlortal-dimethil and pendimethalin for CUPs. Of the historic-use pesticides α-endosulfan and γ-HCH reported the higher concentration of the OCPs with ranges from below detection limit (BDL) to 2185 pg/m3 and from BDL to 1058 pg/m3 respectively. In general, OCPs do not presented a clear difference between the three categories of sites (agricultural, urban and rural). For CUPs, high concentrations (pg/m3) were detected for Malathion (BDL to 3554), Chlorpyrifos-ethly (BDL to 1312) and chlorpyrifos-methyl (BDL to 568). The concentration of CUPs showed a big variation between the different sites probably showing local dynamics. This study shows the feasibility of using PUF disks as time-integrated passive samplers, to asses curent use pesticides levels at local and regional scale.

THPC1-3

Latin American atmospheric passive sampling network (LAPAN) of POPs

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A Latin American Atmospheric Passive Sampling Network (LAPAN) is been set up to: - monitor atmospheric levels of POPs; - identify areas of concern and local sources; - provide data for studies of global distribution and temporal trends; -and, evaluate the effectiveness of the control actions adopted by the countries members of the Stockholm Convention to eliminate the release of POPs to the environment. This network endeavor to install at least 80 XAD-2 based PAS in the Latin American countries, covering different environmental gradients, such as remote - urbanized areas, latitudinal, longitudinal and, possible, altitudinal. So far, approximately 46 PAS were already installed: 5 in Antarctica, 13 in Argentina, 16 in Brazil, 7 in Chile, 2 in Equator, and 3 in Peru.

THPC1-4

Biomarkers of oxidative stress in pine needles as a tool for the assessment of atmospheric pollution in Patagonia, Argentina

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Persistent Organic Pollutants (POPs) are of environmental concern due to their recalcitrance, global distribution, and toxicity. Pollutants are known to generate Reactive Oxygen Species (ROS) formation, however, organisms have evolved multiple antioxidant defense wich include both enzymatic and non-enzymatic complex system. The aim of this work was to assess different oxidative stress biomarkers in pine needles in response to atmospheric pollution in the Patagonian region of Argentina. Levels of lipid peroxidation (MDA), glutathione-S-transferase (GST) activity and total antioxidant capacity (ACAP) were evaluated in pine needles (Pinus sp.) at longitudinal (Rio Negro watershed) and latitudinal (Patagonia) scale by performing a network of 20 sampling stations that were selected on the basis of previous work about atmospheric pollution monitoring. The Rio Negro watershed showed higher ACAP and GST activity than latitudinal sampling stations. Particularly, V. Regina station settled in the upper Rio Negro, showed the highest biomarker values. These results are in agreement to the fact that V. Regina constitutes an intensive fruit production area known to be a hot spot for POPs like DDTs, endosulfans and PBDEs. Moreover, a decrease gradient in the biomarkers levels from west to east was observed which was expected considering data about atmospheric POPs levels. In the latitudinal transect, the northest sampling station (Bahia Blanca), presented the highest ACAP, GST and MDA levels with decreasing values to the southest station. As was observed in V. Regina, results suggest a relationship between biomarkers and POPs levels. Although they could be mainly attributed to the insecticide endosulfan the influence of other pollutants such as polycyclic aromatic hydrocarbons should not be discarded. On the contrary, in the southest station (Rio Gallegos) a non direct relationship between biomarkers and POPs concentrations was observed. In conclusion, the results suggest that the studied biomarkers would be a useful tool in the assessment of atmospheric pollution associated with POPs occurrence. Considering the wide range of temperature variation among stations, future research focusing on the analyses of pine needle lipid classes, and temporal monitoring of POPs and biomarkers will be carried out.

THPC1-5

Indication of regional sources affecting polycyclic aromatic hydrocarbon levels in Antarctic snow

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Antarctica is an almost unpopulated continent, but anthropogenic pollution by sulfur, heavy metals (Boutron and Wolff, 1989; Graf et al., 2010) and persistent organic pollutants (POPs) (UNEP, 2002) has been verified. The main anthropogenic sources of sulfur in the region are suspected to be tourism and research stations (Graf et al., 2010). Intercontinental atmospheric transport to Antarctica has been proposed as a source of various POPs and polycyclic aromatic hydrocarbons (PAHs) (Klánová et al., 2008).

Firn samples attributed to the period between 2002 and 2005 were collected from a snow pit at Neumayer research station. Samples were analysed using SPME-GC/MS and dated according to the firn depth in the snow core and using records on snow precipitation.

PAH concentrations in snow were found within the range of 26-197 ng L-1. The most prevailing substances were determined to be naphthalene, 1- and 2-methylnaphthalene, acenaphthylene, acenaphthene and phenanthrene with naphthalene accounting for an overall mean of 82% of total PAHs. The PAH content was compared to levels of inorganic contaminants as well as methylsulphonic acid in order to distinguish between summer and winter samples. Five time intervals were selected for the investigation of long-range PAH transport and the determination of sources.

Potential emission sources of PAHs in snow were studied using air mass back-trajectory statistics and available emission data of combustion sources in and around Antarctica. The distance to the sources (ships and research stations) in this region was found to control the snow PAH concentrations. The results for the five studied periods did not indicate intercontinental transport or marine sources of PAHs.

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THPC1-6

Persistent organic pollutant micrometeorological air-surface exchange flux measurement

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Together with mercury, persistent organic pollutants (POPs) require a unique regulatory framework as compared to other air pollutants not only because of their long presence in the environment, but also because they are capable of undergoing successive air-surface deposition-volatilization-transport cycles with condensed media. Therefore, to be appropriate, regulatory frameworks for POPs on local, regional, and global scales are highly dependent on knowledge of emission, advection, and deposition fluxes in air and water. Measurement of such fluxes is particularly demanding in air, where turbulent dispersion dominates over diffusion, and the timescales at which these transport processes occur (e.g., 10 Hz) cannot be measured by eddy covariance because sensors that measure POP concentration at high frequency are not available. Understanding of these transport processes has thus been limited by a lack of tools with which to observe them. Fluxes are commonly inferred using model parameterizations based on measured concentrations collected over long timescales, and these parameterizations are largely unvalidated. For example, the so-called "grasshopper effect"in which POPs undergo successive deposition-volatilization-transport cycles is purported to result in global transport of POPs. It has been inferred primarily from observations of POP concentrations in locations in which they were thought to be previously absent, e.g., in Arctic air, water, and wildlife, and observations of so-called "global distillation", the physico-chemical property dependent differentiation in POP concentration with latitude and/or altitude. Micrometeorological methods to measure air-surface exchange fluxes of POPs that do not require use of high-frequency sensors, including the modified Bowen ratio and relaxed eddy accumulation techniques, are available, and in this presentation we summarize these techniques. POP collection/extraction concentration measurement techniques have been developed that enable application of these flux measurement techniques in urban, rural, and remote environments. They will be useful in development of process-based understanding of POP transport.

THPC1-7

Characterization of Chinese coal fly ash and Beijing particulate matter for parent, methyl-, nitro-, oxy-, halogenated-, and high molecular weight polycyclic aromatic hydrocarbons

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Reference materials are homogenous mixtures of an environmental matrix used to evaluate analytical methods for selected compounds. The purpose of this work was to 1) prepare and characterize an in-house Beijing particulate matter (PM) reference material (RM), and 2) apply newly-developed analytical methods for several classes of polycyclic aromatic hydrocarbons (PAHs) to the Chinese coal fly ash certified reference material (CRM) .

As part of an inter-disiplinary project on PAHs at Oregon State University, an in-house PM10 ambient air RM was needed for toxicology studies. The RM was prepared by extracting 229 twenty-four hour PM10 ambient air filters collected from Peking Univeristy in Beijing, China with hexane:acetone and ethyl actetate using pressurized liquid extraction (PLE). The extracts were combined and characterized for parent and methyl-, nitro- and oxy-, halogenated, and high molecular weight (HMW) (≥ MW 302) PAHs using gas chromatrography mass spectrometry (GC/MS). The total PAH mass in both the hexane:acetone and ethyl acetate ranged from 4067 to 9.88 µg.

The Chinese CRM is composed of coal fly ash collected from a coal-fired boiler and was released in 1995. The certified values for five PAHs including phenanthrene, anthracene, fluoranthene, pyrene, and benzo[a]pyrene and reference values for benz[a]anthracene, chrysene, triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, and benzo[ghi]perylene were previously published. Anaysis of the CRM resulted in concentrations that were 11 to 86% lower than the certified values and 25 to 78% lower for the reference values. This difference may be explained by the particularly strong adsorption of PAHs to coal fly ash surfaces and the use of a different extraction solvent. The total concentrations for 17 parent, 12 methyl- and 22 HMW PAHs were 28, 10, and 3.1 µg/g, respectively.

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(anti)estrogenicity. ET07B-3
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