Algal toxicity of platinum nanoparticles - Implications of NP aggregation, dissolution and shading

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Environmental contaminants from land to sea: continuities and interface in environmental toxicology and chemistry
This book composes the abstracts of the presentations for the platform and poster sessions of the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at La Cité Nantes Congress Center in Nantes, France, from 22 - 26 May 2016. The abstracts are reproduced as accepted by the Scientific Committee of the 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.
SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists as well as managers and engineers others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void. Based on the dynamic growth in the Society’s membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC’s growth has been marked the establishment of geographic units around the world: SETAC Europe in 1989, SETAC Asia/Pacific in 1997, SETAC Latin America in 1999 and SETAC Africa in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters of the geographic units are being considered for a number of countries.

Publications

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.

Integrated Environmental Assessment and Management, launched in 2005 to bridge the gap between scientific research and its application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.

SETAC Books total more than 100, encompassing workshop results and other scientific studies.
**Keynote Abstracts**

**K1**

**The Plastic Tide**

Thomas Maes, Cefas, UK

**BIO:** Thomas Maes is a recognised science leader with nearly 10 years’ experience in many aspects of coastal and ocean monitoring, ranging from chemical pollutants and related biological effects to marine litter and other emerging compounds. He has good interpersonal skills and is an effective communicator in different languages. Thomas is Cefas’ International and National Monitoring Programme Coordinator who joined Cefas in 2008 after an international career as environmental manager for large marine development projects. He coordinates and develops Cefas’ international monitoring programmes and provides advice to UK Government issues related to human activities such as contaminant and other emerging pressures. He is involved in the assessment of data and adaptation of monitoring programmes and further implementation of the MSFD. Thomas is currently also chairing the OSPAR monitoring and assessment group (MIME) and acting as the UK expert on Marine Litter in the European task groups. He manages large, multi-disciplinary research projects and monitoring programmes including leadership of several EU-funded programmes in relation to contaminants and marine litter (e.g.: MICRO, MARLISCO,…..).

**K2**

**Climate Change and Environmental Contamination by Chemicals: Do They Affect Ecological and Human Health?**

Arja Rautio, Thule Institute, University of Oulu, Finland

Non-emerging contaminants, persistent organic pollutants (POPs) and toxic metals are transported to the Arctic from regions at lower latitudes. During the last twenty years the Arctic Monitoring and Assessment Programme (AMAP) has followed the levels of POPs and metals in environment, wild-life and human populations, especially pregnant women. Most POPs and metals are declining in many Arctic regions, but there are some areas and populations where levels remain high. Elevated concentrations of toxic metals (usually mercury) and environmental contaminants (dioxins and PCBs) have been measured in Greenland and Canada from seafood, like marine mammals and fish, and dietary advice is needed for users of traditional and local foods. Interactions between climate change and contaminant transport have the potential to change human exposure in the Arctic significantly. It is not possible to estimate the likelihood and magnitude of the health impacts and the changes in exposure. The incidence of many Western type of diseases, like diabetes, heart diseases and cancers, has increased globally, and high increase in numbers have been found among people living in the Arctic. It has been assumed that environmental pollutants may have a contributing effect especially in some heavily exposed Indigenous populations. Epidemiological studies have shown the connections between contaminant exposure (especially mercury) and changes in foetal growth and child development and cardiovascular effects. However, investigations on the precise impact of environmental pollutants on human health are difficult to study, because there are many factors, which affect simultaneously and varying degrees. These include genetic background, age, sex, diseases, exposure history and environment. Environmental factors include mixtures of contaminants and other chemical compounds to which individuals are commonly exposed. Knowledge about the effects of mixtures is mainly missing and difficult to study at population levels.

Exposure during the foetal period is in special attention in the epidemiological and health research, because there are many factors, which affect simultaneously and varying degrees. These include genetic background, age, sex, diseases, exposure history and environment. Environmental factors include mixtures of contaminants and other chemical compounds to which individuals are commonly exposed. Knowledge about the effects of mixtures is mainly missing and difficult to study at population levels.

**K3**

**Environmental Assessment of Cosmetics and Personal Care Products: Challenges and opportunities**

Marc Leonard, L’Oréal Advanced Research, France

Despite a frequent image of futility, cosmetic and personal care products provide strong societal benefits. For centuries, their use has been motivated worldwide by the continuing need for hygiene, beauty and self-esteem. Nowadays, cosmetics and personal care products (CPCP) are most frequently used in the bathroom. After rinsing they flow down the drain where they mix with wastewaters. In industrialized countries they are directed to sewage treatment plants, but in many developing countries wastewaters may directly be discharged into rivers or the sea shore. In addition, certain products such as sunscreens may be directly released by swimmers in lakes, rivers or the ocean.

The exposure of the environment to cosmetic and personal care products is relatively low compared to contaminants from other industrial sectors but nonetheless, the environmental risk assessment of their constituents is done in a similar manner. Assessing their potential environmental impact faces significant methodology challenges because of their extremely diverse composition, from single ingredients to heterogeneous complex mixtures such as natural extracts and essential oils.

In parallel, there is a worldwide trend to move away from animal testing for the human and environmental safety assessment of cosmetics and personal care products. In this regard, fish which are key aquatic models in environmental risk assessment, fall into the scope of several international regulations for the protection of animals used for scientific purposes. As a consequence, replacing animal testing for the safety assessment of cosmetic and personal care products faces additional challenges when addressing environmental issues such as fish long term toxicity, environmental endocrine modulation and bioaccumulation, where fish BCf data are still required for regulatory BPT/vPvB classification. Cosmetic companies share with authorities the desire to inform the public which products have the best environmental profile. Thus, several projects are under study at national (e.g. French) and international (e.g. EU) levels for consumer products environmental footprint labelling, including cosmetics and personal care products. They plan to assess the impact of products on aquatic ecosystems with the USEtox model, developed for Life Cycle Assessment (LCA). But some deviating results are obtained when using other methodologies such as the Critical Dilution Volume (CDV) calculation, which is required to award the European Ecolabel for cosmetics products. Concordant and relevant methodologies are needed and progresses are being made in this direction.

Nonetheless, this challenging context provides opportunities to develop alternative methodologies to anticipate potential short and long term adverse environmental effects of cosmetic and personal care products. Benefits are even expected from the field of human toxicology screening, where aquatic models such as the fish embryo are gaining much interest.

The purpose of this presentation will be to present the trends and advances in these fields.

**BIO:** Marc Leonard is presently head of L’Oréal Research & Innovation, Environmental Research Department. He obtained his doctorate in Veterinary Medicine from the Veterinary School of Maisons-Alfort (France), with a specialization in environmental toxicology from the Centre des sciences de l’Environnement - Metz University (France). The main activities of his department concern two domains : (1) Environmental Assessment of raw materials and cosmetic preparations with applied research aimed at developing cost-efficient assessment tests as potential alternatives to the corresponding OECD guidelines. Domains cover acute and chronic aquatic toxicity (soft and marine waters), bioaccumulation and biodegradation assessment. (2) Invertebrates and Fish Embryo models as potential alternative methods in Human Toxicology and Pharmacology. He has been co-chairman of the HESI (Health & Environmental Sciences Institute) Committee on the Emergence of Animal Alternative Needs in Environmental Risk Assessment. He was member of the OECD Fish Embryo Toxicity Expert Group, and OECD Expert Group on Invertebrates Reproduction. He has hosted and co-organized several international workshops on the Fish embryo model with international partners such as HESI and UFZ (Helmotz Center) and contributed to publications in these fields.
Contaminants of Emerging Concern in the Environment and their Management (I)

1 Solutions for present and future emerging pollutants in land and water resources management

W. Galus, Helmholtz Centre for Environmental Research UFZ / Effect/Directed Analysis; R. Altenburger, UFZ Centre for Environmental Research / Bioanalytical Ecotoxicology; D. Bunke, Öko-Institut e.V. / Sustainable Products Material Flows Division; W. Busch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; G. Engelen, Flemish Institute for Technological Research VITO; B. I. Escher, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology; B. Gawlik, European Commissions Joint Research Centre IRC; M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; C. Lindim, Stockholm University; L. Liska, International Commission for the Protection of the Danube River ICPR; J. Münthe, IVL Swedish Environmental Research Institute Ltd.; P. A. Neale, Griffith University / Smart Water Research Centre; L. Posthuma, RIVM / Centre for Sustainability Environment and Health; T. Schulze, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; F. Sleenwaart, Flemish Institute for Technological Research VITO; J. Slobodnik, Environmental Institute; J. van Gils, DELTARES; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health

Despite significant efforts the good ecological status required by European Water Framework Directive (WFD) could not be achieved in the majority of European surface waters. Emerging pollutants such as pharmaceuticals, biocides, personal care products and many others are hypothesised to play a key role for water quality. The 5-year Project SOLUTIONS addresses complex mixtures of potentially toxic compounds in the water cycle including legacy, presently used and future chemicals with monitoring-, modelling- and scenario-based approaches focusing on the assessment and prediction of the impact of these mixtures, on the identification of drivers of mixture toxicity focusing on deriving candidate River Basin Specific Pollutants (RSPs) and on the identification of priority mixtures. For the identification of priority mixtures with respect to specific targets different criteria are used to establish chemical fingerprints based on common sources, modes of action, fate etc. The impact of mixtures in the environment is addressed by the development and demonstration of a set of effect-based tools following the philosophy of adverse outcome pathways from key events on a molecular level via cellular and organism responses up to the community. These tools are evaluated and demonstrated in large scale case studies such as Rivers Danube, Rhine and Ebro. Results from the River Danube indicate low to moderate effects on a large range of endpoints, most large scale case studies such as Rivers Danube, Rhine and Ebro. Results from the River Danube indicate low to moderate effects on a large range of endpoints, most large scale case studies such as Rivers Danube, Rhine and Ebro.

2 Lagrangian tracer aided sampling approach in assessing the fate of wastewater-related contaminants in rivers

G. Guillet, Tübingen University / Applied Geoscience; J. Knapp, Eberhard Karls Universität Tübingen / Hydrogeology Center for Applied Geosciences; S. Merel, Eberhard Karls Universität Tübingen / Environmental Analytical Chemistry Center for Applied Geosciences; O. Cirpka, Eberhard Karls Universität Tübingen / Hydrogeology Center for Applied Geosciences; C. Zwiener, Eberhard Karls Universität Tübingen / Environmental Analytical Chemistry Center for Applied Geosciences; P. Grathwohl, Eberhard Karls Universität Tübingen / Hydrogeology Center for Applied Geosciences

Emerging contaminants are constantly released by Wastewater Treatment Plants (WWTPs) into surface waters. The persistence of these compounds influences how far they will spread within the river network, raising concern on the extent of their environmental impact and the issue for drinking water exploitations. To study the fate of wastewater-related pollutants in rivers, a common approach is using a Lagrangian sampling scheme. It consists in sampling the same parcel of water repeatedly as it flows along a selected river stretch. Where this approach allows monitoring concentration changes according to residence time, it may include uncertainties that can be misinterpreted as removal – dispersion of WWTPs' effluent coupled with inaccurate travel time estimation and dispersion effects. A new sampling approach combining Lagrangian sampling with an artificial tracer was applied to study the fate of wastewater-related compounds in an impacted river segment. The tracer fluorescein was injected upstream of a 1.7 km long stretch of the Steinlach River, southwest Germany, downstream of a WWTP effluent. The segment was controlled by four measuring stations during a day and a night experiment. Normalizing the compounds concentrations to the tracer isn’t possible in the context of wastewater-related compounds, constantly released unlike the tracer. Instead, breakthrough curves were deconvoluted to identified prefer functioning between each measurement. Concentration time series were then applied to concentration measured from a grab sample. Different behaviours from conservative to very reactive could be observed for the 43 investigated compounds. Compounds like the pharmaceuticals diclofenac or tramadol showed a strict phototransformative character. Other compounds were attenuated at a similar strength during day and night (atenolol, valsartan) and others displayed both behaviours – with a higher elimination during day time (bisoprolol, DEET or TAEED). Part of the compounds list was investigated in a previous experiment also performed in the Steinlach but at only two control planes in 2013. Similar behaviour was found and also found with a better attenuation in the last experiment. Finally, unlike 2013 sampling, 2015 experiment could supply decay profiles, particularly interesting for compounds like DEET.

3 Abiotic and biotic fate of lamotrigine N2-glucuronide in wastewaters and surface waters

B. Zonja, S. Perez, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAB-CSIC / Dept Environmental Chemistry

Lamotrigine, an anticonvulsant, is a xenobiotic which is extensively and predominantly transformed by phase II metabolism to its N2-glucuronide before it is finally excreted from the human body. Both parent and metabolite have been detected in the wastewater system, surface and groundwater. In this work, we tested a suspect-screening approach in order to detect lamotrigine and its related compounds like other human metabolites, impurities and phototransformation products in the aquatic system. Environmental samples that were preconcentrated using organic solid-phase extraction coupled online with a better attenuation in the last experiment. Finally, unlike 2013 sampling, 2015 experiment could supply decay profiles, particularly interesting for compounds like DEET.

4 Micropollutants - how to cope with the residues?

A. Zenker, University of Appl. Sc. Northwestern Switzerland / Institute of Ecoprevention; T. Wittgens, University of Applied Sciences Northwestern Switzerland (FHNW), School of Life Sciences, Institute of Ecoprevention; J. Lowenberg, CSD Ingenieure AG; J. Svojitka, R. Hochstrat, University of Applied Sciences Northwestern Switzerland (FHNW), School of Life Sciences, Institute of Ecoprevention; S. Schmidt, University of Applied Sciences Rapperswil, Institute of Environmental and Process Engineering; M. Burkhardt, UMTec, HSR Hochschule für Technik Rapperswil

Micropollutants in surface waters in most countries worldwide has changed its Water Protection Ordinance demanding about 100 wastewater treatment plants (WWTP) to upgrade with an additional treatment step. The additional step should help reducing 80% of micropollutants' load of the influent over the whole sewage treatment process. Micropollutants are present at low concentrations in aquatic environment ranging from ng/l - pg/l, but their negative effects on biota have already been demonstrated. Ozonation and the adsorption of micropollutants by powdered activated carbon (PAC) are currently the most promising techniques to eliminate micropollutants in WWTPs. An overview of different techniques to eliminate micropollutants in waste water, stormwater and drinking water will be presented. Different adsorbent materials like PAC (SAE Super, Sorbopor MV 125, Carbosell AP, Pulsox W-S, WR) were tested in combination with filtration, deep bed filtration and
ultrafiltration to reduce micropollutants. These settings were tested under laboratory conditions, in pilot scale and subsequently upscaled in the treatment plants themselves. To monitor the removal lead substances from 48th composite samples including pharmaceuticals such as carbamazepine, diclofenac, sulfamethoxazole, the herbicide mecoprop and the corrosion inhibitor bonteztriazole were determined. Measurements were carried out on a high performance liquid chromatography coupled to triple quadrupole mass spectrometer. It was found that the combination of PAC and filtration or PAC and flotation are suitable technologies to remove nearly 80% of the micropol lutants’ load of urban and industrial wastewater. During the operation duration of several months the removal was robust and less prone to faults.

5 Do pharmaceuticals bioaccumulate in marine molluscs and fishes from a coastal lagoon? V. Ledín, R. Moreno-González, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia; S. Rodríguez-Mozaz, Institute for Water Research (ICRA) / Water Quality; B. Huerta Buitrago, Catalan Institute for Water Research (ICRA) / Institute of Environment Health and Societies; D. Barceló, IDAEA-CSIC / Department of Environmental Chemistry

The pressure and impact of organic pollutants are greater in coastal areas than in the rest of the marine environment, since these are where many human activities are concentrated and direct and indirect discharges occur. In the marine environment, pharmaceuticals have predominantly been found in seawater, but less so in sediment. However, field data of the bioaccumulation of pharmaceuticals in marine organisms exposed to environmental conditions is rather scarce and concentrations of pharmaceuticals in marine fish have only be determined in a small number of areas. The aims of this study were to determine the concentration of pharmaceuticals in cockle, noble pen shell, sea snail, golden grey mullet and black goby in spring and autumn; and to assess the bioaccumulation in cages clams in 4 sites with different exposure grade to El Albufera de Valencia, Spain. A method of 20 multi-class pharmaceuticals in fish tissues (adapted for grey golden mullet muscle and liver and for the following molluscs: clam, noble pen shell, and sea snail. Eighteen out of the 20 compounds analyzed were found at low ng g⁻¹ in these species throughout the lagoon. Hydrochlorothiazide and carbamazepine were detected in all species considered. The bioaccumulation of pharmaceuticals was heterogeneous in the lagoon, with a higher number of pharmaceuticals being detected in fish (19) than in wild molluscs (10), particularly in golden grey mullet muscle (16). The bioaccumulation of pharmaceuticals was lower in sea snail than in bivalves, and in black goby than in golden grey mullet. Psychiatric drugs preferably bioaccumulated in fish muscle, while citalopram did so in molluscs. Carbamazepine and hydrochlorothiazide were detected in all species in this study. The higher detection frequency and concentrations found in golden grey mullet (mussel) suggested that mugilids could be used as an indicator of contamination by pharmaceuticals in coastal areas. Acknowledgement. This work was supported by the Seneca Foundation (Region of Murcia, Spain) through the ‘BIOMARO’ project (15398/P10), the Spanish Inter-Ministerial Science and Technology Commission through the ‘IMPACTA’ project (CICYT, CTM2013-48194-C3-1-R) and by the European Union through the European Regional Development Fund (ERDF). It was also partly supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for Water Research 2014 SGR 291).

6 Impacts of compound properties and sediment characteristics on the sorption behavior of pharmaceuticals in aquatic systems Q. Abdullah, University of York / Environment; A. Boxall, University of York / Environment Department

Sorption is a key factor in determining the persistence and attenuation of pharmaceuticals in sediment and will therefore affect the impact that a pharmaceutical has on aquatic organisms. However, our understanding of the relationship between pharmaceuticals characteristics and sorption behavior is lacking as most studies into the sorption behavior of pharmaceuticals focus on their behaviour in sewage sludge and soil. In this study, the sorption behaviour of fifteen different pharmaceuticals in ten sediments from the UK and Ireland, with different properties, was assessed. Batch sorption studies with the pharmaceuticals at a single concentration of 500µg l⁻¹ were performed and the sorption affinity for all sediments was found to increase in the order: fenamethacon acid < cimetidine < enalol < amitriptyline < diltiazem. An existing predictive model for estimating the sorption of ionisable compounds was evaluated against the experimental data. The model tended to over-predict the sorption of the basic compounds and under-predict the sorption of the anionic compounds. Multiple linear regression analysis was therefore used to develop new models for estimating the sorption (Kd) of the study pharmaceuticals from sediment properties. Sediment and pharmaceutical parameters used in the models were: (a) log Dow (lipophilicity corrected for pH) for amitriptyline, (b) cation exchange capacity for atenolol, (c) clay and organic carbon content for cimetidine, (d) log Dow and exchangeable Ca²⁺ for diltiazem and (e) aquatic carbon content for fenamethacon acid. The validity of the proposed regression equations was tested using independent data and gave good results for

Microplastics in the environment: Sources, Fate and Effects

7 Micro- and mesoplastic in Atlantic cod (Gadus morhua) from the Norwegian coastal environment d.p. eidvoll, Norwegian Institute for Water Research (NIVA); I. Nerland, NIVA Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; C.C. Steindal, University of Oslo / Museum of Cultural History; K.V. Thomas, NIVA Norwegian Institute for Water Research / Product Metabolism; T. Krem, Norwegian Institute for Water Research / Marine Chemistry

Microplastics can affect marine organisms in several ways. This study documents microplastics (< 5mm) and mesoplastics (> 5mm) in stomachs from cod (Gadus morhua) one of the most common and economically important marine fish in Norway. 302 fish stomachs were examined from six different locations from the coast of Norway. Ten individual stomachs had items in them identified as synthetic polymers, eight of these were from one location (Bergen). All objects found in the stomachs were Fourier Transform Infrared Spectroscopy (FTIR) scanned and subsequently compared with FTIR libraries to confirm identity of the removed items. In this study all stomachs where plastics were found, were full. Empty stomachs had no presence of plastic. This study presents a record of plastic polymers being identified in the stomachs of cod in three out of six locations. We found the presence of polyethylene terephthalate (PET or PETE) and polyester, polyvinyl chloride (PVC), polypropylene (PP), polyurethane (PS), acrylic and nylon (Other). We identified a hot spot for plastic ingestion in Bergen. Our findings indicate that plastic pieces are more prevalent inside fish with a full stomach content versus those with empty stomachs. Therefore, pieces large enough to be visually identified through a stereo microscope may be flushed out of the stomach region of the GI tract. Further work is needed to establish the sources and potential consequences of microplastics.

8 Fragmentation of plastic items into secondary microplastics under environmental conditions J. Klasmeier, University of Osnabrueck / Institute of Environmental Systems Research; A. Reuwer, Osnabrück University / Institute of Environmental Systems Research

Marine plastic debris of all size classes contaminates marine waters and coastlines worldwide. Larger plastic items are suspected to be fragmented under external friction exerted by water currents, rocks or sediments. Weathering, abrasion or disruption of plastics may result in an increasing number of secondary plastic debris. Main goal of this research was the systematic investigation of the fragmentation behavior of plastic material under conditions typically experienced in the marine environment. PE bottles (1 L) were filled with 1 kg pre-cleaned sand (beach conditions), and half of the samples were additionally spiked with 300 ml artificial seawater (breakwater conditions). Plastic items of different polymer material (PE, PP, PS, PET) and form (cups, small bottles, bottle caps, thin foils, film strips) were added and shaken for 30 days in an overhead shaker. After 30 days, intact items and larger plastic fragments were sorted out by hand. Samples were extracted for microplastic particles using a simplified adaptation of a flotation method developed in our lab. Extracted particles were visually inspected under a microscope and sorted into different size classes. Some randomly selected particles from each size class were analyzed with FTIR (Bruker Vertex 70) to identify the polymer material. All investigated items bear visible traces of mechanical abrasion. Film strips and bottle caps did not fragment, but abrasion of material from the surface had obviously occurred. Thin foil sheets were partly perforated and fragmented into a number of mesoplastic and large microplastic particles of elongated shape. The other investigated items were fragmented into many small, less smaller particles. Micro- and mesoplastic fragment analysis of the PS cup sample resulted in four mesoplastic fragments, 35 large microplastic particles (1 – 5 mm) and 32 small microplastics.

9 Analysis of microplastic in environmental samples using thermal analytical methods U. Braun, E. Duemichen, A. Barthel, BAM- Federal Institute Materials Research and Testing; N. Becker, C. Bannick, UBA Umweltbundesamt

The increasing amount of polymers in practical use with their excellent material properties, such as low weight, easy processing and high stability against various chemical attacks, will also increase the unfavoured presence of plastics in the environment. These plastics and small-degraded plastic particles, called microplastics, are almost ubiquitary in nature. Up to now, no verified data exist about the source, the fate and the amount of various plastics in water, soil, air and biota. The reason for this is that no fast and easy available method exists, which detect the small amount of synthetic polymers being a large amount of natural
macromolecules in various environmental matrices. Known, microscopic-spectroscopic methods are limited due to very large measurement times, high sophisticated evaluation knowledge or method specific limitations such as sample preparation in infrared analysis or fluorescence of environmental matrix in Raman. Furthermore, no quantitative value can be determined by these methods. Quantitative values, which include not just whole range of synthetic polymers, are a prerequisite for administrative regulations. A practicable alternative is the thermal extraction desorption–gas chromatography-mass spectroscopy (TED-GC-MS) (12). In this method, the advantages of TGA (high sample loadings and an easy cleaning) are combined with the advantages of GC-MS (easy identification of various hydrocarbon products with high certainty). In TGA, a large amount of sample is decomposed. The formed volatile decomposition products can be trapped on the surface of an adsorber material located at the outlet of TGA and collects a representative content of hydrocarbon decomposition products can be trapped on the surface of an adsorber material. The investigation of various spiced environmental samples for the presentation of this new method, we present data of real environmental samples. These are air and water filtrates from various sources as well as samples from composting plants and streams. Samples are measured as received or after treatment with chemical solutions. (1) D. Jämrich, A.-K. Barthel, U. Braun, C. G. Bannick, K. Brand, M. Jekel and R. Senz, Water Research 2015, 85, 451-457.

10 Role of microplastic beads on the uptake of silver in zebrafish E. Khan, Roskilde University / ENSPAC; D. Boyle, University of Plymouth / College London; K. Syberg, Roskilde University / Department of Environmental Social and Spatial Change; Y. Shashoua, The National Museum of Denmark; N. Bury, Kings College London / Division of Diabetes and Nutritional Sciences. This study aimed to determine whether the uptake and localization of Ag in zebrafish was affected by the presence of polylethylene microplastic beads (PE MPBs). Zebrafish were exposed to 1 μg Ag L⁻¹ (radiolabelled with 109Ag) for 4 and 24 h in the presence or absence of PE MPBs (10, 100 or 1000 MPBs mL⁻¹), and one treatment in which MPBs (1000 MPBs mL⁻¹) were incubated with Ag to promote adsorption. The presence of MPBs, at any of the tested doses, had no effect on the uptake or localization of Ag. However, exposure to the Ag-incubated MPBs (75% of the Ag bound to MPBs) significantly reduced Ag uptake at both time points and also significantly increased the proportion of intestinal Ag. This study demonstrates that microplastics can alter the bioavailability and uptake route of a metal contaminant in a model fish species. A second study was undertaken to investigate the fate of Ag transported into the gut along MPBs. This second study utilised in vitro gut sac preparations, but showed minimal differences in the internal fate of Ag regardless of its interaction with the MPBs.

11 Comparison of tissue preparation procedures to perform microplastic analysis; application to mussels (Mytilus edulis) from the Atlantic coast (Pays de la Loire, France) N.N. Phuong, Nantes University; A. Zalouk-Vergnoux, L. Poirier, Université de Nantes / MMS; A. Kamari, Université de Nantes / MMS; J. Rouxel, R. Sassarellu, F. Pouvreau, IFREMER; A. Chatel, Université Catholique de L'Ouest / Science Environment; C. Mouneyrac, Université Catholique de L'Ouest / UNAM Université Nantes Angers Le Mans Mer Molécules Santé MMS; f. lagarde, Institute of molecules and materials of Le Mans / Institute of Molecules and Molecules of Le Mans IMM UMR CNRS Plastics is a generic name to encompass most of the synthetic organic polymers exhibiting the property of plasticity. These products are characterized by many advantages which explain that they are massively used in our everyday life. Since the middle of the last century, several million tons of plastics have been produced. Ten percent of produced plastics is estimated to end up in the ocean. Once in the environment, macro debris undergo mechanical (erosion, abrasion), chemical (photo-oxidation, temperature, corrosion) and biological (degradation by microorganisms) actions. All these degradation processes lead to their fragmentation into microplastics (MPs) which accumulate in the environment. According to the last ten years of research, it appears that all natural habitats from polar to tropical are affected by microplastics presence. In these researches, many different procedures were used for MP quantification and characterization in environmental samples. Consequently, the aim of this study was to compare different tissue preparation procedures found in the literature to select the more appropriated. For that, counted MPs of two different forms (fragments and fibers) and types (PE and PP) were identified in Mytilus edulis. After a digestion step implementing different solvents or mix of solvents. Then, the filtration step was performed using different types of filters. The efficiency of the digestion step was calculated from the masses of the filters related to the initial mussel masses and the preservation of the MP integrity was evaluated by using microscopy coupled to Raman and Infra-Red micro-spectrometers. The ratios were compared with the results of HNO3. Nevertheless, the MP integrity was not preserved for all types of MPs added to the mussels since PE fibers were totally dissolved despite good recoveries for PVC and PE fragments. The determination of the better sample preparation procedure is quite difficult since it involves a compromise between efficient digestion of organic matter from mussel tissues and the preservation of the integrity of MPs. HNO3 was however selected for the following analyses since the lower organic matter amounts ensured better microplastic preservation and, eventually this procedure was applied for the evaluation of MP contamination of mussels (M. edulis) from the Atlantic coast (Pays de la Loire, France).

12 Methodological barriers for extraction and characterization of microplastics in biological matrix L. Paul-Pont, LEMAR UMR 6539 CNRS/UBO/IIRD/Iffremer; A. Dehaut, Agence nationale de sécurité sanitaire de lalimentation, de lenvironnement et du travail (Anses) / Laboratoire de sécurité des aliments LSA; A. Cassone, LEMAR UMR 6539 CNRS/UBO/IIRD/Iffremer; L. Friez, LEMAR UMR 6539 CNRS; C. Himber, L. Hermabessiere, Agence nationale de sécurité sanitaire de lalimentation, de lenvironnement et du travail (Anses) / Laboratoire de sécurité des aliments LSA; C. Lambert, P. Soudant, LEMAR UMR 6539 CNRS/UBO/IIRD/Iffremer; A. Huet, Iffremer, LEMAR UMR 6539 CNRS/UBO/IIRD/Iffremer; G. Duflos, Agence nationale de sécurité sanitaire de lalimentation, de lenvironnement et du travail (Anses) / Laboratoire de sécurité des aliments LSA. Pollution of the oceans by microplastics, defined as plastic particles of size below <5mm by the NOAA, represent a major environmental problem worldwide. From an ecotoxicological point of view, the ingestion of microplastics by a wide range of marine organisms leading to substantial impacts on major physiological functions has been shown for different marine vertebrates. To date, only a few studies have investigated the levels of contamination of marine organisms collected in situ, partly due to technical difficulties in isolation and characterization of microplastics in biological samples. The crucial step is the identification of solvent(s) or chemical(s) that efficiently dissolve organic matter and biological tissues without degrading plastic polymers, and this in a time and cost effective way. Most published papers, as well as OSPAR recommendations for the development of a common monitoring protocol for plastic particles in fish and shellfish at the European level, used protocols containing nitric acid to digest the biological tissues, despite reports of substantial degradation of some polyamide types (also known as nylon) with this solvent. In addition, testing a wide range of plastic polymers, and especially those commonly found in the marine environment is essential to validate a common protocol and avoid an underestimation of plastic contents in marine organisms after tissue digestion. In the present study, we reviewed existing approaches and we compared (i) their efficiency in digesting biological matrixes and (ii) their effects on 5 different plastic polymers. Effects on plastics were evaluated through visual inspection weighing and Raman analyses before and after digestion, while tissue digestates were filtered on 1μm-mesh fiberglass filters and observed using a binocular microscope. More research is currently ongoing in our laboratory on a wider range of plastic types (n=15 in total). The aim is to identify and validate a unique and standardized protocol that could be implemented at the international level to insure relevance and comparison of environmental studies on this topic.

Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stresors in Aquatic Animals

13 Why are avian focal species unaffected by chlorpyrifos applications? Integrating data on foraging-behaviour, exposure, and toxicokinetics M. Fouedoulakis, Dow Agrosciences / ISRA ERS; S. Norman, RidgewayEco; C. Wolf, B. Giessing, Tier3 Solutions GmbH; R. Dittrich, Tier3 Solutions GmbH / Wildlife Ecology; N. N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department; G. Weymann, ADAMA Agricultural Solutions Ltd. Tier 1 avian risk assessment indicates high risk from use of chlorpyrifos (CP) due to its high toxicity to birds when tested in standard laboratory studies. However, when used in agriculture, CP is a non-specific assessment tool that helps to understand the status and ecology of the bird community present in the target crop at field level. In this respect, it is important to estimate basic population parameters of species breeding within the treated fields (long term survival and reproductive performance), their foraging behaviour and feeding ecology, and the spatial and temporal movement of the focal species to evaluate the factors (both natural and anthropogenic) which may influence reproductive performance and community of birds present. In addition it is equally important to collect exposure and mechanistic data to support and explain findings in the field. Several field studies have been conducted towards this aim. To gain information on the bird community within the CP treated fields a variety methods were used depending on the goal of the study and the species in focus, which allows measurement and ranking of any factors influencing the local bird community, including the application of CP itself. The work undertaken over consecutive years gave a good insight into exposure of birds utilizing treated fields/fruit orchards and showed a good correlation between the application of CP and cholinesterase (ChE) activity in the same bird communities, providing a diagnostic evaluation of CP exposure.
In order to support field findings and provide a higher tier risk assessment on birds, mechanistic information were also provided based on body burden model describing Toxicokinetics and Toxicodynamics. This presentation will describe the comprehensive and holistic approach which was followed, the new approaches for bird studies in risk assessment over consecutive years of data collection and will also examine why the high risk of mortality predicted by the Tier 1 risk assessment is not seen in the field.

14 Sex and steroid hormones: Impact of a pervasive endocrine disrupting agricultural pollutant on sexual selection in Fish

M.G. Bertran, Monash University / Biological Sciences; M. Saaristo, T.E. Ecker, B.B. Wong, Monash University / School of Biological Sciences

Environmental contamination with endocrine disrupting chemicals (EDCs) threatens human and wildlife populations globally. The sub-lethal effects of endocrine disrupting pollutants on environmentally realistic levels are receiving increasing attention, including the impacts of exposure on behaviours influencing survival and reproductive fitness. Despite this, understanding of how these biologically active pollutants impact processes of sexual selection is rudimentary. In this study, we investigated the effects of EDC-exposure on male mate choice, a key component of sexual selection and a driver of evolution by natural selection. Specifically, the aim of this study was to examine the impacts of short-term (21-day) exposure to an environmentally relevant concentration of 17b-trenbolone (measured concentration 6 ng.L-1)—an androgenic growth promoter used in the cattle industry globally that has been repeatedly detected in freshwater systems—on male choice in the guppy (Poecilia reticulata). The guppy is a sexually dimorphic fish with males inseminating females using their modified anal fin (the gonopodium) as an intromittent organ. Male guppies are choosy and prefer larger females as mates, and therefore gain fitness benefits as female guppy fecundity (brood size) increases with body size. Given that male mate choice is a key component of sexual selection, regarding not only whether to mate but also the allocation of reproductive investment between each mate or mating, this male choosiness is key to reproductive fitness. To test the effect of exposure to 17b-trenbolone on male guppy preference for female size, we used a free-swimming experimental design, within which a pair of male and female guppies were tested in one of four treatments as follows: (1) unexposed male with ‘large’ stimulus female, (2) exposed male with ‘large’ stimulus female, (3) unexposed male with ‘small’ stimulus female, and (4) exposed male with ‘small’ stimulus female. We measured the time and frequency of male courting behaviours, as well as male structural colouration and morphology. The results of the mating behaviour trials, as well as colouration and morphological analyses, will be discussed in reference to the broader ecological and evolutionary consequences of EDC exposure.

15 Effects of the NSAID Diclofenac on the survival, health and behaviour of embryonic and juvenile brown trout Salmo trutta f. fario

S. Schwartz, University of Tuebingen / Animal Physiological Ecology; H. Schmieg, Tübingen University / Animal Physiological Ecology; M. Scheurer, Water Technology Center TZW Karlsruhe; H.-O. Köhler, University of Tuebingen / Institute of Evolution and Ecology Animal Physiological Ecology; R. Triebkorn, University of Tuebingen / Animal Physiological Ecology

While the presence of various phytotoxic or antibiotic organism in surface waters has frequently been shown, the ecological impact of the detected substances is still hardly assessable. Within the project “Eff-Pharm”, new mode-of-action based in vitro systems for the monitoring of non-steroid anti-inflammatory drugs are developed. Simultaneous in vitro studies on diclofenac, a common and widely used representative of this pharmaceutical group, provide further information on possible effect concentrations. The brown trout has been investigated as a species of high local environmental relevance for Central European stream ecosystems. Our aim was to investigate two supposedly sensitive stages of trout life history: the embryonic development from fertilized egg to hatching stage and the post-hatch period. The exposure of trout embryos and sac-fry stages was conducted according to OECD guideline 212, and included five test concentrations (0.1, 0.5, 1.0, 10 and 100 µg/L diclofenac) plus control - tested in triplicates for 127 days at 7 °C. Recorded endpoints were mortality, time until eye development, time to hatch, number of gill rakers, heart rate, heart rate and histological condition of the fry. Juvenile trout (approx. 6 months post hatch) were exposed in a semi-static three-block design (control, 0.1, 1, 10, 100 and 200 µg/L diclofenac per block) for 25 days at 7 °C. Besides examining mortality, biometric parameters and behavioural abnormalities, additional biochemical analyses determined stress protein level. In addition, pathological and histological examination was conducted in liver, gill and kidney tissues. None of the investigated parameters was altered in the embryos and fry. In contrast, juvenile fish showed increased mortality already at 0.1 µg/L diclofenac. Mortality rate was concentration dependent and reached up to 43 % in the highest test concentrations, while it did not exceed 10 % in the control. Furthermore, fish exposed to 10 µg/L diclofenac or higher showed a decrease in swimming efficiency of bites marks, a sign of increased territorial behaviour. Further histological analyses will give us a closer insight into the health state of the surviving individuals. Overall, the study showed alarming results on the toxicity of a commonly used NSAID. It also stresses out the importance of investigating different life stages to fully assess the potential effect of chemicals in the aquatic environment.

16 Can zebrafish larvae behaviour be used for on-line contamination detection in water distribution systems?

L. Naesser, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; E. Salomon, Technion Israel Institute of Technology / O. Skulovich, Technion - Israel Institute of Technology; G. Weyman, ADAMA Agricultural Solutions Ltd.

In TGA, a large amount of sample is decomposed. The formed volatile products enables the fast identification of various polymers. Beside the advantages which explain that they are massively used in our everyday life. Since 1985, the product was used in the cattle industry globally that has been repeatedly detected in freshwater systems—on male choice in the guppy (Poecilia reticulata). The guppy is a sexually dimorphic fish with males inseminating females using their modified anal fin (the gonopodium) as an intromittent organ. Male guppies are choosy and prefer larger females as mates, and therefore gain fitness benefits as female guppy fecundity (brood size) increases with body size. Given that male mate choice is a key component of sexual selection, regarding not only whether to mate but also the allocation of reproductive investment between each mate or mating, this male choosiness is key to reproductive fitness. To test the effect of exposure to 17b-trenbolone on male guppy preference for female size, we used a free-swimming experimental design, within which a pair of male and female guppies were tested in one of four treatments as follows: (1) unexposed male with ‘large’ stimulus female, (2) exposed male with ‘large’ stimulus female, (3) unexposed male with ‘small’ stimulus female, and (4) exposed male with ‘small’ stimulus female. We measured the time and frequency of male courting behaviours, as well as male structural colouration and morphology. The results of the mating behaviour trials, as well as colouration and morphological analyses, will be discussed in reference to the broader ecological and evolutionary consequences of EDC exposure.

17 The mechanism of oil sands process-affected water on feeding-behaviour toxicity in Daphnia magna

E. Lari, D. Steenkey, E. Mohaddes, G.G. Pyle, University of Lethbridge / Biological Sciences

Oil sands process-affected water (OSPW) is produced as a byproduct of the surface mining oilsands industry in northeastern Alberta. Responsible remediation and returning OSPW to natural environment is a big concern of both oil and gas companies and governments. The return of OSPW to the pristine environment presents a threat to the drinking water and aquatic ecosystems. In our previous studies we showed that OSPW decreases the feeding activity of Daphnia magna. To understand the mechanism of OSPW’s effect on feeding behaviour we exposed D. magna to three substances (filtered OSPW (chemical portion), filtered OSPW plus added clay particles (whole OSPW), and clay particles suspended in solution (particulate portion)) and we used Daphnia magna culture water as an exposure control. We fed the animals similar quantities of their food algae, and gave them 24 h (in darkness) to graze. After 24 h we measured the food consumption in all treatments and the exposed D. magna were used for further experiments to understand the mechanism of effects of the components of OSPW on feeding activity of D. magna. For the behavioural bioassay, using a behaviour chamber designed for this study we examined the thoracic limbs and mandible beating as indicators of feeding rate of D. magna. We measured the activity of twodigestive enzymes (amylyase and trypsin) as representatives of digestion of D. magna. Our results from all studied biological levels showed whole OSPW significantly affects thoracic limbs and mandible movement, gut clearance, and digestive enzyme activity of D. magna. Particulate matter component of OSPW is most effective and chemical components are less effective in the observed reduction in the feeding behaviour of D. magna.

18 Comparative behavioral toxicity of two common fish models

B. Steele, Baylor University / Biomedical Studies; L.A. Kristofco; J. Corrales, Baylor University / Environmental Science; B.W. Brooks, Baylor University / Environmental Health Science Program Department of Environmental Science Behavioral responses in model species to experimental insults were examined by comparing the similarity of molecular (MIES) that propagate to physiological...
changes in individuals. Behavioral thresholds for a wide range of contaminants often manifest at lower levels than those eliciting mortality and other standardized adverse outcomes. Zebrfish larvae are common models in behavior, developmental toxicology, neurotoxicology and other biomedical studies. Whereas the fathead minnow is a common model for aquatic toxicity research and regulatory programs, they have received comparatively little attention in behavioral studies. We employed the zebrfish and fathead minnow models to define toxicant induced swimming activity alterations during interchanging photoperiods. We specifically examined behavioral response patterns among compounds specifically targeting various receptors/enzymes compared to response profiles for chemicals eliciting toxicity through narcosis. Following OECD FET and EPA WET experimental guidelines, zebrfish embryos and fathead minnow larvae were exposed for 96 h to each compound, then observed using a digital behavioral analysis system (ViewPoint). Behavioral observations occurred for 50 minutes (10 minutes acclimation, two 10 minute dark periods, two 10 minute light periods). In comparison to fathead minnow larvae, zebrfish generally displayed greater behavioral sensitivities to a number of the investigated compounds. Furthermore, zebrfish displayed more pronounced responses to changing light and dark photoperiods than the fathead minnow model. Such photomotor response profiles for compounds with defined MEI’s are being used to examine industrial chemicals for which behavioral responses are poorly understood.

**Fate, Effects and Risk Assessment of Chemicals in Aquatic and Terrestrial plants (I)**

19 Spatial and temporal distribution of pesticides and their metabolites in tomato plants

M. Kubicki, TU Dortmund / Institute of Environmental Research; M. Lamshoef, Bayer CropScience AG / RD; S. Zülke, M. Spittel, TU Dortmund / Institute of Environmental Research

Knowledge about translocation of plant protection products (PPP’s) in plants is important to understand the uptake via the root system. Imaging mass spectrometry (IMS) has been widely used in life science and has proven to be a powerful tool to combine histological data with specific molecular information. So far phospholipids, peptides, drugs and plant metabolites were imaged in a number of tissue samples at a spatial resolution of 5–10 μm². The IMS data for all these experiments showed excellent correlation with histological staining evaluation. Thus, IMS allows exploration of both PPP’s and their metabolites distribution in plant tissues and provides new knowledge concerning behaviour during plant growth. Within a single experiment two-dimensional spatial maps of parent compound and metabolites can be generated. We report here the use of a matrix-assisted laser desorption/ionization (MALDI) imaging source working at atmospheric pressure which is coupled to an orbital trapping mass spectrometer for new insights into molecular processes inside the plants. Therefore, spray application onto the tissue using selected matrix solution has to be optimised, supporting desorption and ionization of target compounds by means of a focused laser. This sample preparation is important to improve sensitivity and spatial resolution of the IMS-measurement and finally to generate the images. Here, we report the use of MALDI-high resolution-IMS to map the distribution of two selected PPP’s (the phenylamide fungicide metalaxyl and the triazole fungicide tebuconazole) and their metabolites in roots, stem and leaves of tomato plants in high-spatial resolution with minimised sample preparation. The combination of autoradiography of 14C-labelled compounds, LC-HRMS, and MALDI-IMS provides comprehensive information of distribution, localization and dynamics of PPP’s uptake via the root system of tomato plants.

20 Toxicity testing with the willow tree transpiration test - 15 years of results

S. Trapp, Technical University of Denmark / DTU Environment; L.P. Clausen, Technical University of Denmark / Department of Environmental Engineering

Due to the large size of trees, their slow growth and the related difficulties, few toxicity tests for trees have been developed, and no standard test is available. A toxicity test using the transpiration of willows is in use since 15 years [1]. The willow tree toxicity test was employed for toxicity testing, for phytoremediation purposes and for process studies in the soil-plant system. Genetically homogenous willow cuttings are grown under artificial light in 500 L Erlenmeyner flasks filled with spiked nutrient solution (ISO 8962), contaminated soils, wastewater or other test substrate [1]. The toxic endpoint is the change of transpiration, which is measured daily by weighing the whole flask, compared to the transpiration of control trees. In EC50-values of more than 50 compounds obtained under various conditions. EC50-values < 1 mg/L were obtained for Cu(2+), Zn(2+), As (arsenate and As(III)), and Sn(2+) using the plant system tested. The most toxic was the weak acid ibuprofen with an EC50-value of 1.7 mg/L, but only at pH 4, and much less toxic at higher pH. Dicyclofenn (100 mg/L) and paracetamol (3000 mg/L) were less toxic in this test. The bivalent base chloroquin had an EC50 of 2.5, 10, 35, 40 mg/L at pH 9, 6, 7, 6, respectively [2]. The toxicity of chloroquinolines declined from 1.1 mg/L (2,5-DCP) to 5.8-10 mg/L (2,4-DCP) to 32-84 (4-chlorophenol) and 400 to 800 mg/L (phenol), The toxicity of cyanide depended very much on the concentration, from rather non-toxic 1000 mg/L Prussian blue (ferriroycyanide, FeIII-FeII-complex) to quite toxic with EC50 at 2 mg/L for KCN. Toxicity to willows grown in sand was generally lower, for example, EC50 = 20 mg/L for KCN in sand. Little toxicity (contrary, growth stimulation at lower dosages) was observed for mono-TG2 (0.15 mg/L) and zero-valent iron nanoparticles (around 1000 mg/L) in solution. A user manual has been developed and is freely available (http://homepagina.ehu.ehu/kt/)

Overall, the willow tree test is probably the most widely used toxicity test with trees and could be seen as a candidate for a standard toxicity test for woody terrestrial plants. [1] Trapp S, Zambrano KC, Kusk KO, Karlson U. 2000. A phytotoxicity test using transpiration of willows. Arch Environ. Contam. Toxicol. 39(2):154-160

**21 Uptake of pharmaceuticals from wastewater into plants - Novel carbamazepine metabolites in tomato plants grown under hyporonic conditions**

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Water scarcity is one of the main challenges in arid and semiarid regions. Therefore, the use of treated wastewater (TWW) for crop irrigation is a necessity. Under these circumstances pollutants of emerging, with low removal efficiency in wastewater treatment plants, are transferred into the river ecosystems and may be transferred from irrigation water into edible plants and thus enter the food chain [1-3]. However, until now little work has been done to investigate the metabolism of PPCPs after uptake into plants. We studied the uptake and metabolism of PPCP from wastewater into plants with carbamazepine (CBZ) as model compound, an antiepileptic drug which is a commonly detected pharmaceutical in wastewater, surface water and even groundwater [4]. Tomato plants grown in hyporonic cultures were analyzed with both targeted analytical methods (LC-MS/MS) that offer higher sensitivity and liquid chromatography-high resolution mass spectrometry (UPLC-Q-TOF-MS) to identify unknown metabolites. A total of 10 phase-I and three phase-II metabolites could be detected from the one contaminant CBZ. Considering these known CBZ metabolites the overall concentration was about 1.5-fold (for tomato leaves and fruits) higher than that of CBZ alone. This example illustrates that, due to plant metabolism, the concentration of contaminants found in plants can be significantly higher than the concentration of the parent compound. However, further study is needed to identify the potential toxicological effects of these metabolites and the metabolic pathways for the safe reuse of treated wastewater in agriculture. In addition, further research is required to better understand and possibly to predict the metabolism of compounds within plants in terms of potential consequences for modelling. References: [1] Goldstein M, Shenker M, Chetbetz F. 2014. Environ. Sci. Technol. 48:5593-5600. [2] Macherius A, Eggen T, Lorenz W, Moeder M, Ondruschka J, Reemtsma T. 2012. Environ. Sci. Technol. 46:10797-10804. [3] Shenker M, Harush D, Ben-Ari J, Chetbetz F. 2011. Chromosoma 82:905-910. [4] Ternes TA, Bonzer M, Herrmann N, Teiser B, Andersen HR. 2007. Acknowledgement: Financial support of this study by the Deutsche Forschungsgemeinschaft (DFG, Bonn) through the project PECTake (Re1290/7-1) is gratefully acknowledged.

**22 Aquatic macrophyte modelling - Increasing the realism of risk assessments**

S. Heine, Bayer CropScience / Environmental Modelling; K. Kuhl, A. Solga, Bayer CropScience AG; E. Bruns, Bayer CropScience AG / BCS D ETX

For the registration of plant protection products, the risk on aquatic macrophytes has to be assessed. This is done by specifically evaluating the trophic transfer pattern of a plant protection product in several surface water systems considering its specific agricultural application. To assess the risk on aquatic macrophytes, as a first step, the maximum concentration that is predicted throughout the year is compared to toxicity endpoints, such as EC50 values. In most cases, these toxicity endpoints are based on studies considering constant concentrations of the respective plant protection product. A method to increase the realism (higher tier) of the risk assessment is to use toxicity studies that are based on exposure patterns similar to those predicted in surface water systems. To date, this approach is mostly applied to short term exposure patterns, having a duration of less than a week, due to the time range of standard macrophyte toxicity study endpoints. This study demonstrates ecological modelling methods, in terms of toxicokinetic and toxicodynamic (TK/TD) growth models of Lemna spp. and Myriophyllum spicatum that quantify adverse effects caused through entire and complex exposure patterns. Special emphasis is on the toxicity data needed to reliably calibrate the models. Modeling results showed a broad variation of predicted effects strongly depending on the specific trophic transfer pattern of the plant protection product. Another focus of this contribution is the presentation of semi-field sets.
growth data of M. spicatum. The monitoring of plant growth was initiated to identify the spatial capacity limit of plant growth and to obtain long-term growth data that are otherwise hardly available for aquatic macrophytes. The maximum capacity of total plant length was approximately 150 m for the containers with their specific spatial dimensions.

23 Plant community modelling as a means to assess herbicide effects on plant reproduction using the IBC model approach

J. Rees, University of Potsdam / Plant Ecology and Nature Conservation; T. Schad, T. Preuss, Bayer CropScience / Environmental Modelling; C. Mihan, Bayer CropScience AG; F. Jeltsch, University of Potsdam

Risk assessments of plant protection products include testing effects on non-target terrestrial plants (NTTPs). To address the protection of plant communities at landscape scale, several researchers state that the full plant life cycle should be assessed since reproductive end points (e.g., seed production, fruit, and terminal soil metal results) may be more relevant for the continuation of the populations. It remains unclear, which effect extent might be tolerable to still preserve plant populations. During a follow-up SETAC workshop on NTTPs held in Wageningen on September 21 and 22 2015, the need for reproductive studies was discussed and extrapolation from existing studies appears meaningful. The framework of Xplicit-IBC offers an excellent opportunity to address this task. It aims to analyze herbicide drift effects on non-target terrestrial plant communities and populations within a landscape community context by utilizing an individual-based spatiotemporally explicit plant community model (IBC-grass). IBC-grass is an individual-based stochastic plant community model for grasslands using the plant functional type approach to create a wide parameterized for a typical herbaceous field boundary community in Europe. The regional species pool was based on an extensive literature research and was classified into plant functional types (PFTs). Due to the individual-based approach of IBC-grass, we were able to address potential herbicide effects on relevant reproductive attributes of plant individuals. In a first model test phase, we conducted unrealistic worst-case simulation experiments in which we differentiated the relevant attribute(s), effect extents and the affected PFTs. These simulations indicate that effects on reproductive endpoints can have complex impacts on PFT populations. Individual-based plant community modelling can provide valuable tools to clarify the need and - if necessary – the design of future experiments. Further investigations will analyze in greater depth the dependency of effects on plant trait characteristics (e.g., type of reproduction) and the sensitivity of potential endpoints. In these studies herbicide exposure will be simulated in a more realistic pattern to investigate real-world situations (e.g., variable herbicide exposures) and will be upscaled to a landscape level by integrating the herbicide exposure model Xplicit.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (I)

24 Modelling chromium(III) binding to humic substances and its speciation in soil solution

J.E. Groenenberg, Alterra Wageningen University and Research Centre / sustainable soil management; E. Temminghoff, Wageningen University / Soil Quality

Chromium exists in natural environments in the Cr(III) and Cr(VI) redox state. Humic substances can reduce the toxic Cr(VI) to Cr(III). Cr(III) binds very strongly to organic matter in soils which reduces its availability and protects it against oxidation. Binding of Cr(III) to humic substances is however largely quantified and binding constants for Cr binding to humins in ion binding models are based on very limited experimental data. Modeled Cr(III) speciation in soil solution using the NICA-Donnan model in combination with generic model parameters deviated strongly from speciation measurements in soil solution extracts with the Donnan Membrane Technique (DMT). To quantify Cr binding to humins we studied the binding of Cr to an isolated fulvic and humic acid using the DMT. Based on these results new model NICA-Donnan model parameters for Cr binding to humins were derived, using the coupled PEST-ORCHESTRA modeling tool. Additional information other than macroscopic adsorption data were necessary to make scientifically sound choices with respect to the parameterisation.

25 Understanding the behaviour of silver in surface and wastewater environments: Experimental validation of WHAM

A. Peters, wca; G. Merrington, wca consulting; R. van Egmond, Uitlever; S.D. Smith, Wilfried University / Department of Chemistry; S. Lofft, Centre for Ecology & Hydrology / Shore Section

Exposures to ionic silver are known to result in adverse effects in laboratory test organisms at concentrations in the low parts per billion range. Yet, environmental exposures are rarely in the ionic form and so are less ecotoxicologically relevant. It has long been established that dissolved organic carbon (DOC) and sulphur compounds in freshwater and estuaries can bind with silver mitigating ecotoxicity and influencing the behaviour and fate. Concentrated samples of natural organic materials were collected from four freshwater sites in Canada using reverse osmosis. Standard samples of natural organic matter (NOM) concentrations, collected using a similar reverse osmosis method to that employed for the field samples, were also obtained from the International Humic Substances Society (IHSS). The sludge from a raw sewage sample was also tested as a binding matrix. Silver binding to the organic matter concentrates, including raw sewage, was determined by titration using a silver ion selective electrode. Organic matter samples showed varying levels of silver binding at the same metal to carbon ratios. Various properties of the organic matter samples were determined in order to identify the factors affecting silver binding strength between different samples. The properties investigated included fluorescence, specific UV absorption, thiol concentrations, and chromium reducible sulphide concentrations. Chromium reducible sulphide concentrations in the organic matter samples correlate with the degree of silver binding and may be used to refine WHAM predictions of silver speciation.

26 Electroanalytical techniques to determine free concentrations and lability degrees in Indium solutions.

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Indium is a critical element present in electronic devices, from which it can eventually leach to natural waters. The large hydrolysis processes of Indium (e.g., an increase by 0.1 units in pH in precipitated systems decreases the free concentration by a factor of 4) can be accurately predicted using model and availability with most conventional techniques and, so, there are many unresolved aspects of the behaviour of free Indium in various systems. AGNES (Absence of Gradients and Nestman Equilibrium Stripping) is an emerging electroanalytical technique designed to determine free metal ion concentrations in solutions, such as seawaters, river waters, dispersions of nanoparticles, extracts of soils, etc (see recent review). The implementation of AGNES with mercury electrodes requires amalgamating elements such as Zn, Cd, Pb, Sn or In. Due to some irreversibility of Indium on the Hg electrode, a new calibration strategy has been developed. Speciation results in the system Indium+Nitrioltriacetic acid with AGNES agree with computations using database NIST 46.6. However, the evolution of free Indium for increasing amounts of the ligand oxalate indicates that the values of the stability constants in NIST 46.6 are not so accurate as those of Vasca et al. A new strategy, called Accumulation under Diffusion Limited Conditions (ADLC), can be applied to compute the lability degree of the complexes. The lability degree is strongly linked to the availability of this element as it describes the contribution of the complexes to the overall uptake flux. The use of highly labile complexes (such as those of oxalate) allows for a dramatic reduction of the deposition times, especially for huge gains (i.e. preconcentration factors). For instance, in the system oxalate+H₃PO₄ at pH 3, equilibrium for a gain larger than 300000 was attained in just 25 seconds retrieving [In]₃+=7.1×10⁻¹⁰ M. In precipitated solutions at pH 5.6 (where the free Indium concentration is fixed despite the addition of the helping oxalate), [In]₃+=1.87×10⁻¹⁰ was determined. References: (1) Tuck, D. G. Pure Appl.Chem. 1983, 55, 1477. (2) Galceran, J. et al. Electroanal. Chem. 2014, 722-723, 110. (3) Pinargar, J. M. et al. Bulletin de la Societe Chimique de France 1984, 3-4, 115. (4) Vasca, E. et al. Dalton Trans. 2003, 2698.

27 Colloidal metal speciation in historically contaminated sites studied using centrifugal FFF

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Many indications exist that natural colloids are often vectors for many metals, presenting large discrepancies between modelled and measured behaviour, particularly for those metals having relatively high partitioning coefficients (Kₐ values), such as lead (Pb). Centrifugal Field Flow fractionation (FFF) and sequential filtration were used to analyse the colloidal speciation of several metals released during unsaturated leaching of intact cores of historically contaminated sites in an irrigation chamber. It was also investigated whether this speciation changing during simulated extreme rainfall events. FFF – ICP-MS complemented sequential filtration data the former technique gave a much more detailed picture of the association between several elements simultaneously. Most metals of interest were found as dissolved species and/or associated with relatively small colloids (< 200 nm). The speciation of lead was spread over iron oxides, organic matter and an aluminium bearing phase, but it was unclear whether the latter phase was a clay fraction or aluminium oxides. Sb occurred in relatively larger particles only in one particular soil from an industrial area without associations with other elements. As and Cr appeared mainly dissolved, but partly associated with iron oxides. Differences between the two rainfall scenarios were, in general, limited. The partitioning of metals over dissolved phase and differently size colloids has implication for modelling their fate and bioavailability in realistic systems,
because $K_r$ values may not always provide the most accurate prediction. The relative importance of a kinetic versus thermodynamic approach is discussed.

28 Specific parameterization of WHAM improves the prediction of copper competitive binding on plant roots

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The relevance of the default humic acid (HA) in the Windermere humic aqueous model (WHAM) for modelling the binding of metal cations such as copper exhibiting high affinity for plant roots is questionable. We thus compared the ability of the default parameterization of WHAM and a specific parameterization for terrestrial higher plants (WHAM-THP) to model the competitive binding of copper on wheat (Triticum aestivum L.) and tomato (Solanum lycopersicum L.). Roots. WHAM overestimated by twofold the binding of copper on roots under varying ionic strength on concentration of competitive cations (potassium and zinc). With a single set of parameters for both wheat and tomato, the specific parameterization of WHAM-THP improved the goodness of the fit of copper competitive binding ($\log_{10}$ of the root-mean-squared residual $= 0.15$) without any systematic bias. We put forward proposals for the practical application of WHAM-THP in predictive ecotoxicology.

29 Speciation and localisation of gadolinium in root tissue of Zea mays

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The group of rare earth elements (REE) consists of 17 economically critical elements, the lanthanides and Sc and Y. Their physico-chemical properties make them useful for many high tech applications. The lack of economic recycling ways might result in the ambient concentration of REE in the environment, which might negatively affect organism like plants as primary producers. Bioavailability of REE was shown to be high, especially for fine ground materials like tailings [1]. Earlier studies [2] also showed high concentrations of Gd in the root tissue, with low transport to shoot tissue, therefore, speciation and localisation of Gd in root tissue was conducted. ToF-SIMS, as a surface analytical technique, offers high sensitivity and can generally detect all elements and isotopes. Gd was chosen for a hydromin solution culture using a culture crop Zea mays (cv. Rohndalino). It was applied in a concentration of 10 mg L$^{-1}$ as nitrate. The plants were grown in a greenhouse in eastern Germany during the warm and sunny month April. After 14 days the plants were harvested. The roots were used for the investigations presented here. Thin sections ranging between 8 and 20 µm were analysed for the location and species of Gd by ToP-SIMS (ION TOF). Total concentrations of dried bulk samples were measured by ICP-MS (Agilent 7700) after digestion. High content of Gd was found in the dried bulk samples of the roots after aqua regia digestion, 8.43 ± 0.95 kg kg$^{-1}$. Gd was analysed in spots of high concentrations at the epidermis of the root. The typical isotope pattern was identified for Gd as well as for Gd species bound to oxygen. The expected Gd-phosphates could not be found in these spots. The occurrence of the spots in the outer epidermis might be an explanation for the low transfer rates. For the first time, ToP-SIMS analysis with a spatial resolution of 10 µm was used to locate these spots and give information about the species of the Gd in the plant root. J. Mittermüller, M. Saatz, J. Daus, B. A, A sequential extraction procedure to evaluate the mobilization behavior of rare earth elements in soils and tailings materials, submitted. 2. Saatz, J. Vetterlein D. Mattusch, J. Otto, M. Daus, B., 2015, The influence of gadolinium and yttrium on biomass production and nutrient balance of maize plants. Environ Pollut., 204: 32-4.

Natural toxins: an on-going challenge for environmental research, monitoring and management

30 Cyanobacterial toxins: from genome mining to risk assessment

C. Pancrace, J. Humbert, Institute for Ecology and Environmental Science - Paris / Community diversity and ecosystem functioning; M. Gugger, Institut Pasteur / Microbiologie Collection des Cyanobactéries Cyanobacteria are photosynthetic prokaryotes proliferating in many ecosystems worldwide. This phylum is also known to produce a wide range of bioactive natural products, notably cyanotoxins linked to animal deaths and human health hazards through drinking and recreational waters. Cyanobacterial natural products are complex peptides, polyketides and hybrid metabolites such as the well-studied hepatotoxin microcystin. Current strategies for cyanotoxin-related risk assessment are based on detection of the known producers or of the already characterized toxic compounds. This strategy does not take into account the larger cyanobacterial potential recently uncovered with genomic and metagenomic as well as metabolomic analysis. Furthermore, emerging toxins are not monitored or regulated. We studied the blue-green forming genus Planktothrix genetic potential dedicated to the biosynthesis of natural products. Diversity and distribution was established by in silico genomic analysis of newly sequenced isolated strains of diverse origin. Natural products were also characterized by mass spectrometry analyses to detect variants of the known peptides and novel natural products of this cyanobacterial genus. This multidisciplinary analytical approach could be broadened and extended with cell-free biologics tools for early detection of toxic potential of HABs in environmental samples. Most of the time even in the case of known compounds such as the toxin microcystin, the cellular and ecological function of the metabolite remains unknown. The understanding of regulation of natural product synthesis, as well as the cyanoHABs internal population regulation is only at its first steps. The use of genome mining strategies on NGS environmental and experimental samples will lead to a more systematic survey of potential hazards. Although challenging, closing this knowledge gap between producers and their genetic potential, regulation and metabolites detection will give rise to a better risk assessment in freshwater ecosystems.

31 Structure-dependent effects of selected cyanobacterial peptides on immature oocytes

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Cyanobacteria produce many biologically active metabolites synthesized via nonribosomal synthetic pathways. These are classified into several families, according to their structural features, and include also cyclic microcystins (MCs) and linear aeruginosins (AURs). Although MCs are toxico-logically known for their potent toxic effects and able to modulate cell function. In contrast, aeruginosins have been studied in much lesser extent but recently isolated new aeruginosin-865 (AUR865) showed some anti-inflammatory potential. The present study aimed to investigate in detail the effects of three different MC variants and AUR865 on macrophages, which represent one of the key effector cells within the innate immune responses. Specifically, our study includes investigation and comparison of several structurally different cyanobacterial peptides on macrophage activation, associated with production of cytotoxic and cyostatic products such as nitric oxide (NO), as well as proinflammatory mediators (e.g. tumor necrosis factor α, TNFs and interleukin 6, IL-6). Although MC-LR was able to significantly affect the macrophages, other most common MCs (-RR, -YR) and AUR865 did not alter immune responses. Our recently published results on affected molecular pathways (doi: 10.1021/acs.est.8b02049) provide an interesting mechanistic explanation of some adverse health outcomes associated with toxic cyanobacteria. The research was supported by the projects of the Czech Ministry of Education L0214 and LM2011028, and by the ANR COST action (EU COST ESI105).

32 Occurrence of emerging marine toxins in coastal areas of the Catalan coast

C. Bosch, IDAEA-CSIC / Environmental Chemistry; J. Sanchez, IDAEA-CSIC / Water and Soil Quality Research Group; M. Farre, IDAEA-CSIC; D. Barcelo, I+QAB-CSIC / Dept Environmental Chemistry Marine toxins are natural substances synthetized by phytoplankton and other organisms. Main concern about these compounds is due to its potential toxic effects, especially when a harmful algal bloom (HAB) takes place. During this phenomenon, high amounts of toxins are generated; its accumulation in the particulate can favour the eutrophication of the waters and on the other hand, zooplankton, fish and shellfish that can suffer intoxication with poisoning or lethal effects. Moreover, it has been evidenced that the concentration of toxins can bioaccumulate along the food chain, suppose a potential risk for human health. Monitoring of these compounds has become an important issue during last decades. New analytical methodologies are required with the aim of determining the distribution, transport and behavior of marine toxins with enough accuracy and sensitivity. In this study, an analytical methodology has been developed and validated for the detection of different families of the most frequent marine toxins in marine waters and estuarine environments. A unique strategy is based on solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) coupled to high resolution mass spectrometry (HRMS). In order to assess the occurrence and distribution of these marine toxins, the developed methodology has been applied in real samples from the western Mediterranean Sea and the Ebro’s delta basin. Samples were collected in the same areas for different periods of time in order to characterize the occurrence of marine toxins in different seasons. Preliminary results show the presence of some marine toxins in similar concentration for all seasons and in non-algal bloom conditions. These results highlight the importance of further studying the degradation patterns, distribution and chronic toxic effects of these substances in order to properly carry out a risk assessment.

33 Characterization of the voltage-gated sodium channel and its isoforms in the Pacific oyster Crassostrea gigas: link with its sensitivity to paralytic shellfish toxins produced by Alexandrium minutum

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UBO IRD Ifremer; A. Bidault, P. Boudry, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer / UMR CNRS UBO IRD Ifremer; P. Soudant, C. Fabioux, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer French coasts are regularly affected by blooms of the dinoflagellate *Alexandrium* spp. These micro-algae can produce paralytic shellfish toxins (PST), such as saxitoxin (STX), which binds to voltage-gated sodium (Nav) channels and block conduction of action potential in muscle and nerve fibers. Filter feeder marine organisms, such as oysters, can accumulate high levels of PST, making them toxic and unsuitable for human consumption. The toxin load in *Crasnostrea gigas* oysters, has been shown to vary between individuals, sampled from the same location and processed independently. These results suggest a high variability in PST tolerance. The purpose of our study was (i) to characterize Nav channel and its isoforms in *C. gigas* and (ii) to determine whether Nav channel isoforms play a role in the sensitivity of *C. gigas* to PST. The tissular and cellular expression pattern of Nav was characterized by real-time PCR and in situ hybridization. Nav appeared mainly expressed in neurocytory cells of the visceral ganglia, in nerve fibers of the striated muscle and in epithelial cells of the labial palp and mantle. First sequencing analysis of Nav cDNA in muscle and visceral ganglia revealed three alternative splicing transcripts. These transcripts have a specific tissular expression. Only one transcript is found in the striated muscle, whereas the two other transcripts are expressed in the visceral ganglia. This alternative splicing could be a source of the differential sensitivity to PST in *C. gigas*, as demonstrated in some insects for resistance to pyrethroids, and consequently could lead to variable toxin load in oysters exposed to *A. minutum*. As a result, we will analyze the presence/absence of each splicing variant as well as their expression level in muscle and visceral ganglia in oysters experimentally exposed to *A. minutum* to test the correlation between Nav variant expression and toxin accumulation.

34 Identifying phytoxins of environmental concern
J. Bücheli, Agroscope ART / Environmental Analytics Natural Resources and Agriculture; J. Hollender, Eawag / Environmental Chemistry; M. Scheringer, K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering Today, environmental scientists, regulators and decision makers are well aware of the presence and potential risk of anthropogenic chemicals in the aqueous environment. In contrast, natural toxins from plants (phytoxins) have not yet been perceived as potential aquatic micropollutants. A number of arguments lead us to hypothesize that phytoxins might indeed play a role in this respect, the most important of which are: 1) there are thousands of toxic plants, containing a variety of even more chemically very diverse phytoxins, 2) exposures in anthropogenically managed/affected (agro-)ecosystems can be orders-of-magnitude higher than in the pristine environment, 3) phytoxins are toxic by definition, although their mode of action is often unknown, 4) effects of phytoxins (on non-target organisms) have been shown in case of allelochemicals, invasive plant species, and biopesticides, and 5) to properly evaluate the risks of (emerging) anthropogenic contaminants, the background stress/toxicity potentially exerted by natural toxins should be known. Therefore, we propose a systematic assessment of the aquatic environmental exposure to phytoxins in Switzerland. This is accomplished with a sequence of iteratively refined work packages, including: 1) compilation of a database of phytoxins, based on the spatial distribution and chemical fingerprint of toxic plants in Switzerland, 2) elucidation of physicochemical properties of phytoxins using literature data or in silico estimations, 3) prediction of the phytoxins’ environmental fate and behavior and likely recipient matrices by means of in silico multi-compartment mass-balance models, 4) surface water sampling in collaboration with respective monitoring networks, and 5) target and suspect analysis of phytoxins in the collected water samples. This way, we aim to identify among the many thousands of phytoxins those – probably rather few – that actually may be considered as aquatic micropollutants.

35 Environmental fate and ecotoxicological impact of leptospermone, a natural β-tritketone herbicide in soils
J. Barthes-Méan, S. Rodmmand, University of perpignan / CRIOBE BAE; M. Deveres-Lamrani, INRA Institut National de la Recherche Agronomique / UMR Agroecologie; C. Calvayrac, University of perpignan / BAE; F. Dayan, Colorado State University / Department of Chemistry and Molecular Biology; U. Pesquet, University of Perpignan / CRIOBE; C. Cooper, University of perpignan / CRIOBE; I. BATISSON, CNRS-Clermont Université, Université Blaise Pascal / Laboratoire Microorganismes Génome et Environnement; F. Martin-Laurent, INRA / Agroécologie Most agricultural production relies on the use of chemicals to maintain high crop yields. The use of these chemicals in farming practices is viewed as an integral part of the agricultural system. The use of pesticides in farming, however, has been linked to the production of micropollutants. When applied to agricultural lands may affect non-target organisms and contaminate soil and water media. Increasing public concern about the impact of pesticides on the environment, and European legislation has led to develop some strategies to evaluate and to prevent the potential impacts of different land management practices. In this context, we propose an environmentally dangerous molecules, such as lower impact pesticides, biopesticides and natural product-based pesticides have been introduced. Despite this, a lot of work has to be carried out to evaluate the possible risks and adverse effects of such compounds on environment and humans. In the frame of the TRICETOX project, we study the β-tritketone herbicide family, a post-emergence maize selective herbicides belonging to this new generation of molecules. Inside this family, we focus on leptospermone, an allelopathic compound isolated from the bottlebrush plant *Callistemon citrinus*. Since few data are available for this molecule, we want to gain new insights by addressing its soil behaviour, by studying its impact on soil microbial communities, by studying its biotic and abiotic degradation and by testing its toxicity. Then, multidisciplinary approaches were applied to assess the fate and bioactivity of leptospermone in two different soil types. Our results showed that in the two tested soils, leptospermone has a similar fate to that of the synthetic β-tritketone herbicides, such as sulcotrine or mesotrione. Interestingly, leptospermone exhibits a 10 fold lower toxicity, determined with Microtox® than the two synthetic counterparts. Although leptospermone was rapidly dissipated from the soil, it has a significant ecotoxicological impact on the abundance and diversity of soil bacterial community. This ecotoxicological impact was transient and when leptospermone was fully dissipated, the bacterial community recovered its initial composition and diversity. However, the recovery cannot be reached in soil microcosms where leptospermone remained. Characterization of a bacterial isolate able to degrade leptospermone is in progress. Further work need to be done to test the ecotoxicological impact of this natural β-trit ketone on a wider range of soil classes.

Advances in exposure modelling: bridging the gap between research and application (I)

36 Screening level Environmental Exposure modelling Of Engineered Nanoparticles: An analysis using SimpleBoxNano
J. Meesters, Radboud University Nijmegen / Environmental Science; D. van de Meent, Radboud University / Department of Environmental Science In order to sustain the development of nanomaterials by industry, a robust risk assessment framework is needed. A screening level environmental exposure model is one of the first steps of such a framework currently in place in Europe and the United States, but this needs adaptation for use with nanomaterials. For this reason, SimpleBoxNano was developed as a first step in developing a screening level exposure model that can be used as part of such risk assessment frameworks currently in place for conventional chemicals. Here we present a further analysis of the SimpleBoxNano model in order to continue its development towards a robust screening level exposure assessment model for nanomaterials. First an analysis is done of the uncertainty and variability in output exposure concentrations of three metal oxide ENPs (TiO₂, ZnO and CeO₂) in relation to the uncertainty and variability of the emission rates and a broad selection of input parameters, including environmental properties such as e.g. ambient particle concentrations and temperature. Second, a sensitivity analysis is done for the nano-specific properties: nanoparticle size, density, dissolution rate and attachment efficiency while still accounting for variability of the environmental properties in order to more clearly understand the significance of these specific nanomaterial properties compared to natural variability.

37 A Kinetic Environmental Fate Model for the Risk Assessment of Engineered Nanomaterials
J. Perez Holmberg, University of Gothenburg / Chemistry and Molecular Biology; R.F. Domingos, Institut de Physique du Globe de Paris / Équipe de Géochimie des Eaux; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology; E. Dumont, CEH Wallingford; M. Matze, Centre for Ecology & Hydrology (NERC) / Molecular Ecotoxicology; R. Vanhauten, ThinkWork B.V.; S. Lofts, Centre for Ecology & Hydrology / Shore Section; V. Keller, Centre for Ecology & Hydrology; G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology An integrated exposure model is proposed for the prediction of the environmental fate of nanomaterials (NMs) in various natural and man-made compartments. A semi-mechanistic approach is used, wherein the kinetic nature of the NM fate processes takes focus. Environmental matrix interactions depend on NM properties and relevant parameters, such as organic matter and ionic conditions, and are calculated on a temporal scale in each compartment. The model is therefore based on the calculation of the calculated maximum temporal NM bioavailability and existing hazard information. The goal of this approach is to apply the model for most currently-produced and possible future NMs, also aiming to find a compromise between mechanistic accuracy and operational simplicity. In the current model, fate descriptors are detailed for the following compartments: soil, surface water, sediments and wastewater treatment plant (WWTP). The WWTP is the main entry point for NMs emitted with domestic and industrial liquid waste. Partitioning of NMs in the WWTP, based on their affinity to sludge, determines the amounts that are transported to the surface water (in the effluent) and the soil (via sludge). The physicochemistry of the surface waters, including fresh- or estuarine-, and marine water, and the properties of the NMs are among the factors...
that affect its bioavailable concentration in the water column and, after possible sedimentation, in the sediment within a certain time. The kinetic model includes rates of the following key processes: dissolution, sulfidation, heteroaggregation, and sedimentation. MNs arrive in topsoils via the annual deposition of sludge. The time-dependent concentration in the pore water (liquid phase) and on the mineral surface (solid phase) was estimated by adjusting a dual chamber model, allowing both MN release and (ir)reversible attachment. The time-dependency of chemical species concentrations is essential since it evokes realistic time-dependent exposure scenarios whereas available hazard values are almost always considered static. This information is relevant in assessing the bioavailability and eventual risks of MN release and transport. This kinetic fate model is a key component in the web-based tool for the assessment and management of risks associated with MN-enabled consumer products, under the GUIDEnano Project (EU FP7). This work also contributes to the NanoFASE Project (EU H2020).

38 The importance of temporal variables in environmental modelling: Rationalizing measured concentrations of cyclic volatile methyl siloxanes in an Arctic lake
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Cyclic volatile methyl siloxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. In this study, we describe a holistic evaluation of the environmental fate of cVMS in an Arctic lake. Lake Storvannet is situated in the north of Norway, in an area characterized by cold and dynamic environmental conditions and intermittent wastewater emissions. Sewage, lake water, and sediment samples were collected and analyzed for cVMS concentrations in 2014. While measured cVMS concentrations in lake water were below detection limits, concentrations in sediments were comparable to concentrations observed in other aquatic systems. Storvannet sediments were dominated by cVMS in the shallow layer of the sediment, indicating a significant over-prediction of cVMS concentrations in sediment. This resulted in a significant over-prediction of cVMS concentrations in water compared to actual measurements and to concentrations in the water column. Inverse modelling was employed using the steady-state QWASI to estimate the average rate of emission from the measured concentrations of cVMS in sediment. This study illustrates the use of the software to estimate inventory data for the production of MNs.
Life Cycle Data and Modeling Developments - From Data Collection to Usage (I)

42 Towards a Global Network of Interoperable LCA Databases

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1. Introduction International LCA dialogue has been formalized between countries within the "Network for the exchange of process data" (NEXPO) and the "Open LCA" (http://www.openlca.org). The common objective is to facilitate the implementation of Sustainable Consumption and Production policies. Within this dialogue, cooperation on and access to global data has been identified as a must in order to reduce the cost and increase applicability of LCA studies in a context of a global economy. Therefore, the countries, with the support of UNEP, launched the Global Network of Interoperable LCA databases (the "Global Network") and agreed on a shared ambition that by 2017 the LCA user has a global and easy access to the main LCA databases with assessment of "fitness for purpose". 2. Materials and methods The Steering Committee is the governance body of the initiative and it is supported by the working groups, who are the operational units, created for a limited duration and focused on particular tasks producing deliverables. Three working groups (WG) have been launched: i) "Network Architecture and Technology" and ii) "Nomenclature and iii) "Metadata descriptors". Working groups are co-chaired by experts and gather more than 50 experts worldwide. Each working group has defined a workplan, timeline and corresponding budget for the duration of the project. 3. Results This presentation will provide the latest update following up the 2016 face-to-face meeting, scheduled in Brasilia from 14 to 16 March 2016. As of now, the following deliverables are planned to be completed: Network Architecture and Technology Review of existing examples and technologies for interoperable databases Development of functional requirements of the interoperable network of LCA data First discussion on a list of viable network options: Nomenclature

Agreed elementary flows mapping file ready for review Critical review of existing nomenclature mapping systems Metadata descriptors Identification of data quality assessment needs, with a focus on meta-information 

4. Conclusion In conclusion, enhanced data accessibility and interoperability will benefit the whole community and improve inter-systemic applicability of LCA, and is therefore fundamental for key sustainability initiatives. Policy makers are relying on it for e.g. developing sound SCP policies. Industries will be able to base their innovation and strategic sustainability decisions on more robust information.

43 BONSAI recommendations and procedures for implementation of an open source database

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The BONSAI database (bonsai.uns.org) is an open source community initiative, using semantic web technology and data harvesting to increase the free access to structured LCI data. This presentation describes our recommendations and current work on implementing the BONSAI core database and system. We implement the core database as an RDF store. The RDF store is designed for storing the supply-chain LCI data and is a native format for the balancing properties of flows between activities, further specified by location, time and macro-economic scenario (relevant for forecasted data). Each datapoint consists of a value together with an unit and an uncertainty estimate. Non balanceable flow-properties and metadata can also be stored with the supply-use framework. We recommend a separate matrix to store systems as a complementary tool to substitutability between products. Besides the core database, BONSAI will store algorithms and software code for estimating missing data, automatic data harvesting and accessing and data manipulation. From the supply-use data, the algorithms will allow the calculation of direct requirements tables and their Leontief inverse matrices in order to estimate the product footprints. The barrier for adding data to the BONSAI database should be kept as low as possible, and

conflicting data sources allowed to co-exist. Instead, data quality should be scored and reviewed subsequently, allowing algorithms for quality-scoring and consolidation to provide thresholds for temporary inclusion/exclusion and to derive preferred datapoints. The integration of data at many different levels of detail requires algorithms for automatic disaggregation and gap-filling, using global product and activity breakdowns based on industry data sources and data from countries with detailed data. The first public beta-version should demonstrate proof-of-concept of the key aspects that differentiate BONSAI from other similar databases. This includes the modular approach to software design, the low barrier to data entry with subsequent open review, the completeness (integrating MRIO and detailed data), the natural language footprint query interface and the transparency of the underlying data and routines. This also implies that the data needs to be provided with a fully open license (CC-BY) that allows also commercial use.

44 Coupling process simulation and life cycle assessment: applications to bio-based process investigation

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Simulation of manufacturing processes combined with environmental impact assessment gives engineers the opportunity to improve the environmental performances of the production system. Process simulation tools were used with LCA in order to study the influence of operating parameters and to estimate missing data for life cycle inventory. This work presents, through two illustration examples, the performances of the Aspen Plus™ software for inventory data estimation and for environmental performance optimization. The first case illustrates the use of the software to estimate inventory data for the production process of H2 from biochar reforming based on an anaerobic digestion plant inventory from the literature. The second example addresses an optimization of biofuel steam reforming process to provide accurate conditions for sustainable H2 production. A variation in process operating parameters was also performed to illustrate the environmental performance sensitivity and provide guidance as to research and development effort focus for the process improvement. The variation of the parameters was performed using two methods: intuitive approach, where the levels of all parameters are fixed except one varying, and a factorial Design of Experiments (DOE) method. The different process configurations were simulated in Aspen Plus™ to provide foreground inventory data for the LCA of H2 production system. For both systems, H2 from biochar reforming or from bioethanol, the substrate production greatly influences the environmental performances of the whole system, especially when the system comprises an end-of-life management scenario (first case). The second investigation (H2 from bioethanol reforming) indicates that the increase of reforming temperature improves the environment performance of the H2 production system. Whereas steam to carbon ratio has a marginal effect on the environment performance. The main outcome of this DOE method is the development of mathematical models that predict how changes in the reformer operating temperature and steam to carbon ratio affected the environmental performances. The systematic integration of simulation tools into the elaboration of environmental assessment of processes will bring scientific legitimacy to environmental evaluation by LCA. The simulation-based LCA can be very helpful to develop more sustainable processes and products. This axis should be further developed, for instance, by sharing the software databases.

45 LCA Cloud: enhancing LCA data collaboration

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Data exchange is an important concern in LCA due to the high variety of existing data formats, nomenclatures, data quality criteria, etc. Some of the mentioned challenges have been already overcome in the free, open source LCA software openLCA by integrating the most complete import and export features, which include all the commonly used LCA software formats. The main challenge has been the lack of import and export capabilities for the most widely used data formats, such as EcoSpold2, etc.), as well as by the harmonization of elementary flows for the different LCI databases available in openLCANexus. As a further step towards facilitating LCA data creation and exchange, a new web service providing cloud functionalities was developed and integrated into openLCA. This "LCA Cloud", which was developed as a proof of concept supported by the US Department of Agriculture (USDA), allows the upload and download of data sets into/from a data repository. Moreover, a merge tool was also implemented in openLCA to identify and solve conflicting changes in the data sets retrieved from the server. The RESTful web service for storing and providing LCA data in a uniform way was developed as a standalone service prototype supported by the US Department of Agriculture (USDA). Data can be exchanged between the server and the openLCA software. This format is recommended by W3C for linked data and it can directly be parsed as RDF triples and, therefore, be directly linked to ontologies. Moreover, the web service contains also other functionalities such as querying of data sets by user defined criteria, validity check to avoid corrupting databases, user and repository management systems and access protection procedures. Additionally, the web service, a merge tool was developed and integrated into openLCA in order to
identify and manage conflicting changes to the same dataset. This functionality is specially useful when several users work in parallel in the same data. A text comparison between the two versions of the dataset is done for each of the fields included in it (e.g. descriptions, locations, amounts, units, etc.). The differences found are then displayed highlighted in the tool’s editor and the user has the possibility of accepting all or none of the modifications addressed. “LCA Cloud” and the merge tool are powerful features for data sharing using the LCA software openLCA, which enable not only loading and retrieving data from web repositories managed by the users or by external hosts but also the identification of differences within several versions of the same data set.

46 SuBoot - Sustainability Bootstrap Project
J. Hildenbrand; A. Civoth; M. Cinelli, University of Warwick; V. Gjorgjoski, Granta Design

Oftentimes individual data collection for a case is considered as one of the more time consuming stages of a life cycle assessment, while on the other hand data is available but needs to be processed from all kinds of repositories. A novel approach to rapidly create a reliable and sound data base is tested within the SuBoot project, which is built around three key ideas. (i) The request from consumers to consider sound, reasonable information about the sustainability of products, at point of sale; (ii) Opportunities for businesses to increase their revenues and market shares if they focus on green and ethically produced goods; Inclusion of alternative sources for sustainability information, which are “untapped” today. The aim of SuBoot is, therefore, to be a system to massively collect life cycle and sustainability data from primary sources, and make it available in supply chains and to end users: empowering reliability by providing life cycle sustainability information at the point of sale. These aspects are integrated in a smart and smooth software system, including one or several data nodes, an app for end consumers, and a B2B application. The system will be presented for a practical case to highlight challenges and solutions.

47 Water supply mix: spatial, temporal and user variability at European scale
S.O. Leão, IRSTE A Montpellier / UMRITAP ELSA; M. Núñez, National Research Institute of Science and Technology for Environment and Agriculture (IRSAT) / UMR ITAP ELSA; E. Loiseau, National Research Institute of Science and Technology for Environment and Agriculture - IRSIA; E. Peron, Suez Environment SAS; A. Sgard, Société du Canal de Provence et daménagement de la région provençale; G. Junqua, Ecole des Mines d'Alès / LGEE; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - IRSIA / UMR ITAP

Usually, the water used by a particular user (domestic, industrial, agriculture, etc) does not come from a single source but rather from a mix of local and sometimes imported water sources. Depending on the origin of the water abstracted (river, lake, aquifer, sea, etc), the location (water abundant or poor), the volume being extracted and the season of the year (wet/dry), the resulting environmental impacts will differ. It is therefore very important that Life Cycle Inventory (LCI) databases contain information on these aspects for proper subsequent impact assessment. However, current LCI databases neither differentiate (sub-)watersheds nor include temporal aspects, as required by ISO 14046 for a proper water footprint and impact assessment. The differentiation between surface and groundwater and the consideration of unconventional sources such as increasingly used desalinated or reused water remains limited. Furthermore, information on the combination of water sources supplied to a specific user is very scarce, while for background processes the actual origin of water is usually unknown. The aim of this work is to develop a spatially and temporally specific water supply mix for different water user needs. As a starting point, the geographical scope is Europe. The water mix builds on 1) the well established concept of the electricity production mix in Life Cycle Assessment (LCA), 2) specific literature on inclusion of water in the LCI and the Quebec tap water supply mix, already integrated in ecoinvent v3.1. The water mix proposed includes: water origins, water users, spatial and temporal specification and technologies (water abstraction, water production and water distribution). Data collection on water abstraction was done at national and regional (i.e., watersheds) levels per European country. A great spatial variability of water origins for different water users has been observed. There is also a temporal variation throughout the year that should be considered. The water supply mix, a mix of water sources and related technologies to meet a user need at a specific time (season) and location (sub-watershed), provides reliable water use profiles for background (and foreground) systems in LCA and will allow for LCIA methods to distinguish between the trade-offs of the various impact profiles of a given local mix. This project will enable routine assessment of water-use impacts just as straightforward as the use of the electricity supply mix.

Improving the usability of ecotoxicology in regulatory decision-making: findings from a SETAC Pellston® Workshop

48 How to improve the quality of ecotoxicity studies
J.W. Green, DuPont / Applied Statistics Group

This presentation will address how we can go about ‘improving the quality and reporting of ecotoxicology studies’. By advancing these two aspects, we should be able to increase the number of studies that can be used in the risk assessment process. Overall, there is consensus around what constitutes a well-conducted ecotoxicity study; and that these factors should be required for publication in the peer-reviewed literature. These include, but are not limited to, confirmed exposures, minimum replication, appropriate controls and control performance, and data transparency and sharing. Implementation of these recommendations by journals, and adoption of these practices by researchers at the study performance level, will facilitate the evaluation of the quality of work by all scientists, and draw more defensible data into the regulatory decision-making.

49 The student perspective
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry

50 Panel discussion
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry

Moderated panel discussion focusing on questions that the panel members get in advance. Panel members: Allen Burton (University of Michigan, USA/editor Et&C), Thomas-Benjamin Seiler (Aachen University, Germany/chair of SETAC Europe Advisory Group on Science and Risk Communication), Natalie Burden (NC3Rs, UK), Henrik Sundberg (Swedish Chemicals Agency, Sweden)

51 Questions

52 Evaluating reliability and relevance of ecotoxicity studies
C. Morrendorf, RIVM / Centre for Safety of Substances and Products

This presentation will address the following questions: What types of problems can be anticipated when evaluating ecotoxicity studies for regulatory risk assessment? What are the appropriate reliability and relevance evaluation criteria for peer-review publications? Can the available evaluation methods be used for regulatory risk assessment? e.g., Klimisch, CRED, EPA Soils (EcoSSLs), or other guidelines for evaluating ecotoxicology data, and methods of other disciplines – toxicology, evidence-based medicine, etc. It also will highlight frameworks that can be used to better design studies so they can be used in the regulatory process and what is needed for these studies to be evaluated for reliability and relevance to risk assessment and risk management.

53 Weight of evidence evaluation
T. Hall, Bayer CropScience / Environmental Toxicology and Risk Assessment

In order for sound risk assessment and risk management decisions to be made, the Weight of Evidence (WoE) procedures must be objective, systematic and transparent. We reviewed the current quantitative and qualitative methods used by regulatory agencies and those found in the general literature. The goal was to develop a method that allow the weighing of the lines of evidence in a prospective risk assessment that then demonstrate the reliability, relevance, adequacy and consistency of the information. The general obstacles and difficulties when performing WoE evaluations in regulatory risk assessment were discussed. Through this evaluation a series of recommendations and approaches have been developed that allow for all scientific evidence relevant to a hypothesis to be considered and evaluates the strengths and value of the ecotoxicology data in the risk assessment.

54 Panel discussion
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry

Moderated panel discussion focusing on questions that the panel members get in advance. Panel members: Anu Kapanen (ECHA, Finland), Jose Tarazona (EFSAs, Italy), Jason Snape (AstraZeneca, UK) Discussion points: 1) Comments on the results from the SETAC Pellston workshop. 2) What is needed from researchers to
increase the use of peer-reviewed studies in regulatory risk assessments? 3) What can regulatory agencies and industry do to ensure that all relevant and reliable data is included in dossiers? 4) What are the barriers that exist, and potential solutions, to making industry-sponsored studies more transparent? 5) Future needs? 6) How can SETAC contribute?

55 Questions

Contaminants of Emerging Concern in the Environment and Their Management (II)

56 Transformation products found in full-scale wastewater ozonation and biological post-treatment

C. McAndell, Eawag / Department of Environmental Chemistry; M. Boechler, Eawag / Department of Process Engineering; E. Borowska, M. Bourgin, Eawag / Environmental Chemistry; J. Fleiner, Eawag / Process Engineering; J. Hollender, Eawag / Environmental Chemistry; C. Kienle; R. Teichler, Eawag / Environmental Chemistry; H. Siegrist, Eawag / Process Engineering; U. von Gunten, Eawag / Water Resources

In Switzerland, a new water protection act was approved in spring 2014 which asks for the upgrade of around 100 out of 700 wastewater treatment facilities with additional ozonation to eliminate micropollutants within the next twenty years to enhance surface water quality. The WWTP Neugut in Dübendorf is the first facility in Switzerland where ozonation has been installed before sand filtration for the abatement of micropollutants. As the contaminants are not fully mineralized during ozonation, transformation products are produced. The elucidation of these transformation products and their potential adverse effects for the environment is of concern. In the scope of the study was: (i) determination of reactivity of compounds with ozone and identification of ozonation products (OPs) of selected compounds, (ii) investigation of the formation of OPs in the ozonation at the full scale facility in the WWTP Neugut and of their fate in different biological post-treatments, and (iii) investigations of different effects by bioassays in the laboratory and directly on the WWTP in a flow-through systems. In the study pharmaceuticals which occur in significant concentrations in WWTP effluents were investigated in detail. Species-specific second-order rate constants for the reactions of the molecular and dissociated forms of eight compounds with ozone were determined. The investigated compounds were found to represent a wide range of reactivity. For three compounds, different reactivity OPs were proposed from measurements with high-resolution mass spectrometry, MS2 spectra analysis and expert knowledge on ozone reaction mechanisms. Several OPs could be confirmed with available standards. N-oxides were quantified with high yields as the primary OPs for tertiary amines. In the bacteria bioluminescence assay performed on ozonated and non-ozonated samples of the three compounds cetirizine, fexofenadine, and hydrochlorothiazide, the toxicity was slightly increased during the ozonation of cetirizine, however, only at cetirizine concentrations higher than what is found in wastewater treatment. The assessments of the ozonation effluent of WWTP Neugut confirmed the results from lab studies concerning reactivity with ozone and the formation of OPs. Several OPs from the literature were also found after ozonation at this plant. The evaluated OPs were mostly stable in the different biological post-treatments investigated after ozonation.

57 BIOMONITORING AND SOURCE TRACKING OF DIOXINS IN THE NETHERLANDS

A. Arkenbout, Toxicowatch Foundation

In the region of Harlingen, situated in the north of the Netherlands, people are becoming very concerned about adverse health effects related to the possible emission of dioxins and other POPs by a waste incinerator installed in 2011. A monitoring programme using backyard chicken eggs, shows a serious pollution with dioxins and dl-PCBs in the environment of Harlingen harbour. In order to find the source of this pollution, the preliminary results of analysis of a long-term sampling of the flue gas of the incinerator will be discussed in relation to the congeners found in the backyard chicken eggs.

58 Fate, metabolism and transformation of wastewater-borne pharmaceuticals and their transformation products in the aquatic environment and fish in European rivers

S. Perez, IDAEA-CSIC / Environmental Chemistry; J. Aceña, M. Hannemann, J. Peña, IDAEA-CSIC; D. Barcelo, IQAB-CSIC / Dept Environmental Chemistry

The presence of pharmaceuticals residues in the aquatic environment has caused concerns about potential adverse effects on exposed wildlife. Pharmaceuticals prior to entering WWTPs have already been metabolized in humans which leads to bioconcentration products with different chemical structures and physico-chemical properties compared to their parent compounds. After their excretion in conjunction with their human metabolites and their discharge into the sewer, they enter in WWTPs where they can be further transformed by secondary treatments and advanced treatments generating transformation products (TPs). Several studies have been reported the occurrence of pharmaceuticals, their human metabolites and their TPs in wastewater treatment plants (WWTPs), surface waters and different fish tissues, but still very little is known about their fate in fish. Fish have been shown to possess hepatic detoxication systems capable of metabolizing xenobiotics taken up from polluted waters. In this context, we propose to screen surface water samples and fish for a large number of TPs formed in WWTPs and rivers in order to have a rough idea about the transformation of pharmaceuticals detected in surface waters and their metabolism in freshwater fish a suspect screening of pharmaceuticals, TPs and their predicted metabolites was applied in all samples from around Europe. With this approach several pharmaceuticals and TPs, more than 30 compounds, were detected in surface samples. In fish more than 15 phase I metabolites were detected corresponding to different hydroxylation reactions, and phase II metabolites such as glucuronide and taurine conjugates were tentatively identified. The suspect analysis of muscle allowed the detection of more than ten different pharmaceuticals. The analysis of fish bile has allowed for the detection of several phase I and phase II metabolites and suggests that the occurrence of drug metabolites in bile can be used as a surrogate for exposure of fish to pharmaceuticals. Currently, the identification of several TPs and metabolites still requires confirmation. In accordance with the literature, anti-inflammatory, antibiotics and psycho-active drugs have been the most commonly detected drugs. Thus this approach highlights that UPLC–HRMS is a powerful tool for simultaneous quantitative and qualitative analysis, allowing the search for suspected compounds, TPs and metabolites, their identification and the quantitation of target compounds.

59 Removal of contaminants of emerging concern (CECs) during primary, secondary and tertiary wastewater treatment steps

Z. Baalbaki; T. Maere, P.A. Vannolleghem, modelEAU - Université Laval / Département de génie civil et de génie des eaux; V.V. Yargeau, McGill University / Chemical Engineering

Contaminants of emerging concern (CECs), including pharmaceuticals and hormones, are secreted by humans and end up in wastewater treatments plants (WWTPs) that are not designed to treat them, resulting in the occurrence of CECs in water bodies. This study consists of both experimental and modelling work and aims at understanding the removal of 19 target CECs, including hormones, pharmaceuticals and some of their main metabolites during primary, secondary and tertiary treatment. The WWTP studied is the Guelph WWTP, ON, Canada (120,000 PE). The treatment train consists of primary clarification, activated sludge, rotating biological contactors and sand filtration. Sampling campaigns were carried out on four consecutive days in the summer, where 24-hour composite aqueous and sludge samples were collected. Aqueous and sludge samples were extracted by solid phase extraction and accelerated solvent extraction, respectively, and the extracted samples were analyzed by LC–HRMS. Conductivity was measured throughout the plant and used as a tracer for the calibration of the hydraulic model of the treatment plant that was built using the wastewater simulation software WEST (MikeByDH.com). The hydraulic model was used to obtain load fractions, which were used along with experimental CECs concentration data to obtain reliable removal levels of CECs. Results showed that for some CECs, such as carbamazepine and codeine, the average mass loading in the effluent was as high as 20 g/d. It was also found that the majority of CECs had low removal rates in the primary clarifier stage and most of their removal took place in the activated sludge unit, with removal rates >70% for some of the target CECs. Concentrations of CECs in primary sludge ranged from 2 to 200 ng/g. The removal of CECs in rotating biological contactors, for which limited data is available in literature, and sand filtration was found to be <30% for most of the CECs, although a few of them were efficiently removed by sand filtration. The experimental removal rates were used to calibrate a model that predicts the fate of CECs during primary and secondary treatment. This model can be used as a design and optimization tool in WWTPs.

60 First Swiss wastewater treatment plant with full-scale ozonation - Ecotoxicological assessment of ozonation and several post-treatments

C. Kienle; H. Besslink, Biodetection Systems BV; S. Fischer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; S. Flückiger, Xenometrix AG; B. Ganser, S. Gut, H. Hasler, Swiss Centre for Analytical Toxicology; M. Langen, Eawag / Applied Ecotoxicology Eawag-EPFL / Chemical Engineering; L. Schlüter-Vorberg, ECT Oekotoxikologie GmbH; C. Thiemann, R. Triebskorn, University of Tuebingen / Animal Physiological Ecology; B. van der Burg, Biodetection Systems BV; E. Vermeiren, Oekotoxzentrum Eawag-EPFL / Chemical Engineering; M. Langer, Oekotoxzentrum Eawag-EPFL / Chemical Engineering
Analysis ITAS; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology

Emerging pollutants in wastewater, such as pharmaceuticals and personal care products, can be eliminated by advanced wastewater treatment techniques. Thus the discharge of these substances into freshwater ecosystems can be diminished and drinking water resources can be protected. Previous studies have shown that the discharge of these substances is a promising technique to eliminate micropollutants, even though it can produce reactive and potentially toxic transformation products. To remove these transformation products, which are often biodegradably or bind to particles, post-treatments like sand filtration or granulated activated carbon are recommended by the Federal Office for the Environment (FOEN). The wastewater treatment plant (WWTP) Neugut is the first in Switzerland to apply a full-scale advanced treatment of wastewater with ozone followed by sand filtration. In the present study, the efficiency of ozonation and a number of post treatments to reduce ecotoxicological effects of biologically treated wastewater were assessed using a set of bioassays. Post-treatment methods were: a full-scale sand filtration, pilot-scale granulated activated carbon (GAC), and pilot-scale fixed and turbulent fluidized beds. In vitro bioassays were applied in the laboratory and in vivo bioassays with living organisms were performed in the laboratory and on site. The investigations revealed that the wastewater treatment plant with ozone resulted in significantly reduced ecotoxicological effects, improved growth and photosynthesis of green algae, decreased toxicity to luminescent bacteria, and decreased estrogenicity, compared to effects measured in the waste water from the secondary clarifier. The post-treatments partially lead to a further decrease of the effects. Only the reproduction of water flea and oligochaetes was decreased with ozone.

Introduction

ECOSYSTEM ANALYSIS; S. Küppers, Forschungszentrum Jülich GmbH

The presence and occurrence of emerging flame retardants (FRs) in the environment, in indoor air and dust however, to the best of our knowledge, has been less studied than that of classical flame retardants. Flame retardants are chemicals that are added to many consumer products such as plastics, electronics, textiles, and foams for furniture, automobile interiors and many others. The most widely used flame retardants are polybrominated diphenyl ethers (PBDEs), although others have been used, including halogenated forms of diphenyl ether (PBDEs). Hexabromobenzene (HBB), allyl 2,4,6-trimethylphenyl ether (ATE), 1,3,5-trihexabromobenzene (TBBz), anti- and syn-dechlorane plus (anti-DP and syn-DP) were dominant FRs found at two GLB sites, namely Point Petre on Lake Ontario and Burnt Island on Lake Huron, with levels mostly higher than PBDEs. Similar to the PBDEs, lighter FRs in air showed a strong seasonality with higher concentrations in the summer months. Higher FR air concentrations were found at Point Petre, which is closer to urban developments, than at Burnt Island. While air concentrations of PBDEs were declining in Great Lakes air between 2005 and 2013, which may be related to control efforts, no consistent changes in air concentrations were observed for most emerging FRs (2009-2013).

Electrochemical oxidation of fluoroquinolone antibiotics: Mechanism, residual antibacterial activity and ecotoxicity

L. Zhu, Forschungszentrum Jülich GmbH, ZEA / ZEA; B. Santiago-Schübel, Forschungszentrum Jülich GmbH

Greenhouse gas emissions are of increasing concern and their reduction is targeted in the European Green Deal. In this paper, we address the reductions of greenhouse gas emissions from the shipping sector by the introduction of new technologies. The European Union has set targets for the reduction of greenhouse gas emissions from the shipping sector. These targets are challenging and need to be met by the shipping industry. In this paper, we explore the potential of new technologies to reduce greenhouse gas emissions from the shipping sector. We review existing technologies and propose new ideas for reducing greenhouse gas emissions from the shipping sector. The potential for reducing greenhouse gas emissions from the shipping sector is significant, and new technologies can play an important role in achieving these reductions.
levels, sources, and movement of flame retardants in the Turkish environment. In this study, rep sources of certain OPFRs in ambient of Bursa, Turkey that was obtained during the course of a 1-year passive sampling study.

65 Monitoring levels of legacy and emerging flame retardants in indoor environments: a multi-location study between Norway and the UK K. Kademoglou, University of Reading / geography and Environmental Sciences; F. Xu, University of Antwerp Toxicological Center / Toxicological Centre Dept of Pharmaceutical Sciences; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center Dept of Pharmaceutical Sciences; L.S. Haug, Norwegian Institute of Public Health; C.D. Collins, Reading University / Soil Research Centre Living in the post-PBDE era entails commercial production, use and gradual replacement of fire-inhibiting compounds with newly manufactured chemicals also known as “novel” or “alternative” flame retardants. Such compounds include TBB and BEH-TEBP (Penta-BDE replacement), BTBPE (Octa-BDE replacement), DBDPE (Deca-BDE replacement), Dechloranes (Dps) (Deca-BDE replacement) or organophosphorus flame retardants (PFRs; TPHP, TCPP etc.). The main objectives of the present study are: a) To assess the occurrence of legacy and emerging alternative FRs across two European countries (the UK and Norway) with respect to human exposure via dust ingestion. No data have been reported yet on emerging PF Rs, also known as novel PF Rs (NPFRs), in dust from residential or occupational microenvironments neither for the UK nor for Norway. In addition, this is the first study reporting significant levels of NPFRs including isocyclodiphenyl phosphate (IDP) and tricyclodiphenyl phosphate (TCP) in indoor microenvironments. b) To identify possible FR sources with respect to the collection source and country of origin, taking into consideration source variability and its impact on usage of emerging FRs in consumer products. Dust samples (n=22) from vacuum cleaner bags (10 houses and 12 stores, offices, libraries) in the UK were collected during August 2013 – December 2013. Dust samples (n=10) were collected from vacuum cleaner bags (houses) in Norway during November 2013 – April 2014 as a part of the A-TEAM cohort sampling within the framework of the ‘Advanced Tools for Exposure Assessment and Biomonitoring’ (A-TEAM) project, a Marie Curie Initial Training Network aiming to establish for human exposure biomonitoring of emerging FRs. In the present study, a broad range of legacy and emerging FRs were studied, including PBDEs, novel BFRs, PFs and novel FRs. A commercial shift and gradual replacement from the phased-out FRs to emerging FRs is evident. We are the first to identify and report on NPFRs, such as iDPP and TCP in indoor dust originating from residential and occupational environments, suggesting extensive usage of NPFR-coated consumer products in the UK and Norway. This project is financially supported by the European Commission FP7 Marie Curie Initial Training Network “A-TEAM” grant number 316665.

66 Biomagnification study of hexabromocyclododecane isomers in freshwater fish and invertebrates M. Lauzen, LPTC-EPOC, University of Bordeaux / UMR EPOC LPTC; P. Labadie, CNRS; S. Augagneur, K. Le Menach, University of Bordeaux / UMR EPOC LPTC; A. Ferrari, PROBIEN, CONICET -UNCOmab; M.P. Babut, Irstea / Water; H. Budzinski, University of Le Havre, UMR EPOC LPTC Since 2011, hexabromocyclododecane (HBCD) is subject to authorization (Regulation 143/2011 EU) and considered as persistent, bioaccumulative and toxic (PBT); this compound is included in Annex A of the Stockholm Convention since 2014, and became a priority substance for water in Europe since 2013 (Directive 2013/39/ EU). As for several other priority substances, the Environmental Quality Standard (EQs) for HBCD is set for biota (i.e. fish), meaning that compliance checking will have to refer to fish. HBCD is an additive brominated flame retardant (BFRs), used especially in the building sector, thermal insulation, textiles, electrical equipment. In this context, the aim of this work was to determine trophic magnification factors (TMFs) in various rivers and streams in France, and to examine their spatial-temporal variability. HBCD isomers (α, β and γ) were analysed in fish and benthic invertebrate tissues. These samples were collected from five study sites representing different river conditions (catchment size and anthropogenic pressure). All the concentrations found in fish and invertebrates were below the Environmental Quality Standard EQmax (167 ng g-1 wet weight (ww)). α-HBCD displayed the highest trophic magnification factor and significant differences were observed between sites. The determination of TMFs by 3 different models (linear regression, censored regression and GLMM) has shown the importance of the regression model used for TMF assessment. It is therefore important to consider the different aspects of data treatment (e.g. heterogeneity in the number of samples), all the while knowing that each model has its limitations.

67 Fishing of unknown halogenated environmental contaminants in environmental sentinel based on isotopic pattern and mass defect through targeted high resolution mass spectrometry R. Caron, Oniris-LABERCA / Oniris Laboratoire dÉtude des Résidus et Contaminants dans les Aliments; A. Léon, E. Omer, LUNAM Université / Oniris Laboratoire dÉtude des Résidus et Contaminants dans les Aliments; G. Dervilly-Pinel, B. Le Bizec, Oniris-LABERCA / Oniris Laboratoire dÉtude des Résidus et Contaminants dans les Aliments Bioaccumulation of Persistent Organic Pollutants (POPs) is predictably associated with the compounds characterized by high lipid solubility that can accumulate in the fatty tissues of living organisms for long periods of time. POps increase in concentration in the food chain and are typically halogenated organic compounds. In the case of known substances, targeted approaches mainly based on mass spectrometry (MS) are available to characterise human exposure (environment, food) as well as the metabolism of individuals. Such approaches do not permit however to detect non yet described exposure markers, i.e. degradation products of known substances or unknown/existing substances. Last generations of high resolution mass spectrometers (HRMS, e.g. orbitrap or TOF) open the way to untargeted full scan footprints as a new generation of screening tools. A first study has been carried out to search for such HRMS couplings with chromatography from which the extraction of the useful information stands as a central question, thus requiring the development of fit-for-purpose intelligent data processing, analysis and visualisation tools, supported by appropriate biocomputing solutions. In our work, we discuss the proof of concept of a new methodology capable of seeking unknown halogenohalogenated compounds in environmental sentinels. Our strategy relies on an automated peak picking step based upon the centroid wave function (xcms package, R environment), a VBA script developed for pairing features according to the exact mass difference between Cl and Br isotopes to filtering potential organohalogenated clusters among full scan HRMS datasets. H/C ratio mass defect datasets were used to find potential metabolites, using the database of the HRMS. The filtering script was successfully applied to an LC-HRMS untargeted profiling dataset generated from an eel muscle sample extract obtained after pressurised liquid extraction and a non selective liquid-liquid partition between concentrated sulphuric acid and hexane. From 9,789 initial features observed in the eel muscle from various extraction, 1,994 features were paired in 589 clusters containing at least an M+2 contribution (indicating a Cl or a Br atom), allowing for realistic fundamental investigations of filtered clusters. Hexabromocyclododecane, chlorinated paraffin series and other candidates were fished and for some of them identified.

Microplastics in the environment: Sources, Fate and Effects (II)

68 What, when and where? Assessing temporal and spatial trends in microplastics>10µm in coastal waters west of Sweden T.M. Karlsson; M. Hasselov, University of Gothenburg / Department of Marine Sciences; F. Norén, IVL Swedish Environmental Research Institute Comparable datasets are a luxury within marine litter research, due to the novelty of the field. These golden glimpses of information provide valuable insights in trends of abundance and characteristics, to understand sources and pathways and can also help us plan sampling and monitoring to achieve and increased accuracy in future comparisons. On the Swedish west coasts measurements of microfibers>10µm have been carried out at 14 locations using comparable methods at 4 occasions during 3 years. In a recently initiated project these datasets are evaluated to achieve a detailed overview of differences in composition and abundance. Preserved samples of commonly found anthropogenic microscopic litter such as blue, red and white particles and fibers are additionally being analyzed using FTIR. Initial comparisons of the datasets show that a consistently higher amount of particles were found on the smaller sized filters (10µm). Interestingly the composition here also differed, with a higher amount of fibers and black particles on the smaller filters in relation to the classic polyolefin and expanded polystyrene microplastics on the filters with a wider pore size (300µm). The industrial harbor area by Gallerön separates from the others on account of the relative high composition of synthetic fibers, red particles and black particles in the range of 50-100 µm. FTIR analysis is being carried out to further investigate these relationships as well as the potential relationship to activities in the area. The city areas of Alvsborgshon, Skalkargorna and Dafnbe show some separation form the others, which is clearest in the results from 2013. The strongest separation is for Alvsborgshon which is also the sampling point closest to the urban areas of Gothenburg which fits earlier observations where cities have been identified as a source of microplastic. The compositional differences here show a high amount of black particles with a probable origin in incineration processes, it also shows a high composition of plastic particles and to some extent also blue particles. The initial results of the ongoing analysis show higher amounts but also clearer trends in composition for the >10 µm samples than for the samples collected with a 300 µm net. It is therefore suggested that sampling and monitoring of smaller size classes of microplastic is important to understand temporal and spatial differences in microplastic abundance and composition.

69 Neustonic microplastics in the Bay of Brest (Brittany, France): composition, abundance and spatial distribution
Since a decade, microplastics represent an increasing concern for science and society with many studies establishing their fate and their impact in aquatic ecosystems. Many studies have been reported microplastic occurrence throughout the world ocean and in different environmental matrix such as water, sediment and biota. Some of the studies have focused on contaminant bioavailability but few have done a complete spatial and temporal assessment. Microplastics were investigated in April 2014 from surfaces water of the Bay of Brest (Brittany, France), which is divided in three zones (north, central and south) characterized by different hydrodynamics and anthropogenic pressures. Microplastics particles were of the following classification: 0.3-1 mm, 1-2 mm and 2-5 mm. A combination of static image analysis of particles and automated Raman micro-spectroscopy was used to obtain the counting, size, shape and chemical composition of a large number of particles for each sample. 35 % of visually sorted particles were not identified as plastic polymer, so morphological criteria such as color, shape or texture are insufficient to assert that a particle is a plastic polymer. Eight of the nine stations contained microplastics particles and a microplastics concentration (particles per cubic meter) was estimated. Only 65 % of particles collected at surface water were identified as plastic suggesting that visual sorting is not enough. A majority of polyethylene (76 %) was identified. The spatial distribution of microplastics in the Bay of Brest seems to be different between the three zones: the central zone exhibited the highest value (5 500 particles/m³) followed by the north zone (2 210 particles/m³), whereas only few microplastics were collected in the south part (0.049 particles/m³). The south zone was mostly polluted by an important concentration of fibers but analyses are currently ongoing to identify their chemical composition and confirmed their plastic nature.

**70 Abundance of microplastics and adhered contaminants in the North Atlantic Ocean**

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Marine plastic contamination has increased steadily over the last decades, and plastic debris account for more than 90% of the marine litter encountered by marine wildlife. The majority of the plastic litter can be characterized as microplastics (MP). Those plastic types that have a density below that of seawater will float on the sea surface for a prolonged period of time, and will therefore be transported with currents. This has led to a concentration of plastic debris in oceanic gyres. One of the five major oceanic gyres is situated in the North Atlantic Ocean and elevated concentrations of MPs have been reported in this area. Apart from direct physical effects such as clogging of gills and entanglement, plastic may also serve as a vector for other lipophilic contaminants, which can bind to and be transported with the plastic. The importance of such vector effects are not fully understood and are currently being discussed in the scientific literature. The current study reports findings and analyses of microplastic samples along a transect from the Caribbean Sea into the North Atlantic Gyre conducted in spring 2015. The aim was to: (i) quantify abundance of MP ii), estimate relationships between size and distribution of MPs, iii) determine concentrations of lipophilic contaminants adhered to the MPs and iv) generate a GIS based map where data are included, that subsequently can be expanded to include data from other sources and thus be used for the marine plastic pollution. The studies showed that MPs dominated by PE and PP are abundant in surface waters of the North Atlantic Gyre and that smaller fractions were more dominant then larger. The analyses further documented that PCBs and PAHs can bind to the MPs at concentrations higher than those observed on biota. This illustrates that plastic can be used as a vector for other contaminants. The GIS makes a very useful communication tool that can be used to inform the public and policy makers, and thus facilitate measures to prevent future pollution. The GIS is a map useful for updating the data and displaying them for the scientific community as well as the public and policy makers. It may therefore be an effective tool in the work towards promoting mitigation measures.

**71 Microplastics in inland waterways and coastal waters - origin, fate, and impact**


The accumulation of plastic debris in aquatic environments is one of the major but least studied human pressures on aquatic ecosystems. Under environmental conditions, larger plastic items degrade into smaller particles, so-called microplastics (MP) (< 5 mm in diameter). MP resulting from degradation of polymers and flame retardants were identified. For this reason, it is very likely that microplastics will float on the sea surface for a prolonged period of time, and will therefore be transported with currents. This has led to a concentration of plastic debris in oceanic gyres. One of the five major oceanic gyres is situated in the North Atlantic Ocean and elevated concentrations of MPs have been reported in this area. Apart from direct physical effects such as clogging of gills and entanglement, plastic may also serve as a vector for other lipophilic contaminants, which can bind to and be transported with the plastic. The importance of such vector effects are not fully understood and are currently being discussed in the scientific literature. The current study reports findings and analyses of microplastic samples along a transect from the Caribbean Sea into the North Atlantic Gyre conducted in spring 2015. The aim was to: (i) quantify abundance of MP ii), estimate relationships between size and distribution of MPs, iii) determine concentrations of lipophilic contaminants adhered to the MPs and iv) generate a GIS based map where data are included, that subsequently can be expanded to include data from other sources and thus be used for the marine plastic pollution. The studies showed that MPs dominated by PE and PP are abundant in surface waters of the North Atlantic Gyre and that smaller fractions were more dominant then larger. The analyses further documented that PCBs and PAHs can bind to the MPs at concentrations higher than those observed on biota. This illustrates that plastic can be used as a vector for other contaminants. The GIS makes a very useful communication tool that can be used to inform the public and policy makers, and thus facilitate measures to prevent future pollution. The GIS is a map useful for updating the data and displaying them for the scientific community as well as the public and policy makers. It may therefore be an effective tool in the work towards promoting mitigation measures.
one with the washing product only, and the last one as a regular wash. Microplastics in WWTP influents and effluents were also analyzed during 3 campaigns. Conventional 24-hour averaged samples were collected. Atmospheric fallout and rainfall were collected in two different sites, one urban (1 year) and one sub-urban (a half year), through a collection funnel. Stormwater samples were collected at the outlet of a sub-urban stream events. The two first samples of washing machine effluents (empty machine and without clothes) contained between 120 and 150 particles/L. When clothes are washed, concentrations between 8850 and 18700 particles/L were encountered, confirming the large contribution of the clothes. High levels of fibrous plastics are found in WWTP influents (260 – 320 particles/L) while the concentrations in the effluents are in the 14 – 50 particles/L range. A microplastic removal rate between 83 and 95% have been estimated. Throughout the year of monitoring (urban site), an average atmospheric fallout of 110 ± 96 particles/m²/day (mean ± SD) was encountered. On the suburban site, a 6-month monitoring shows an atmospheric fallout around 53 ± 38 particles/m²/day. Throughout all the monitoring period, a significant difference between the atmospheric fallout on the urban and the sub-urban site was found. By considering the rainfall volume collected on the atmospheric fallout funnel, a median concentration of 40 particles/L can be estimated. In runoff, the first results indicated that the microplastic concentrations lies in the same order of magnitude, i.e. between 39 and 60 particles/L. To date, further work is required to evaluate the contribution of total atmospheric fallout to stormwater pollution.

Fate, Effects and Risk Assessment of Chemicals in Aquatic and Terrestrial plants (II)

74 Ecosystem services approach to pesticide risk assessment and management of non-target terrestrial plants: recommendations from 2 SETAC Europe workshops

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The registration of Plant Protection Products (PPPs) in the EU falls under Regulation 1107/2009, which recommends a tiered approach to assessing the risk to non-target terrestrial plants (NTTPs). However, little information is provided on how to perform and implement higher tier studies or how to use them to refine the risk assessments. Therefore, two workshops (April 2014, Sept 2015) were organized with the aim of: developing a framework for a higher-tier approach to assessing the risk of PPPs to NTTPs; providing expert opinion and advice as input for the ongoing revision of the terrestrial ecotoxicology guidance document and NTTP risk assessment procedures. The recommendations agreed to by the first workshop relate to the three main themes, i.e. specific protection goals, risk assessment and mitigation. The participants of the workshop adopted the European Food Safety Authority (EFSA) approach of using an ecosystem services framework for identifying specific protection goals. First, delivery and protection of ecosystem services were discussed for in-crop, in-field and off-crop, and off-field areas. Second, lower and higher tier risk assessment methods, including modelling approaches, were evaluated and the benefits from these options were addressed. Third, options for risk mitigation of spray drift and run-off were discussed and evaluated. A number of concerns were raised during the workshop and literature reviews were performed and data collected in order to reduce uncertainty. These actions focussed on the protective power of standard test species for wild species; the protective power of regulatory endpoints for reproductive endpoints; the methods and endpoints for multispecies or field-studies; and the importance of different factors to structure risk assessment. The second workshop built upon the results of these literature reviews, the recommendations of the first workshop and the EFSA opinion on risk assessment of PPPs for NTTPs. The main charge questions identified for the second workshop were: how to address reproductive endpoints; how to mitigate risks; how to conduct higher tier tests. A higher-tier framework for NTTP risk assessment was proposed. Recommendations from both workshops will be presented.

75 Sensitivity of NTTPs to plant protection products - vegetative and reproductive endpoints: Literature review and analysis for SETAC AG Plants

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A literature review was performed comparing vegetative and reproductive endpoints for non-target terrestrial plants (NTTPs) aiming to test the hypothesis that reproductive endpoints are more sensitive than the standard vegetative endpoints currently used to inform the pesticides risk assessment. In addition the EFSA Scientific Opinion [1] was considered, in which, among others, also reproductive endpoints were discussed to be more sensitive than the currently assessed vegetative endpoints. The dataset listed in [1] was assessed in parallel to the full data set (that also included confidential industry data). In addition current proposals to move from ER10 to ER50 endpoints were considered. Although cases are reported of reproductive endpoints considerably lower than vegetative endpoints, based on the data assessed here the difference between the two was found to be minor, on average just a factor of ca. 1.5. Even if both endpoints (vegetative and reproductive) were always determined and the lowest of the two was used in the RA, the conclusive impact on protective power would just be a factor of ca. 2 (based on the EFSA dataset). In contrast, the difference between ER50 and ER10 was greater than a factor of 6. A change of the effect level used in the RA (ER50 to ER10) would thus increase the conservatism of the tier1 RA for NTTPs considerably. The ecological relevance of a 10% reduction at the population and landscape level, advantages and disadvantages of the proposed change in effect level, a potential alternative by using the ER25 (or ER50 with an additional assessment factor), and if there is a need for an increase in conservatism of the tier1 risk assessment will be discussed.

76 What information can we gain by including trait-based endpoints for plants in ecotoxicological tests? Case studies with Myriophyllum spicatum, the new OECD test organism for rooted aquatic plants

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In plant ecology, the trait concept provides functional markers for plant functions. Plant functional traits (morphological, physiological, phenological) reflect ecological strategies and determine how plants respond to environmental factors. Trait variability can reflect adaptations or acclimations at the intraspecific level. We hypothesize that morphological and physiological traits changes in the presence of certain pollutants, Leaf, stem and root mass fractions (LMF, SMF, RMF), dry matter content (DMC) and stomiometry, the content in carbon, nitrogen and phosphorus and resulting C:N:P molar ratios in plant tissue are traits that can provide information about effects at the individual level or allow extrapolations on ecological interactions and ecosystem processes. We tested our hypotheses exposing sediment-grown Myriophyllum spicatum, the new OECD test species to arsenic or mixed herbicide. Experiment 1 had a cross-factorial design with two CO2-levels (low and high, LC and HC) and four arsenate levels. Experiment 2 exposed plants to isoproturon (IPU) or metsulfinuron-methyl (MSM) alone or in varying combinations. After two weeks, we measured final plant length, fresh and dry weight and dissected a part of the shoots into leaves, stems and roots, and determined C, N, and P contents. In experiment 1, arsenate and CO2 availability affected morphological and physiological plant traits. SMF decreased in arsenate treatments, and was higher in the HC treatments. RMF declined to one third at high arsenic levels. LMF was affected both by CO2 availability and arsenic levels. Arsenic level affected the DMC of leaves, stems and roots, but only roots exhibited a lower DRCM under LC treatments. Arsenic affected the N and P content and resulting C:N and C:P ratios in the whole plant, while CO2 availability affected C content and CF and NP ratios. In contrast, we observed very little effects on C, N and P content in leaves. In experiment 2, IPU alone had no effect on LMF, but with increasing proportion of MSM, the LMF declined strongly. At the same time, SMF increased strongly with increasing MSM. With increasing MSM, the LDMC increased strongly compared to solvent control (TCA). We conclude that incorporating different plant traits as endpoints with Myriophyllum spicatum can provide valuable information useful to predict ecological effects of pollutants. Our experiments have shown that such changes in plant traits are frequent.
Effects of pulsed mixtures and recovery in Lemna minor based on POCIS field measurements.

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Pesticide contamination, in particular herbicidal loading, has been shown to negatively affect Luxembourgish river quality. Such pressure can have direct impacts on autotrophic species, and wider indirect impacts on the freshwater ecosystems. In order to identify the toxicity of these chemical mixtures, we conducted a series of experiments to determine the best approach for the evaluation of pesticide mixtures in the environment.

However, it is difficult to incorporate the variety and combination of compounds sold, used, and detected in surface waters into bioassays. Literature regarding pesticide mixture toxicity shows the validity of the concentration addition (CA) concept for mixtures of compounds with similar modes of action, and the independent action (IA) concept for mixtures of dissimilarly acting compounds. However, a mismatch exists between real field exposure patterns and typical laboratory tests. This study attempts to bridge this gap by directly using spiked mixtures of pertinent compounds based on whole field extracts. Mixtures were applied in pulsed scenarios determined from passive sampling campaign results, in order to determine the toxicity of these real environmental mixtures to a representative autotroph. High risk compounds were selected based on sales, use, and exposure (4–6 days), but at trough exposure with toxicity values and comparison characteristics. Herbicidal loading was determined using passive samplers (POCIS) over eight months, spanning two years and twelve representative streams in Luxembourg. LCMS analysis of these samples indicated the time-weighted average herbicide load over the exposure period. Seven day bioassays using the floating macrophyte Lemna minor were then performed to determine the toxicity of six high risk compounds and spiked artificial mixture based on whole field extracts. Bioassays were performed in pulsed scenarios mimicking exposures determined from POCIS results. Early results indicate metabolite toxicity to be the most toxic of the selected compounds (EC50 0.0106 mg/L). Further tests will clarify the toxicity of these compounds in mixtures and will provide 42-d data to the floating macrophyte Lemna minor. Presented here are the results from: (i) L. minor bioassays using both whole spiked mixture, and pulsed mixture scenarios (ii) use of CA and IA concepts to fit L. minor pulsed and spiked mixture bioassays results.

Pesticide mixture toxicity to algae in agricultural streams - field and laboratory studies

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Contamination of surface waters with multiple chemical pesticides is well documented, but little is known about their concerted effects on aquatic communities. We collected water samples from four streams in small agricultural catchments in southern Sweden during a whole year. In addition to pesticide analysis, we used POCIS to sample compounds to expose to algae with Pseudokirchneriella subcapitata. In addition, we identified worst-case samples observed in long-term monitoring of pesticide water concentration (≈ 80 pesticides) and used these to run tests with reconstituted pesticide mixtures in algal growth medium to specifically test their combined effects on algal growth. Both for bioassays with in situ water samples and mixture toxicity tests we used controls that consisted of standard growth medium. In situ water samples were spiked with nutrients to rule out confounding effects of nutrients. Long-term monitoring data showed more than 10 pesticides in 63% or more of the 902 collected water samples, with 90-percentiles ranging 21–29. The average number of pesticides in a single sample ranged 9–18, while the maximum was 46. These results stress the complexity of exposure by pesticides in agricultural streams.

However, the multitude of pesticides in a sample, toxicity was frequently set by one or a few dominating compounds that contribute to more than 90% of the mixture's toxicity. For long-term monitoring data exceeding 0.1 on 28 occasions, 70% of which occurred between May and July, i.e. the period when spraying is most intensive. Bioassays with in situ water samples showed a significant inhibition of algal growth in 49% of the 61 samples that were run during a whole year. On four occasions algae grew better in in situ water than in controls. There was no relationship between growth inhibition and estimates of pesticide toxicity (i.e. PTI and SUA toxic), suggesting that also chemical stressors other than pesticides could have contributed to the observed inhibitions. Tests with reconstituted medium showed that significant inhibition of algal growth occurred in the range of 1–10x the SUA toxic observed in monitoring data. For all mixtures but two, calculated EC50-values were between 3 and almost 10-fold higher than literature values for the dominant pesticide. The calculated EC50-values for most of the mixtures were 2–17 times higher than those estimated by the model of concentration addition.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (II)

80 Investigating fractionation and speciation of metal(loid)s in sediment pore water samples

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In aquatic environments the fate and behaviour of inorganic and organic substances is often driven by biogeochemical gradients at the sediment water interface (SWI), like gradients of e.g., the oxygen concentration, the redox potential or the pH value. Important transport processes at the SWI are sedimentation and resuspension of particulate matter and diffusional fluxes of dissolved materials. Beside this, the speciation of elements at the SWI is important to assess relevant reactions as well as potential effects. To investigate the factors and processes that govern the distribution and fluxes of nutrients, metals and metalloids (metal(loid)s) at the SWI, the analytics of interest in the sediment pore water have to be studied in parallel to different sediment parameters. Several methods for an active (e.g., suction, squeezing or centrifugation), or a passive (e.g., dialysis or DGT/DDE) pore water sampling are available. These often require the installation of the sampling devices at the sampling site and/or intensive preparation procedures (e.g., slicing and centrifugation or re-elution from/digestion of the accumulation gels), probably impacting the environmental conditions at the area studied and/or the characteristics of the samples taken.

The authors developed a micromorphological (abiotic contact) and a mixture of sampling methodologies that enable to study element distributions in sediment pore water at the SWI at different spatial resolutions (sub-mm to cm) and depths (2 cm – 20 cm within the sediment). By combination of this micro or meso sampling system with a micromorphological profiling system (Missy/Messy) and different sample preparation procedures, it was possible to address the fractionation (size dependent and micelle mediated) as well as the speciation of metal(loids) in parallel to different sediment parameters (O2, redox and pH) along sediment depth profiles. The profiling experiments were complemented by slurry reactor experiments enabling to simulate specific conditions by controlling the redox potential. The vision of the experimental approaches is to address a variety of parameters in parallel and, therefore, to help to deliver a statement of how anthropogenic caused processes that govern the fate of substances at the SWI.

Within the presentation, the results of different Messy and Missy experiments will be given. The different approaches will be discussed, especially with regard to their potentials and limitations and in comparison to other methods.

81 Chromium speciation and release from mining waste in Barro Alto and Crominia, Goiás state, Brazil

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Chromium is abundant and naturally present in ultramafic rocks and serpentinite responsible for producing branched amino acids. Given that growth is a time-rated process, large growth inhibitions exerted during short periods are comparable to lower inhibition over more prolonged periods. In this series of studies, we demonstrated that the inhibitory activity of the sulfurylurea Myriophyllum scutatum follows Haber’s law. Two distinct exposure regimes (1- and 2-d) at a fixed concentration with toxicity values and comparison characteristics, were not statistically different. Either 1- or 3-day exposures of Myriophyllum scutatum to the four SUUs resulted in similar growth rate values for shoot length, and wet and dry shoot weights measured at either 3, 7, and 14 days after the beginning of exposures. There is previous data on metsulfuron methyl showing that EC50 values for a single minor exposure were not similar to those calculated for 4- and 2-d, but reciprocal (TWA) exposures. Also, a study with metsulfuron methyl on Myriophyllum scutatum showed the same result when comparing the same 21-d TWA, but with different peak exposure concentrations and exposure periods (1, 3, 7, 14 or 21 d). The data presented here further support the validation by EFSAS in relation to the validity of a TWA for metsulfuron methyl and also agrees with data collected for the better-studied Lemna spp. These results allow to recommend that the reciprocity assumption, and therefore allowance for the use of a 7-day TWA concentration, should be considered as a standard refinement for the risk evaluation of sulfurylurea herbicides.
soils. However, anthropogenic activities such as mining, leather tanning, chrome plating and metallurgical industries release an important amount of chromium into the environment. In spite of existing in chemical forms displaying oxidation number from 0 to VI, only hexavalent (Cr(VI)) and trivalent chromium (Cr(III)) are stable enough to occur in the environment. Despite Cr(III) is not toxic and more stable soil under natural conditions, Cr(VI) is known to be highly soluble in water, bioavailable and toxic. In plants the toxicity of Cr(VI) results in the suppression of seed germination, chlorosis of its leaves and oxidative stress (Choppala et al., 2013). In human and animals, the dermal contact is the most important exposure pathway, and as a result Cr(VI) easily crosses physiological barriers, enters into the body, and accumulates in different organs and tissues (Robb and Testa, 2005). In order to determine the potential release of Chromium from mining activities and its impact on the environment, chemical extraction, chemical leaching under controlled physico-chemical conditions and isotopic exchange techniques associated with mineralogical studies has been applied in solid samples (soil, sludge, waste) and in water extracted in two different locations in Goiás State (Brazil). The first site is Barro Alto, Ni mining and metallurgical area, and the second is the Crominia, old chrome exploitation area. The chemical exchangeable Cr(III) and Cr(VI) were extracted with KCl 1M and KH2PO4 0.1 M respectively. Controlled leaching experiments were carried out with a NaNO3 10M and NaNH4 0.02 M leaching solution (S/L ratio of 20:1), controlled pH and monitoring additional parameters (pH, DO, Fe, Anions, etc.). In addition, isotopic exchange was performed with 13C (4.35% of abundance). The samples obtained from the chemical extraction and the isotopic exchange were all analyzed with HR-ICP-MS (ThermoScientific Element-II). Additionally, X-ray diffraction (XRD) analyses were performed on soils powder and shown that the mineralogy of the ores and soils are dominated by micas and oxides. X-ray fluorescence (XRF) analysis inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis were used to assess the total element concentrations of the solid samples. The results of the chemical extraction and isotonic chromatography indicates that exchangeable Cr is mainly under hexavalent form.

82 Do climate conditions affect metal bioaccumulation in earthworms?
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The present study aimed to assess the effects of changing climate conditions (temperature and soil moisture content) on the bioaccumulation of Zn and Cd in the earthworm Eisenia fetida in soils polluted by metal mining wastes. Bioassays were performed in different metal-polluted soils (mine tailing and watercourse) and under different combinations of temperature (20 and 25 °C) and soil moisture content (50 and 30% of the soil holding capacity - WHC). Adult earthworms were individually exposed to the study soils for 21 days and analysed for internal Zn and Cd concentrations at different times. Before and after 21 days of exposure soils were analysed for pH and metal concentrations in porewater. The mining tail soil did not show changes in metal availability (Zn-1, Cd-1). In the watercourse soil, porewater concentrations of Zn and Cd significantly decreased after 21 days exposure to the different climate conditions tested: Zn: from ~3761 to ~1613-2170 μg L-1; Cd: from ~63 to 32-41 μg L-1, especially at 20 °C and 50% of the soil WHC (standard conditions). In the latter soil, metal availability decreased accompanied by a significant pH increase at 20 °C and 50% of the soil WHC. Both metals were accumulated in the earthworms, with Cd accumulation pattern in earthworms, without differences among climate conditions. Earthworms reached the highest internal Zn concentration after 1 day of exposure (~450–1500 μg g-1 d.w.) then remaining constant until the end (typical pattern of essential elements). The Cd bioaccumulation pattern changed when changing the climate conditions. At 20 °C and 50% of the soil WHC the internal Cd concentration continuously increased from ~4 to ~13-19 μg g-1 d.w. (typical pattern of non-essential elements). But when increasing temperature and/or decreasing soil moisture content the pattern changed toward a pattern typical of essential elements due to increased Cd elimation rates (mine tailing: from ~0.11 to 0.24 μg g-1 d.w.; watercourse) collected in two areas located in northern Sweden. During each variable duration, were not statistically different. Either 1- or 3-day exposures of macrophyte demonstrated that the inhibitory action of four sulfonyures on the aquatic shoot length, and wet and dry shoot weights measured at either 3, 7, and 14 days during a whole year. In addition to pesticide profiling experiments were complemented by slurry reactor experiments enabling different spatial resolutions (sub-mm to cm) and depths (2 cm from/digestion of the accumulation gels), probably impacting the environmental interface (SWI), like gradients of e.g., the oxygen concentration, the redox potential, and the Eh. Long-term water samples showed a similar pattern with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis were used to assess the total element concentrations of the solid samples. The results of the chemical extraction and isotonic chromatography indicates that exchangeable Cr is mainly under hexavalent form.

83 Keeping memory of past mining activities: assessment of trace metal bioavailability and ecological risk using active biomonitoring.
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Mining activities are the first substantial source of trace metals (TM) contamination of the whole environment. However, past mining activities are often forgotten or minimized due to the disappearance of visual marks while mining wastes are known to be highly concentrated in TM. Bioavailability of TM and risk assessment linked to past mining and smelting activities are developed in a past mining district in the Vosges Mountains (France) to reconstruct their environmental impact in a forested valley. A bioindication campaign using the land snail Cornu aspersum as bioindicator species was conducted for six weeks along a gradient of soil TM concentration with eight stations comprising two archaeological mining stations, a communal garden, four forested stations and a control station. Three microcosms were placed in each station with fifteen snails per microcosm. Each week, two snails were randomly selected for digestive gland TM analyses. Bioavailability of TM is evaluated by the uptake rate of TM. Bioavailability of TM is high in the eight stations compared to recent industrial sites, highlighting the importance of knowledge of localization of past mining and smelting sites. Moreover, the two archaeological mining sites present the highest bioavailability of TM to snails with, for example, 68 ± 1 mg g-1 d.w for Cr(VI), 4.37 mg g-1 d.w for Pb and 0.07 mg g-1 d.w for Cd. A high bioavailability of TM is observed in the communal garden. These results conduct to assess the risk of this past contamination by using two indicators, i.e. SET and ERITME index which allow assessing the excess of transfer and the toxicological risk linked to TM. Excess of transfer and toxicological risks linked to archaeological mining sites are the highest ever measured with these index. Those calculated for the communal garden are similar to those on recent industrial sites. In conclusion, this study highlights that time elapse since the deposit of contaminated particles from mining and smelting activities is not sufficient enough to strongly catch TM in soils without transfer to trophic chain.

84 Water quality assessment of a catchment affected by historical lead mining
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Historical metal ore extraction and processing have resulted in severe water quality issues worldwide. In England and Wales approximately 20% of water bodies fail the objectives of the Water Framework Directive (WFD).
Environmental agencies from the United Kingdom and the European Union are constantly challenged in the implementation of this directive, therefore applied research and monitoring programmes are essential for the understanding of metal ion speciation, mobility, accumulation and bioavailability in river ecosystems. In northern England an exhaustive study has been carried out in an uplifted catchment polluted by heavy metals from historical mining. This research aims to identify the sources of pollution in the catchment and overall significance to water quality criteria for better management and potentially remediation of surface and ground water. A multi-methodological approach has been applied including water quality monitoring and conventional chemical analyses (Ion chromatography, ICPMS) for determining metal sources and concentrations. Correlation tests and principal component analysis have provided a consistent physicochemical characterization of the catchment, reflecting different sources of minerals that affect its water quality. Results suggest that the major geochemical signals are determined by the contribution of carbonate host rock (Ca2+Mg2+:DIC:EC = 0.9, <0.001), other metals are possibly associated with weathering (Fe2+:Al3+:Pb2+:Cu2+:DOC r=0.2–0.7, <0.001) and erosion of mined ores such as sphalerite (ZnS) and galena (PbS). The major sources of Zn and Cu were PbS and PbO, respectively (Zn: Pb2+:Cu2+:Cd2+:Fe2+:Al3+: r=0.5, <0.001) and PbS and PbO (Zn: Pb2+:Cd2+:Cu2+:Fe2+:Al3+: r=3.84 mg/l, 439.9 mg/l, 47 mg/l, 19.8 mg/l) exceeded the Environmental Quality Standards by 99x, 61x, 313x and 2x, respectively. In addition, the identification of different flow regimes emphasizes their impact on metal discharge. Accordingly, mine channels are considered as important point sources, but a major problem is caused by extensive mine tailings during high flow scenarios becoming significant sources of diffuse metal pollution. Results suggest that a catchment-scale assessment combined with analytical approaches offer reliable information about factors controlling metal inputs (point and diffuse), providing monitoring programmes with a practical way to evaluate heavy metal pollution in headwater catchments.

85 A field-based approach to linking biological responses of freshwater organisms to sediment contamination by metals
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The legacy of contaminated sediment is a serious impediment to rivers meeting the Environmental Quality Standards by 99x, 61x, 313x and 2x, respectively. In conclusion, this study highlights that time elapse since the deposit of contaminated particles from mining and smelting activities is not sufficient enough to strongly catch TM in soils without transfer to trophic chain.

SETAC Europe 26th Annual Meeting Abstract Book
variety of factors can influence the uptake of contaminants, such as metals, from the sediment by biota. These influences can be difficult to accommodate when deriving environmental limits that are relevant to field conditions. Here we have used a GIS approach based on river reaches to match biological data, from Water Framework Directive monitoring programmes of diatoms, invertebrates, fish and macrophytes, to riverbed sediment chemistry. A total of 283 sites were identified with matching sediment and biological data. A threshold biological response to sediment metal concentrations was expected, where the metal would have no influence until a threshold concentration had been exceeded. By modelling the 95%ile biological response thresholds to sediment concentrations of the metals silver, cadmium, chromium, copper, nickel, mercury, lead, tin and zinc, and the metalloids arsenic and antimony were determined. Based on field data describing the response of the relevant BQEs, the rank order of sensitivity to sediment contaminated with metals and metalloids (from most sensitive to least sensitive) was: diatoms < invertebrates < fish < macrophytes. Despite the uncertainties in the data used to produce the datasets, these findings, based on field data, suggest that for some metals the existing sediment quality guidelines may be too precautionary.

Toxicity Testing in Sediments - Bioassays As Link Between Chemistry and Complex Benthic Community Testing for Sediment Quality Assessment


In sediment-contact tests effects caused by sediment-bound pollutants usually are assessed on a macroscopic scale. In this approach a sediment-contact test with rice is enhanced by molecular biomarkers for arsenite contamination. As macroscopic endpoint the inhibition of root elongation was assessed. A dose-response curve for the inhibition of root elongation was characterized and the EC50 was calculated as 13 mg kg–1 dw. Molecular endpoints in form of transcript biomarkers were derived from microarray analysis. For this purpose, rice plants were exposed to two arsenite concentrations, namely 11 mg kg–1 and 15 mg kg–1 on spiked artificial sediments. Differentially expressed genes that are dose-dependently induced were selected as candidate biomarkers. From these genes a subset of five genes was analyzed by means of qPCR in rice roots that were exposed to a broader range of arsenite concentrations ranging from 13 to 23 mg kg–1 dw. On arsenite spiked artificial sediments, all genes showed a dose-dependent increase of expression up to a maximum at 9 or 13 mg kg–1 dw, respectively. While on artificial sediments a distinct inhibition of root elongation was observed, on natural sediments only at the highest concentration of arsenite, an inhibition of root elongation occurred. This finding from the natural sediments contradicts the expectations based on the results of the artificial sediment where an inhibition of the root elongation was observed at lower concentrations. This might be explained by the observation, that rice roots grown on silty natural sediments like, grow thinner, but longer compared to roots grown on sandy artificial sediments. However, one of the five molecular biomarkers reacted clearly on the contaminated sediment. These findings might suggest that the macroscopic endpoint root elongation is less sensitive on silty natural sediments compared to molecular endpoints, if sandy references sediment is used. In sum, it was possible to identify candidate biomarker genes for arsenite stress by means of an omic-approach. On artificial, but especially on natural sediments, the biomarker react more sensitive than the inhibition of root elongation and thus indicated a stress response before adverse effects manifest on a macroscopic level.

87 Evaluation and selection of test methods for the assessment of contaminated sediments in the Baltic Sea - The CONTEST project K. K. Lehtonen, A. Ahvo, Finnish Environment Institute / Marine Research Centre; N. Berezina, Russian Academy of Sciences / Zoological Institute; M. Breiheitz, B. Eklund, Stockholm University / Department of Environmental Science and Analytical Chemistry, K. Jorgensen, Finnish Environment Institute / Marine Research Centre; M. Linde, Stockholm University / Department of Environmental Science and Analytical Chemistry; E. Schultz, Finnish Environment Institute / Laboratory Centre; J. Strand, Aarhus University / Department of Bioscience; K. Ström, B. Sundelin, Stockholm University / Department of Environmental Science and Analytical Chemistry

There is a growing need to standardize reliable, scientifically based assessment methods to assess and classify the degree of contamination in sediments. This is necessary for decision-making concerning regulatory actions towards the reduction of pollution from different sources, removal and re-location of contaminated materials, and on-site remediation measures and the follow-up of these. The purpose of the CONTEST project (“Evaluation and selection of test methods for assessment of contaminated sediments in the Baltic Sea”, 2014-15), funded by the Nordic Council of Ministers and the Finnish Ministry of the Environment, was to test, evaluate and select suitable biological methods for the quantitative and qualitative assessment of toxicity of anthropogenically contaminated sediments in the Baltic Sea marine environment. In the project, a hierarchy of biotests currently in use in toxicity assessments were applied using a heavily contaminated harbour sediment as the test matrix. Chemical analysis of the sediment confirmed the presence of high concentrations of poly cyclic aromatic hydrocarbons, organotins and trace metals. For the toxicity testing, a series of dilutions was prepared by adding reference sediment contaminated on a macroscopic scale. All data used to produce the datasets, these tests, based on field data, suggest that for some metals the existing sediment quality guidelines may be too precautionary.

88 Bioassays in framework of a triad assessment method for brackish sediments K. De Schamphelaere, J. Rervoets, J. Teuchies, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology; T. Ysebaert, IMARES - Wageningen University / Institute for Marine Resources and Ecosystem Studies; P. Meire, University of Antwerp / Ecosystem Management Department of Biology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology

In sediment risk assessment, analysis of the pollutant concentrations is essential in determining the degree and nature of sediment contamination. However, chemical analyses provide no evidence of biological effects as, for example, mixture effects are not accounted for. Bioassays provide valuable information on the toxic effects of sediments, but are carried out under lab conditions and are limited to the testing of a few organisms. An evaluation of the benthic community structure can provide evidence of effects in situ. The sediment quality triad method incorporates measures of various chemical parameters, toxicological effects and benthic community structure in view of conducting an integrated assessment of sediment quality. In framework of developing a triad assessment method for the quality evaluation of brackish sediments, 28 sediment samples were taken along the Scheldt estuary (Sea Scheldt (Flanders) and Western Scheldt (the Netherlands)) and other brackish aquatic systems in Flanders. For these samples, chemical variables (e.g. metals and organic pollutants), toxicological effects and benthic community structure are being assessed. In this study, two sediment contact bioassays and a pore water bioassay are carried out to test their suitability for uptake in a quality triad method for brackish sediments as indicators of ecotoxicological effects. A 28-day sediment contact bioassay with the polychaete worm Hediste diversicolor is performed on a selection of the samples, with growth and mortality being evaluated. A 10-day sediment contact bioassay with the amphipod Corophium volutator and a 24h pore water test with the rotifer Brachionus plicatilis, evaluating mortality, are carried out on all the samples. For the sediments already evaluated mortalities vary between 0%-20% for H. diversicolor, between 1%-20% for C. volutator and between 10%-45% for B. plicatilis. Chemical concentration data are evaluated and compared with existing Sediment Quality Guidelines to assess the expected potential of sediment pollutant concentrations to influence sediment toxicity, and compared with the bioassay results. Through multivariate analysis the chemical, ecotoxicological and biological sediment measures are being analysed to assess the correspondence among the three components.
to assess the ecological risk of sediment-bound contaminants. In this work we study the relationships between the sediment chemistry, the toxicity testing and the status of biological communities, in order to a) assess the degree of (dis)agreement among these three LoEs; b) identify the factors and conditions more likely disturbing the expected cause-effect relationships; and c) suggest improvement measures towards the managed use of dredging operations in the coastal areas of Spain. Data from several studies carried out by AZTI in the Basque coast (N. Spain) for different institutions were considered in this work. Overall, almost 300 sediment samples collected along the Basque coast with chemical information of contaminants (heavy metals and organic contaminants) and biological data (biological LoE: toxicity testing and/or benthic communities) were considered. Most of the metal concentrations in the sediments were well above the ERL, and values above ERM were frequent, suggesting that adverse toxic effects were likely to occur. Statistically significant correlations have been found between variables of the three LoEs: e.g. Sorgonic matter, PCBs, Cr, Ni and Zn Concentra LACO as defined by the International Joint Commission’s Great Lakes Water Quality Agreement. A sediment remediation project took place in the lower 14.2 km of the river where urban and industrial activities impacted the river as a beneficial resource. Sediment was removed based on a surface weighted average concentration model where PCB and PAH levels exceeded targeted levels. This presentation will focus on three biological tools used to assess remediation effectiveness: change in fish, invertebrate and Tetragnathid spider tissue concentrations of PCBs and PAHs, DNA damage in Brown Bullhead and macroinvertebrate biotic condition as measured by Ohio EPA Lacustrine Index of Community Integrity (LICI). From 2009-2013 and again in 2015, pre- and post-remedy sampling of fishes representative of different trophic levels was conducted via electroshocking and fyke net sampling. The study area was divided into three river reaches with distinct hydrogeomorphology. Fish collected from each reach included: Largemouth Bass, Brown Bullhead, White Sucker, Pumpkinseed, Gizzard Shad, Bluntnose Minnow and Emerald Shiner. Blood samples were collected from 10 Brown Bullheads from each reach to assess DNA damage using Comet Assay methods. Multi-plater samples were deployed for collection of macroinvertebrates for tissue analyses and biotic condition assessment. DNA damage in Brown Bullhead increased during dredging then declined in subsequent years. Largemouth Bass, White Sucker, Brown Bullhead, Pumpkinseed, Bluntnose Minnow, macroinvertebrate & spider tissue concentrations showed no change 3-y post dredging compared to pre-dredging. Gizzard Shad and Emerald Shiner showed lower tissue concentrations 3-y post dredging compared to pre-dredging across the entire project area. No difference in the LICI was found based on samples collected before and after dredging. Based on modeling performed during the design phase, it was anticipated that the long-term clean up goals would be met approximately 10 years after the completion of dredging activities, by 2020.

91 A comparative approach using ecotoxicological methods from single-species bioassays to model ecosystems


Effects on natural nematode assemblages to chemical stress (Zn) were assessed in this study in complex sediment microcosms, including indirect food-web effects, and in acute community toxicity tests, considering direct toxicity only. Responses of the various freshwater nematode species in both approaches were compared to effects of Zn on the well-established model organism C. elegans in standardized toxicity tests (ISO 10075). The acute tests were performed with a concentration of 20 mg Zn/L, resulting in a sensitivity ranking of in situ nematode species. The findings only partly reflected classifications of nematode species according to the NemaSPEAR[%]-index but underlined the function of C. elegans representing the sensitivity of freshwater nematodes. The sediment in the microcosms was spiked with 1 mg Zn/L and 15 mg Zn/L dw and 15 mg·kg-1 dw. Observations allowed for more accurate decision making in environmental risk assessment. Data on chemical concentrations, single-species and community toxicity, and in-situ assemblages can be integrated as single lines of evidence in a weight-of-evidence approach. As suitable ecotoxicological tools and ecological indices (NemaSPEAR[%]-index) for nematodes are already available, this organismal group should be used more often in sediment quality assessments.

Advances in exposure modelling: bridging the gap between research and application (II)

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Humans can be exposed to chemicals in consumer products during product use and environmental releases with inhalation, ingestion, and dermal uptake as typical exposure routes. Nevertheless, chemical exposure modeling has traditionally focused on the far-field with near-field indoor models only recently gaining attention. Further, models that are mostly emissions-based, may not necessarily be applicable to all types of chemical release from consumer products. To address this gap, we (1) define a framework to simultaneously account for exposure to chemicals in the near- and far-field, (2) determine chemical product concentrations for various functional use categories, (3) introduce a quantitative metric linking exposure to chemical mass in products, the Product Intake Fraction (PIF), and (4) demonstrate our framework for various consumer product categories. This framework lends itself to high-throughput calculations for characterizing exposure to the vast consumer product chemical space. The chemical mass in products is used as a starting point for quantifying human exposure obtained by multiplying the chemical concentration (e.g. % w/w) in the product with the amount of product used per defined application. Chemical concentrations in products can be obtained from empirical studies, formulations and associations described in databases, or when unavailable, estimated based on chemical-product functions or regulatory framework formulations. Exposure is quantified by estimating the PIF for each product category that is taken in by humans via each exposure pathway, considering specific compartments of entry into the near-field environment (releases of chemicals encapsulated in articles, indoor air spray, etc.). To estimate PIFs, we combined far-field environmental compartments with near-field compartments and exposure pathways in a multimedia matrix of transfer fractions, with columns and rows for each compartment and exposure pathway. The multiple transfers and PIFs (e.g. from chemicals encapsulated in articles to inhalation of indoor air and dermal uptake via skin contact) were obtained by inverting the transfer fractions matrix, yielding cumulative multimedia transfer fractions. PIFs for various chemicals in products were found to be on the order of 1x10-3 for semi-volatile organic compounds (SVOCs) in thick flooring, 5x10-3 for VOCs in indoor air spray, and up to 9x10-2 for ingredients in leave-on cosmetic products.

93 Jungle relationships in modelled and measured concentrations of chemical pollutants in the Danube River

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Persistence is an undesirable property of chemicals that is difficult to measure in the real environment. Jungle relationships describe the correlation between the relative standard deviation of concentrations of chemicals and their residence times. Once calibrated for a set of compounds they can potentially be used to estimate the persistence of pollutants. The parameter b in the Jungle equation is a
measure of the strength of the relationship between variability in concentrations and residence time. [1] In this study we explored Junge relationships relating the concentrations of chemical pollutants to their degradation half-lives in the Danube River. Concentrations of four hypothetical chemicals with biodegradation half-lives of 7, 15, 30 and 90 days were obtained using the STREAM-EU model. [2]. Junge relationships and temporal correlations from variability at the year end each of the stations, and 2) "Spatial" relationships from variability between the stations on each day of the year. Next, a Junge relationship was calculated from the data of the 2nd Joint Danube Survey (JDS) [3] for caffeine and six pharmaceuticals: benzoatulose, carbamazepine, diclofenac, gemfibrozil, glimepiride, and sulphonylurea. The compounds were gathered from literature. We found Junge relationships in the Danube in both modelled and measured concentrations. Results from STREAM-EU show clear Junge relationships for the 4 theoretical chemicals. The parameter b ranges between 0.1 and 0.8 for the spatial analysis and from 0.1 to 0.4 for the spatial analysis. The higher b values in the temporal analysis are found downstream (i.e. Romania), were population and emissions are relatively low. For the spatial analysis, the higher b are found in the month of November. The analysis of pharmaceuticals and caffeine data from the JDS, also shows a Junge relationship with b of 0.25, well within the values obtained in the modelled spatial Junge relationships. Modelling results suggest that Junge relationships are more likely to be found during November and downstream in the Danube, but this has not been confirmed with monitoring data. Measurements within the optimal spatial and temporal conditions defined by the modeling analysis could potentially be used to estimate the persistence of other chemical pollutants. [1]MacLeod, et al. 2013. Chemosphere 93:830-834[2]Lindem, et al. 2016. Chemosphere 144:903-910[3]ICPDR. 2007. http://www.icpdr.org/wq/dihome

94 Application of a spatial model to contextualise monitoring data for risk assessment of down-the-drain chemicals over large scales J.Kilgallon, A. Franco, O. Price, Unilever / Safety and Environmental Assurance Centre SEAC
Regulatory screening level exposure assessments (e.g. EUSES) are based on simple conceptual scenarios at various scales. However, exposure, and therefore risks associated with chemicals, are characterised by high spatial variability. The Scenario assembly tool (ScenAT) is a global screening level exposure model to enable spatially explicit predictions of environmental concentrations of home and personal care chemicals. It uses a simple equation to predict local scale freshwater concentrations (PECs) of chemicals discharged via wastewater, as described in the EU TGD. Calculations are performed for 28,080 spatial units across 88 countries worldwide using GIS data layers for the underlying socio-economic population, GDP and environmental parameters (per capita water use, STP connectivity, dilution factor). Using a probabilistic approach, we incorporate sources of uncertainty in input data (tonnage estimation, removal in sewage treatment plants and seasonal variability in dilution factors) into spatially explicit model simulations for two test chemicals: the antimicrobial trichloroethane (TCS) and the anticoagulant linear alkylbenzene sulphonate (LAS). We then compare model estimates of wastewater and environmental concentrations of TCS and LAS to monitored data available for the UK. This comparison showed that screening-level modeled probabilistic PECs were higher than mean measured data for TCS and LAS by a factor 3.4 and 1.4, respectively. Considering the uncertainty associated with both modeled and monitoring data, results suggest that the use of a probabilistic approach using the ScenAT model for screening assessment over large scales is a valid approach, produces output that is consistent with monitoring data, and thereby represents a robust screening level exposure assessment tool. The combination of modelled and monitoring data enables the contextualisation of available monitoring data, focussed on few specific catchments, over larger scales. Such information can be used to support the refinement of exposure assessments as well as for the identification of areas of high concentration for use in higher tier assessments.

95 Exposure hotspots and source apportionment analysis of home and personal care products chemicals in Asia C. Wanazz, The University of Michigan, Ann Arbor / SPHEHS; A. Franco, J. Kilgallon, Unilever / Safety and Environmental Assurance Centre SEAC; O. Joudac, University of Strathclyde

The increasing use of household and personal care (e.g. HPC) products in developing countries requires new tools to assess the potential ecological impacts of HPCs, accounting for local hydrological and use characteristics. This paper therefore aims a) to develop a tiered set of models to describe the hydrological and multimedia fate of releases associated with the use of HPCs in Asia b) to combine the two model with the emission monitoring data to predict chemical concentrations and to identify of (sub)catchments hotspots, and c) to perform an apportionment analysis to understand dominant near and distanted sources. Geo-referenced emissions scenarios are first determined for the entire region using the ScenAT model and focusing on six different test substances. These emission scenarios are then combined with the regional model results and combined to the Pangea fate and exposure model (tier 2) that has the ability to create scenario-specific multi-scale grids. A more detailed analysis is carried out on the Yangtze and Huai River catchments in China, comparing estimated exposure hotspots with water quality grades. LAS estimated concentrations in freshwater are the highest among the considered HPC chemicals but few LAS monitoring data is available for Asia. The modelled LAS hotspots leads to differences in spatial variation in water concentrations. Of special interest is the contrast between short and long lived chemicals in surface water. For a short-lived substances such as LAS (half-life of 0.29 d), dilution leads to decreasing concentrations in the Yangtze River as we move downstream, whereas concentrations are relatively constant for a less degradable substance. The highest concentrations are observed for the Yang River and tributary of the Huai River, classified with the poorest grade (V+) for macro indicators of water quality in China. For LAS only a few adjacent cells contributed to the concentrations in the terminal cell, 63% of the concentration being due to direct emissions in this terminal cell. In contrast, for triclofen, only 1% of the concentration in the terminal cell is due direct emissions to the river, and the remaining 99% coming from more than 220 cells spread over 2000 km upstream in the catchment. The combination of ScenAT with the multi-scale multimedia model Pangea provides a parsimonious way to identify hotspots and to study them with refined resolution.
of guidance and or guidance-related documents like the ESDs is still ongoing. The following guidance documents have been finalised since entry into operation of the BPR (09/2013): Mesocosm guidance (available as Appendix in Vol. IV Part B) Cut off criteria for groundwater assessment of biocides Volume IV Part B: Guidance on Environmental Risk Assessment – Active Substance Scenario for the biocides and emissions from non-platforms PT11/PT12 for PEC calculation Direct emissions to surface waters in PT 6, 7, 8, 9 and 10 Use scenarios for PT 9 roof membranes Supplement to the ESD for PT 13 Emission Scenario Document for PT 19 PT 21: Fish net scenario in aquaculture The preparation of the following guidance documents is currently coordinated by ECHA, member states are actively involved in the development of the scenarios, the protocols and methods to assess the exposure/risk of wood preservatives Proposals for standard scenarios and parameter setting of the FOCUS groundwater scenarios when used in biocide exposure assessments Leaching to groundwater from paint, coatings and plaster Harmonisation of testing for PT 7, 9 and 10 - test methods.

98 Mixture toxicity assessment of biocides: Experimental verification of the relevance of additives in wood preservative products


The Biocidal Product Regulation requests to consider combined effects of active substances and of active substances and relevant additives in the environmental risk assessment. The present study aimed to inform guidance development regarding the identification of additives as being relevant for such a mixture risk assessment. Further, it aimed to verify whether an additive can substitute for ecotoxicological testing of biocidal products or their residues released into the environment. The hypothesis of the study is that the consideration of the active substances and additives of environmental concern is sufficient to reliably predict the aquatic toxicity of biocidal products using the concept of Concentration Addition (CA). Several wood preservative products were chosen for the experimental verification of the hypothesis using green algae (growth rate after 72 h), Daphnia magna (immobilisation after 48 h) and fish embryos (mortality after 48 h and/or 96 h). To determine how relevant various types of additives are for a protective mixture risk assessment, the Model Deviation Ratio (MDR) was calculated for different subsets of product components/ndl general, the CA prediction was (over)protective for the so far tested products. If this can be confirmed as a general finding, the theoretical risk assessment of products by the CA concept appears sufficiently protective to be used as a substitute for product testing, taking only additives into account that are identified as environmentally relevant. The presentation aims to give an overview of the results of all seven tested products and will also discuss whether such findings can be generalized for the risk assessment of biocidal products in view of the limited variability of wood preservative products available for testing in the present study.

99 Biocidal use of anticoagulant rodenticides results in the secondary exposure of non-target animals

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Residues of anticoagulant rodenticides (AR) in non-target animals were studied in Finland. ARs are used in non-target animals to control targeted rodents. In particular, second generation ARs (SGARs) have been found in non-target animals in many countries. This study focused on species which feed either on rodents or their carcasses and in which ARs have been found in other countries. The animals were either found dead or were shot or trapped for other purposes. Bromadiolone, difenacoum, brodifacoum, flucoumafen and coumatracyl were analysed in 136 liver samples by an HPLC-triple quadrupole mass spectrometric method. One or more ARs were detected in 87% of samples. ARs were commonly found in eagle owls, tawny owls, raccoon dogs, foxes and mustelids. The most commonly found AR was bromadiolone which was also found in the highest concentrations. Bromadiolone was the most frequently used AR in Finland in 2014. The second most common AR present in the liver was coumatracyl followed by difenacoum, brodifacoum and flucoumafen. Overall, the prevalence of ARs in non-target species correlated well with the sales of these substances. A high variation of concentrations was found within and between animals. Bromadiolone was found in significantly higher concentrations compared to SGARs. Our results show that the biocidal use causes frequent exposure of non-target animals, because ARs are authorized only as biocides in Finland and the use for the crop protection can be considered negligible. There are several restrictions on the use of ARs in Finland. The most restricted use is allowed to the governmental bodies. Only terrestrial samples will be used for the distinct selection of substances as well as a possibility to use ARs outdoors. Even the most restricted substances, i.e. those allowed only to indoor use by PCOs were found, but in lower frequency compared to more commonly used substances. Coumatracyl was found surprisingly often despite of its anticipated low use and fast elimination half-life. On the other hand, concentrations of coumatracyl in the bats were several times higher than the total concentration of all other anticoagulants which were assumed to be sublethal, but in 15% of non-target species concentrations were so high that ARs could have contributed to the death of these individuals.

100 The fate of biocides in stormwater pond sediments

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Biocides are added to paints, wood preservatives, plaster, and other building materials to protect them against fouling. Upon application, biocides also ensure local control of pests and biocides are released to surface waters through off- during rain, and enter the stormwater flow, resulting in concentrations that routinely exceed quality standards set by the EC. Stormwater contains a range of other pollutants which are perceived as problematic. To mitigate their impacts, retention ponds are used. Retention ponds are used to treat stormwater prior to discharge into the environment. The objective of this study is to assess how far biocides are sorbed and degraded in the organic-rich sediments of stormwater retention ponds. No study has hitherto reported the fate of biocides in retention ponds, and only few studies exist on pesticides in sediment from comparable systems. The sorption capacity, sorption kinetics, and degradation of Cybatryn, Terbutryn, Dicuron and Carbendazim were investigated in microcosms simulating retention ponds. Stormwater sediments were deposited at the bottom of microcosms, and biocides were added. The removal of biocides from the liquid phase was monitored. After 3 weeks of incubation the sediments were extracted and analyzed for biocides. Parallel hereto sorption kinetics and stoichiometry was determined on the same sediments kept in suspension, the latter based on the relevant OECD standard. The total degradation of biocide was 10-30%, depending on biocide and sediment type. The sorption determined by slurry-tests and the microcosm tests did however not agree. The amount of biocide remaining in the liquid of the microcosms after 3 weeks were up to 10 times higher than what was expected based on the sorption capacities determined by the slurry tests. A fully dynamic diffusion-adsorption-degradation box-model was applied to analyze this discrepancy. The simulations represented the measurements well and allowed dynamic distinguishing between degradation and sorption. The study showed that all biocides underwent sorption and degradation in stormwater sediments to a degree where these processes will affect the final amounts discharged from retention ponds to receiving waters. It was furthermore observed that sorption kinetics of biocides in deposited sediments differed significantly from those of sediment slurries and that kinetics based on slurry experiments significantly over-estimated the sorption to retention ponds sediments.

101 Implementation of an Environmental Monitoring of Biocides to Follow Consequences of their EU Risk Assessment Outcome


The implementation of the Biocidal Products Regulation (No. 528/2012; BPR) causes changes in the application of biocidal active substances and their entry into the environment. For biocides included in the list of approved substances concentrations may increase while decreasing environmental levels are expected for biocides with non-approval or inconclusive data on predicted environmental measures. Such consequences may be proven by an environmental monitoring. However, in most current monitoring programmes biocides are not appropriately covered yet. Traditionally, e.g. in surface waters mainly plant protection products (partly also approved as biocides) and legacy chemicals are monitored. In this context, the German Environment Agency (UBA) initiated a project which aims at developing a comprehensive monitoring concept for biocides which also includes selected monitoring studies. The developed prioritisation approach provides lists for a biocides monitoring in all relevant compartments (e.g. monitoring in surface waters: water phase, aquatic biota, suspended particulate matter/sediments). For the implementation of the proposed biocidal monitoring approach mainly cooperation with existing programmes is recommended. At least some biocidal compounds are already covered (e.g., for surface water monitoring according to Water Framework Directive obligations). All proposed monitoring activities should be organised in a stepwise approach (e.g. screening study, survey in different regions, and implementation on new or improved integrated monitoring programmes). In a further part of the project archived biotic and abiotic samples from the German Environment Specimen Bank (ESB) were used for exemplarily retrospective monitoring studies to identify possible spatial and temporal patterns of selected biocides. As case studies monitoring data from recent investigations covering the ESB covered time periods and specific substances were compared to recent biocidal residues in aquatic suspended particulate matter and rodenticides in fish liver tissue. Time series reveal sporadically occurrence of rodenticides such as brodifacoum in fish and decreasing levels of cybutryne (previously used as preservative in construction materials). Examples support the assumption that changes of the use of biocides after approval/non-approval decisions or as consequence of risk mitigation measures can be followed by an environmental monitoring. Appropriate monitoring data would also allow to check the appropriateness of exposure
estimations of model outputs).

102 Biocidal active substances in households - reasons for the need to promote a sustainable use of biocides
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Households are a possible application site for a wide variety of biocidal active substances that fall under the Biocidal Products Regulation (EU) 528/2012 (BPR). However, the use is not monitored sufficiently. Furthermore, all biocidal active substances present in personal care products are not covered by the BPR and thus are not evaluated under its risk assessment scheme.

The objectives of the work presented here are therefore (i) to identify the biocidal active substances that are found in households and the respective product categories they are used in and (ii) to describe the cases where biocidal active substances might enter the sewage system without being covered by the BPR and thus are not evaluated under its risk assessment scheme.

Face-to-face interviews were conducted in 133 households in predominantly urban, intermediate and predominantly rural study sites in Germany. Members of private households were interviewed using a standardised questionnaire. Additionally, the products that were used in the households were registered with the help of a barcode scanner. Biocidal active substances were present in all households, even though not all possessied biocidal products, as the majority of uses of biocidal active substances was in washing and cleaning agents and personal care products, but not in biocidal products. Around 60 % of the registered applications of biocidal active substances do not fall under the risk assessment of the BPR. These can be active substances present in washing and cleaning agents, which are not assessed or approved for the use as in-can-preservatives.

Furthermore, all biocidal active substances present in personal care products are not covered by the risk assessment of the BPR. The results show that gaps exist in the risk assessment of biocidal active substances. The attempt to solve the problem would require an extensive increase of complexity of risk assessments and their aggregation throughout all legislation. From our point of view, a better approach to reduce possible risks by these substances in general would be to limit their use to in fact essential usages. A sustainable use of biocides should thus be promoted to account for existing gaps in risk assessment.

Life Cycle Data and Modeling Developments - From Data Collection to Usage (II)

103 How to complete LCA studies data collection in order to improve its quality?
E. Lees-perasso, CODDE; A. Roy, Bureau Veritas CODDE; J. Orgelet, Bureau Veritas CODDE / EcoDesign; J. Garcia, P. OSSET, SCORE LCA

As a more and more common and widespread tool used to calculate the environmental impacts of products and services, the importance of LCA studies data collection increases. In parallel, the multiplication of LCA studies as well as the growing complexity of reference documents and analysed systems leads to an increasing need of temporal and human resources to ensure the collection and research of data to assess the systems considered. The time needed to perform this collection and the assessment is greater than the time needed for the model, as some data may be missing, hard to find or even false. Therefore, it is necessary to determine and use methods in order to fill those gaps. These choices are essential and must preserve the relevance and transparency of studies while limiting the need for resources. This presentation aims at introducing the study performed on behalf of the ScoreLCA association concerning how to answer this need for reliable methods of missing data gap completion.

This study aims at answering this need through three steps: Describing existing reference documents requirements in terms of missing data identifying, describing and applying actual or ongoing methods aiming at solving the data missing gap Determining recommendations allowing the selection and application of chosen methods, depending on the studied system typology and the LCA study goals Concerning the requirements, the study enlightened the different logical schemes about their implementation in the different documents, depending on the activity sector, the age of documents, and the involved actors and documents objectives. This study shows that the actual developments go towards a greater harmonization and complexity of requirements. Concerning the methods, the study concluded that the existing ones cover quite well the need for identification, analysis and substitution of missing data. Though, those methods have different application scope and advantages and drawbacks, due to inherent or external reasons, the choice of those methods are allocated to the collection and application of those methods. To go further, the study concluded on the importance to gather and analyse feedbacks from the different stakeholders (practitioners, verifiers, study users), as well as the methods and requirements answer thestudies objectives. This could provide a clear synthesis to be used as a basis for the development or update of future reference documents.

104 How to prioritize data collection based on uncertainty analysis: Application to the regionalization effort
L. Patouillard, CIRIAE- Ecole Polytechnique de Montréal; P. Collet, IFP Energies nouvelles; P. Tirado Seco, CIRIAE; C. Bulle, CIRIAE - ESG - UQAM / Sustainable corporate society; M. Margni, CIRIAE - Ecole Polytechnique de Montréal / Mathematical and Industrial engineering

Life cycle assessment (LCA) practitioners spent a lot of their time on data collection. A smart data collection insures a good quality study with low uncertainty. One of the main challenge for them is to focus effort on data that can reduce the overall uncertainty and that will be the biggest contributors to the result uncertainty and that need further data collection. They have been selected based on a trade-off between accuracy and operationalization. For uncertainty contribution analysis (UCA), tools such as Contribution To Variance based Monte Carlo results and coefficient of variation (CV) are used.

The proposed methodology has been tested on an ecosystem v3 process using the IMPACT World+ LCA method to investigated which aspect of this process should be regionalized. For water use impacts on human health, the relative importance of LCI and LCIA uncertainties are compared based on CV values. Main results show that the effort for data collection should be focus on information to spatialize water EF in order to use less aggregated CF with a lower spatial variability. This methodology is described for regionalization purpose but could be further adapted for global data collection prioritization.

105 Influence of data choices in Life Cycle Assessment of waste management systems
T. Henriksen, Technical University of Denmark (DTU) / DTU Environment; T.F. Astrup, Technical University of Denmark / Department of Environmental Engineering; A. Damgaard, Technical University of Denmark / DTU Environment

Environmental models, e.g. life cycle assessment (LCA), are widely used to support decision-making. An important factor for the reliability and usefulness of the models is to extent which they represent reality, i.e. the system of interest, which is influenced by the representativeness of the input data and the model itself. There are specific challenges for LCA of waste management systems related to the collection of representative data. Site-specific conditions affect the quality of the waste and thus the quality of the impact data. Site-specific conditions are also included in LCI regionalization collection schemes, why there is a high need for site-specific data when modelling waste management systems. The objective of this paper is to evaluate the influence of input data choices in LCAs of waste management systems. The hypothesis is that data choices affect the LCA such that end result can vary with orders of magnitude. This is evaluated by analysing and comparing the spread of results calculated on basis of the modelling of a case study, where data combinations are implemented in the model. The case study is landfilling of Danish residual household waste with the functional unit being landfilling of 1 tonne of waste. A mechanistic landfill model was applied, where sub-processes are represented by models and emissions are linked to the fractional and chemical waste composition. In the model, data choices were varied based on around ranges to represent different options for landfill gas and leachate generation and different types of technical collection and treatment solutions. A total of 864 data combinations were obtained, hence resulting in 864 results per input category. From these 864 results with different data combinations, the model users can choose between the minimum and maximum values of potential impacts of climate change, ozone depletion, ionizing radiation, fossil resource depletion and particulate matters. This highlights that data choices, even individual technology choices, influence the LCA results. By tracking the results back to its data combinations we found that the largest potential impact of climate change corresponded to a combination with high landfill gas generation and limited gas collection and utilization. Based on these preliminary results, it can be concluded that the choice and availability of representative data influence the LCA results, and thus that proper identification of the correct modelling choices is crucial within specific technologies.

106 Comparing models in estimating additive emissions from plastic materials in

26 SETAC Europe 26th Annual Meeting Abstract Book
The introduction of additives in the total societal stock was previously estimated based on trade statistics combined with product lifetime estimates and as detailed information "as possible" about the additive type, down to substances and their concentration in materials. Emissions from the amount was then estimated by two different emission models. The work presented here is a cross-comparison of these two models for calculating emissions of additives in polymer materials during the use phase of the materials. The purpose is to investigate if, at all, a rough model can be considered good enough. The actual product type used for the comparison is not important. An alternative purpose for this comparison is to estimate information for total volumes of wall paint was available through the chemical product registry at the Swedish Chemicals Agency. The substances included are functional additives in paint, i.e. preservatives, biocides, etc. Actually the cross-comparison could have been done also on a theoretical composition of some other product. The purpose of the two models is to estimate the actual emissions of the additives at stake. The two models provide very varying results. When looking at the total emission estimate for the whole stock of plastics in the economy, it becomes clear that the rough model overestimates the emissions of additives when added together across all plastics and all additives. The advanced model, on the other hand, has been shown to provide a reasonably accurate estimate at least for specific substances (DINP from vinyl flooring) also when validated against concentrations in the indoor and outdoor environment. In this particular case the rough model overestimates emissions by a factor of 250. It is however highly uncertain to apply a similar factor for other materials and additives, as the emission ratio varies across several orders of magnitude. The rough model must therefore be considered "not good enough", while the advanced model needs further validation for more material/additive combinations.

**Contaminants of Emerging Concern in the Environment and their Management (III)**

**107 A visual solution to optimize the understanding and exploitation of metadata from Open Data sources in LCA**

B. Ruegge, Luxembourg Institute of Science and Technology (LIST) / Environmental Research

Innovation ERIN; P. Carvalho, B. Othoniel, T. Navarrete-Gutiérrez, Luxembourg Institute of Science and Technology LIST / Environmental Research Innovation ERIN

Metadata is a fundamental piece for the discovery and management of input datasets and methods in Life Cycle Assessment-LCA, Collaborative initiatives among LCA practitioners, database developers, data suppliers and other stakeholders worldwide have brought to several forms of metadata developments to improve the transparency of unit process datasets and impact assessment methods, providing the necessary knowledge on the applicability and relevance of data, helping reduce uncertainties and ensuring the reproducibility of LCA studies. The general impression is that the LCA practice is getting more and more oriented towards Open Access thinking, in particular for the use of life cycle commodity databases. Building on such free knowledge, however, can imply a lack of standardization, and pose additional problems to the consistency and quality of shared data. Hence, efforts to improve and harmonize metadata could reduce such issues. Information to build databases or update characterization models may be retrieved from open access portals, which can be valuable tools. The value of such information can even increase by using and combining datasets coming from different data sources. However, metadata from such sources, when defined, are not always easy to exploit. This intensifies the complexity of searching and filtering datasets, which is one of the major challenges for database developers. Moreover, gathering datasets from different sources complicates the data collection because of different metadata policies, types and languages. While open data portals are more and more tailored to store large amounts of metadata, they are less effective in making data available. Such portals are not usually prepared to allow users downloading datasets, and not all of them use open formats (e.g. CSV and XML), limiting the dataset reuse. Thus, beyond the necessity to increase their readability, metadata need to be better exploited for more comprehensible, replicable and transparent dissemination and use of life cycle data.

To this end, a novel visual technique is proposed here to organize in an effective and friendly ways all metadata related to a group of life cycle datasets. This choice is made because data visualisation techniques allow rapid assimilation and recognition of large amount of information. For validation, a case study is presented with focus on an integrated modelling framework for the characterization of life cycle impacts on ecosystem services.

**108 UNEP technical support on datasets review: a summary of learnings**

B.W. Vignon, SETAC: A. Asselin, UNEP/SETAC Life Cycle Initiative; G. Sonnemann, University of Bordeaux / The Life Cycle Group CyVi; A. Croith; S. CHEN, SIRMIF; S. IRCH; V. POOSHR; L. TAYLOR; F. Laburn, National Metal and Material Technology Center MTEC / LCA Lab; J. Munkskalsi, MTEC; F. Bouremia, L. Mila i Canals, UNEP

Introduction The UNEP/SETAC Life Cycle Initiative has developed the General Guidance Principles for LCA databases [1] that describes guidelines for LCA databases to be followed. The objective of adopting Principles, a review of 40 datasets has been conducted with UNEP support by independent LCA experts. Review process, timeline and criteria The whole effort had to be delivered in a set and limited timeline of six months and covered 40 datasets from emerging economies, 20 from Malaysia, 10 from Brazil and 10 from Thailand. The intent of the review was not to validate or not the sufficient quality of the datasets for publication, but rather to encourage improvements, either prior to publication or as part of a continuous improvement cycle. The developed review process consisted especially in i) developing a set of review criteria for this specific effort, ii) establishing a non-disclosure agreement (NDA) between the data provider and reviewer and iii) providing a review report for each dataset that assesses the presence, magnitude, and implications for qualifying usage regarding deficiencies for data providers and their reviewers.

The review process will be conducted in the given timeframe for the majority of the datasets. Challenges have been faced along the way, and among them: - Agreering on NDA with data providers took more time than expected, due to countries’ specific requirements; - Need for sufficient information: datasets have to be provided with detail of underlying unit processes, accompanied by the detailed description of what has been modelled and how; - The developed set of criteria for this exercise are not final review criteria for datasets: they are acknowledged as a starting point for further debates and improvements that should involve the broad LCA community.

- Another question of interest raised during this exercise is the confidentiality of the data. Conclusions This hands-on exercise provided some valuable insights into the road towards global consensus and guidance on data review. Beyond this time-constrained effort, a broader discussion and consensus at international level on the review of datasets and a continuous effort in reviewing datasets is desirable. References [1] 2011. Global Guidance Principles for Life Cycle Assessment databases Paris, France. UNEP: 160 p.

**109 Investigating the Occurrence and the Fate of UV filters in Swimming Pools**

T. Manssf, B. Coulomb, J. Boudenne, Aix Marseille Université

Chemical UV filters belong to emergent organic compounds that are turning out to be a sticking point for both environmental and health concerns. Today, these compounds are used in increasing quantities and can be found in various environmental media. However, little is known about the occurrence of UV filters in seawater swimming pools and about their reactivity in presence of disinfectants such as chlorine. Determining the concentrations of UV filters in swimming pools and their transformation products is paramount to any risk assessment especially concerning biodegradation of these pollutants before reaching the environment.

This exercise has been conducted in the given timeframe for the majority of the datasets. Challenges have been faced along the way, and among them: - Agreering on NDA with data providers took more time than expected, due to countries’ specific requirements; - Need for sufficient information: datasets have to be provided with detail of underlying unit processes, accompanied by the detailed description of what has been modelled and how; - The developed set of criteria for this exercise are not final review criteria for datasets: they are acknowledged as a starting point for further debates and improvements that should involve the broad LCA community.

- Another question of interest raised during this exercise is the confidentiality of the data. Conclusions This hands-on exercise provided some valuable insights into the road towards global consensus and guidance on data review. Beyond this time-constrained effort, a broader discussion and consensus at international level on the review of datasets and a continuous effort in reviewing datasets is desirable. References [1] 2011. Global Guidance Principles for Life Cycle Assessment databases Paris, France. UNEP: 160 p.
111 Comparison of UV/H2O2 and UV/S2O8 2- photolysis efficiency for the removal of estrogens in treated wastewater: chemical and biological assessment.

B. CEDAT, COMAP/INSA Lyon / LGCIE; h. metivier, C. de Brauer, INSA LYON

Conventional Wastewater Treatment Plants (WWTPs) are not designed to treat organic micropollutants and can only partially degrade them. Amongst hundreds of detected molecules, endocrine disruptors compounds (EDCs) are found at very low concentrations (in the ng/L range) but often sufficient to induce biological effects due to their high estrogenic potency. The natural estrogens, estrone (E1), 17β-estradiol (E2) and the synthetic one, 17α-ethinylestradiol (EE2) are the most active and commonly found estrogens in wastewater. In a near future, WTPs will need to remove a large number of estrogens due to their negative impact on human health and the environment. The study of estrogens degradation products have been done but still not exhaustive. Several studies investigated the formation/degradation of products were investigated using time series experiments. The results have identified efficient degradation pathways for wastewater treatment and highlight the need to focus on degradation products and their potential toxicological effects.

112 Advantages of wastewater treatment plant upgrading with additional activated carbon for ecosystem health

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Aquatic organisms have to cope with a variety of anthropogenic substances continuously released into the aquatic environment. In order to reduce surface water contamination, wastewater treatment plants (WWTPs) were developed during the last years. However, little is known about their efficiency with respect to ecosystem health. The BMBF project SchussenAktivplus examined the efficiency of different wastewater treatment technologies and the resulting advantages for the ecosystem. For this purpose, different chemical and biological analyses were conducted prior to and after the application of new technologies to several wastewater treatment plants and rainwater overflow basins connected to the Schussen River, a major tributary of Lake Constance. Another tributary, the Argen River, served as a reference. The present study is integrated into the follow-up project SchussenAktivplus+ and focuses on the long-term effects of the additional activated carbon filtered wastewater treatment plant Langwiese (Ravensburg, Germany) for the ecosystem of the Schussen River, with special focus on fish health. In order to assess endocrine and toxic effects in fish, different biological analyses were conducted prior to and after the upgrade. Thus, the induction of vitellogenin, the induction of micronuclei in blood cells, EROD activity, and fish development were examined. Overall, the results imply a possible positive impact of the additional activated charcoal stage for the ecosystem of the Schussen River.

113 Biological treatment of micro pollutants in drinking water resources

J. Wittenbol, M.H. Welelmans, Bioclear

Contamination of drinking water resources is becoming a threat that is particularly widespread. Micropollutants are emerging substances in surface and groundwater, causing contamination of drinking water resources and ultimately to closing down groundwater abstraction wells. BIOTREAT is a European project in which sustainable biotechnologies are developed for remediation of drinking water resources based on bioaugmentation, introduction of specific degrading microorganisms or microalgae into existing drinking water treatment plants (WWTPs) or mobile filters. The compound BAM (2,6 Dichlorobenzamide) was chosen as model compound. Based on results of laboratory tests, a total of 6 submerged sandfilter columns were operated, of which 2 non-inoculated controls and two columns that were exposed to 1 µg/L BAM and two to 30 µg/L BAM (all inoculated with MSH1) at a hydraulic retention time of 24h. A total operation time of the experiment was 240 hours. No back-flushing was applied. Results show that in the 1 µg/L BAM exposed columns, BAM degradation up to 72 % was established. In the 30 µg/L BAM exposed columns, 99% degradation was achieved. Well relocation is the cheapest scenario in case of pollution with micropollutants. But the well replacement scenario is only cheaper if the new well is placed within a few kilometers from the water production plant (3.5 kilometers for the 800,000 m³ water production per year). If clean water is more than a few kilometers away, implementing a BAM removal strategy becomes the best solution. For the large drinking water production volumes a GAC filter is the cheapest BAM removal technique. BIOTREAT bio-augmentation technology + carrier is the most attractive technology for small scale drinking water production plants. Compared to traditional physicochemical technologies, biological technologies have the advantage that: Natural resources are used. - No harmful chemicals are used, use of contaminated groundwater for drinking water production is made possible which otherwise might remain unused. - Application is low cost compared to usual traditional physicochemical technologies. - Technology is plug and play. No additional infrastructure needed at waterworks. - Compound specific removal is possible. Based on a production facility of 800,000 m³ per year the operating costs for these technologies, bioaugmentation and bioaugmentation + carriers, are € 0.20/m³ and € 0.11/m³ respectively.

114 Triclosan, triclocarban and parabens in greywater: identification of their sources.

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The use of soaps, shampoos or other personal care products (PCPs) generates discharges of chemicals into the environment through grey- and wastewater. Among these chemicals, parabens (PBs), triclosan (TCS) and triclocarban (TCC) are widely used in the composition of PCPs but also in sportive clothes, pharmaceuticals and food products as antiseptic or conservative.

In a previous study several kinds of greywater from shower, washbasin, manual dishwashing, dishwasher and washing machine have been analyzed. The key lessons highlighted by this work were that (i) greywater strongly contributes to the contamination of wastewater and (ii) at high flow rates, greywater must be treated separately. However, it is unclear if any toxic mechanism(s) are involved. Present study elucidates the internalization process of CNPs in algae. There is evidence of CNP-internalization by C. reinhardtii which again suggest that CNPs could be adsorbed by algae. Monodispersed nanoparticles were used to improve their stability, by inhibiting aggregation) with the aim of identifying their dissolution and shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs (Pt mass/cell) determined using a coulter counter and ICP-MS analysis. The degree of inhibition by PtNPs was observed to be higher than in the case of the standard PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or ultra-pure PtNPs, with inhibition of up to 80% at 0.05 mg Pt/L. This suggests that PtNPs inhibited growth by other means than shading.
115 Tracking nanomaterial targeting and uptake by SUSTU, protein corona surface proteomes.
S. Cristobal, Linköping University / Department of CI and Exp Medicine Cell Biology; A. Farinha, Linköping University / Clinical and Experimental Medicine Cell Biology; N. Bayat, Stockholm University / Biochemistry and Biophysics; J. Kuruvilla, Linköping University / Clinical and Experimental Medicine

The nanosafety of any newly developed nanomaterial should be studied through its evolution and aging upon interaction with environmental or biological samples rather than at the stage of newly synthesized entity. One of those key elements is the understanding of the nano-bio interface. The nature of the protein corona (PC) in contact with different biological environments has been extensively reported [1-3]. Nevertheless, less few studies addressed the evolution of the PC in terms of how the transition from an extra- to intra-cellular environment can modulate its biological identity and physicochemical properties [3, 4]. By understanding the key questions to be addressed from a new synthesized nanomaterial we can focus our analysis in the minimal set of data that would provide that information. Following this shortcut strategy we have developed, SUSTU, a surface proteomic method for tracking nanoparticle targeting, uptake and safety based on nanoparticle protein corona. The objective of this work are: i) develop methodologies that can define the specific enrichment of the PC surface, and ii) integrate MS-based PC data to develop nanointeraction tools for nanosafety. The method developed is highly reproducible between biological and experimental replicates. The key steps has been considered in the method development to be able to compare the data from the entire corona to the surface. By comparing the quantitative analysis of entire protein corona to the surface protein corona, we are able to define how the distribution of proteins in the PC is followed different pattern than in the entire corona. The protein concentrated in the surface are not equally distributed in the internal core of the corona. We could define a set of proteins that could be involved in targeting and other set that are not exposed to interaction with biological systems. We discussed the biological difference among the proteins composing the surface and the entire corona and the biological implication at the level of targeting and uptake capability and nanosafety at destiny. 116 Antibiotic toxicity of platinum nanoparticles - Implications of NP aggregation, dissolution and shading
S.N. Sorensen, DTU Environment / Department of Environmental Engineering; C. Engelskov, Technical University of Denmark / Department of Chemistry; H. Lützhøft, Technical University of Denmark / Department of Environmental Engineering; J. Jimenez-Lamana, University of Georgia / Section of Earth and Environmental Sciences Institute Forel; J. Noe, Environmental Pollution and Emissions; H. Naehr, Technical University of Denmark; F. AlZahraa Alatrakchi, Technical University of Denmark / Department of Micro and Nanotechnology; C. Giron Delgado, Technical University of Denmark; V. Slavikova, University of Georgia / Section of Earth and Environmental Sciences Institute Forel; A. Baun, DTU Environment / Department of Environmental Engineering

Platinum nanoparticles (PtNPs) are used in automotive catalytic converters and emitted to the environment during use. The resulting aquatic toxicity of PtNPs remains largely unknown. Algal growth rate inhibition has been reported, however, this endpoint is substantially influenced by PtNPs shading effects, and it is difficult to discriminate any other mechanisms(s) are involved. Present study addressed the mechanisms involved in the algal growth inhibition caused by PtNPs by comparing different toxicity endpoints in two algal species and media, including influence of NP shading, dissolution, aggregation, and adhesion to algal cells. Algae were exposed to PtCl₄ and 5.7 nm starch stabilized PtNPs in standard growth rate inhibition tests (ISO 6692:2004) with 48h incubation and a 2h C-accumulation test with the green algae P. subcapitata and C. reinhardtii. Shading effects were studied using a double-vidal setup, containing algal cells in inner vials, surrounded by PtNPs in larger outer vials. After 2, 24 and 48h, oxidative stress and cell membrane damage were determined by flow cytometry and body burdens of PtNPs (Pt mass/cell) determined using a couler counter and ICP-MS analysis of Algal captured filters. Characterization included size distribution (AlFIEFF, NTA, DSL), dissolution (ultracentrifugation), sedimentation (UV-VIS) and abiotic ROS generation (fluorescent dye, UV-VIS). PtNPs inhibited growth rates of the algae in the standard test and slightly less in the double-vidal shading setup, suggesting that shading occurs but that PtNPs inhibits growth by other means than shading. In the C-accumulation test, inhibition was similar using the standard or the double-vidal setups, thus inhibition was solely ascribed to shading, making this test inappropriate for testing PtNP-toxicity. If PtNP concentration-response curves were based on dissolved fractions, higher toxicity was found than accounted for by the ionic Pt, suggesting a NP-specific effect. P. subcapitata was more sensitive to C. reinhardtii, which correlates with a greater measured body burden of PtNPs in P. subcapitata, which again might be related to less PtNP aggregation in the ISO media. PtNPs caused extensive oxidative stress, but little membrane damage. P. subcapitata was less affected than C. reinhardtii, which is opposite to the growth rate inhibition tests. Thus, growth rate inhibition and oxidative stress do not appear linked in a straightforward manner. 117 Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae
Z. Wang, J. Zhao, H. Zheng, X. Luo, Ocean University of China; B. Xing, University of Massachusetts - Amherst / College of Natural Sciences

In this work, we systematically investigated the toxicity of graphene-family materials (GFM)s including graphene oxide (GO), reduced graphene oxide (rGO) and multi-layer graphene (MG) to alga (Chlorella pyrenoidosa). GFM s exhibited higher toxicity than other carbon materials (e.g., carbon nanotube and graphite), with the 96 h median effective concentration (EC₅₀) values of 37 (GO), 34 (rGO), and 62 (MG) µg/L. Heteroagglomeration between GFM s and algal cells contributed to algal growth inhibition. rGO and MG were easier to agglomerate with alga than GO. Cell membrane was damaged after GFM exposure as indicated by leakage of K⁺, Na⁺ and DNA and rGO caused the highest damage. Oxidative stress and physical penetration were responsible for the observed membrane breakage. For GO, shading effect contributed approximately 17% of growth inhibition due to its higher dispersibility while the other GFM s did not show any shading effect. All the three GFM s adsorbed surfactontrients (N, P, Mg, and Ca) from the algal medium, leading to nutrition depletion and indirect toxicity. For GO, rGO, and MG, the contribution of nutrition depletion to growth inhibition was 53%, 35%, and 27%, respectively. The information provided in this work will be useful for understanding the environmental risk of graphene materials in aquatic environments. 118 Internalization and toxicological mechanisms of uncocated and PVP-coated cerium oxide nanoparticles in the freshwater alga Chlamydomonas reinhardtii
G. Paludo-Reyes, Universidad Autónoma de Madrid; S.M. Briffa, University of Birmingham / School of Geography Earth and Environmental Sciences; F. Leganes-Nieto, Universidad Autónoma de Madrid / Biology; V. Puentes, Instituto Català de Recerca i Estudis Avançats (ICREA) / Experimental Science and Mathematics; E. Vaismi-Jones, University of Birmingham / School of Geography Earth and Environmental Sciences; R. Rosal, University of Alcalá; F. Fernandez-Puigas, Universidad Autonoma de Madrid / Biology

Cerium oxide nanoparticles (CNPs) nanospecies are increasingly used in industrial applications and may be released to the aquatic environment, where the exposure of aquatic organisms becomes likely. There are contradictory reports on whether nanoceria may act as an oxidant causing toxicity[1] or as an antioxidant being able to scavenge free radicals[2], given that the toxicological behaviour of these nanoparticles is still poorly understood. Moreover, little is known about the internalization process of CNPs in algae. There is evidence of CNP-internalization by Chlamydomonas reinhardtii (C. reinhardtii), but the internalization mechanism and route of uptake are still unknown[3]. In this study, we used an uncoated and different polyvinylpyrrolidone-coated CNPs (the purpose of the coating being to improve their stability, by inhibiting aggregation) with the aim of identifying their internalization and toxicological mechanisms. Monodispersed nanoparticles were synthesized and physicochemically characterized both in distilled water and the exposure media. Nanoparticles coated with PVP, irrespective of PVP molecular weight, had greater growth inhibition to C. reinhardtii than the uncoated CNPs. The CNPs coated with PVP were shown to induce oxidative stress and confer toxicity indicating that oxidative stress might be an important toxicity mechanism. Interestingly, there was evidence of membrane disruption upon nanoparticle exposure, suggesting that membrane permeability might increase; thus, allowing internalization of nanoparticle. At present, the mechanisms of CNP-internalization are under thorough study. 1. Paludo-Reyes G, Rodea-Palomares I, Das S, Sakhthivel TS, Leganes F, Rosal R et al. Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states. Scientific Reports. 2015:5:15613. doi:10.1038/srep15613. 2. Das M, Patil S, Bhargava N, Kang J-F, Riedel LM, Seal S et al. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. Biomaterials. 2007;28(10):1918-25. 3. Taylor NS, Merrifield R, Williams TD, Chipman JK, Lead JR, Viant MR. Molecular toxicity of cerium oxide nanoparticles to the freshwater alga Chlamydomonas reinhardtii is associated with supra-environmental exposure concentrations. Nanotoxicology. 2015:1-10. doi:10.3109/17453900.2014.1002868. Acknowledgement - This research was supported by CTM2013-45775-C2-1-R and CTM2013-45775-C2-2-R grants from MINECO.

119 Silver nanoparticles vs. dissolved silver toxicity to Daphnia magna: Unique modes of action?
K.B. Paul, Blue Frog Scientific Limited / SLS; D. Brown, Heriot-Watt University; D. Carson, Blue Frog Scientific Limited; T.L. Hargreaves, Blue Frog Scientific Ltd / Environmental Science Ecotoxicology; V. Stone, Heriot-Watt University / School of Life Science; T.F. Fernandez, Heriot-Watt University / School of Life Sciences

Disturbances in genes or biochemical regulation of an organism can often be seen at lower doses or on shorter time scales than whole body effects, and are a
investigate the interaction of surface functionalised Ag-NPs with the
subcapitata
TiO(2)-nanoparticles in the environment: The quest for advanced analytics and
From clothing to laundry water: Investigating the fate of flame retardants
interactions) are currently critically limited and are in high demand [1]. This is a
'nanoscale'. Importantly, it is now recognised that due to their small size and high
materials are manufactured at the extremely small size that is referred to as
from cosmetics, medical diagnostic imaging, and forensics, to energy and
Grovenor, The University of Oxford / Department of Materials; E. Lombi,
University of South Australia / Centre for Environmental Risk Assessment and
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in agreement with those previously reported for RDP and TPHP and for other FRs in samples collected on/around electronics. The high levels of RDP, TPHP, and meta-HO-TPHP (reaching the μg/g levels) that were found in some of the dust collected on electronics suggest that dust could be a significant route of human exposure to these compounds via ingestion/dermal absorption. This could happen especially when different electronics are used on TV or computer keyboards. Giving these first results, more data on the presence and potential toxicity of meta-HO-TPHP (and of other RDP impurities) is necessary in future monitoring studies to assess the human exposure and risks of RDP related compounds. Besides, due to its specificity and ubiquity as RDP impurity, meta-HO-TPHP could be a suitable marker/tracer of RDP.

124 Toxicological mechanisms of current flame retardants
B. Kriwokeny, F. Dardenne, University Antwerp; R. Blust, University of Antwerp; Department of Biological and Ecotoxicological Department of Biology; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center/Department of Pharmaceutical Sciences; S. Husson, Systematic Physiological and Ecotoxicological Research Group - University of Antwerp
Flame retardants (FRs) are chemicals produced at high volume that are introduced to nearly all manufactured materials with the purpose to limit fire hazard. Given their persistence and potential to bioaccumulate, the compounds have been found in serum and breast milk. Toxicological data is largely limited to monitoring classical endpoints such as measuring lethal dose values. Therefore there is little insight into their molecular modes of action that may give rise to their toxic phenotypes. Additionally, some previously-used FRs have been shown to elicit a wide range of toxicological effects and have thus been banned from use. Given the structural similarity to their toxic predecessors, their persistence, bioaccumulation, and lack of insight of toxicological and molecular mechanisms, currently-used FRs pose a significant risk. We therefore used a bacterial gene profiling assay to screen 12 currently-used FRs as to obtain mechanistic insights of toxicity. Bacterial bioassays were previously used to detect potential toxicity of compounds since they are particularly useful in compound screening and classification according to mode of action. The assay consists of 12 bacterial reporters (Escherichia coli) responsive to oxidative stress, protein degradation, DNA damage, membrane damage, and growth arrest. Both brominated and organophosphate FRs were tested. Nearly all compounds showed significant inductions in a majority of stress genes when compared to control treatments. When observing only raw absolute induction levels, the stress genes ClpB, RecA, and MicF were the only genes exhibiting fold inductions of greater than 2, indicating that these compounds result primarily in protein, DNA, and membrane degradation. However, accounting for the different induction potentials for each stress promoter and clustering using hierarchical and k-means algorithms, clusters corresponding to growth arrest and oxidative damage were also observed. This indicates that FRs may result in a loss of protein, DNA, and membrane integrity with generation of reactive oxidative species as the potential underlying mechanism, in agreement with other studies. Finally, the lack of any notable gene induction following DOP treatment even at extremely high concentrations, along with its excellent fire-retardation supports its increasing interest as an alternative to halogenated FRs.

125 Species differences in steroid hormone receptor responses
P. Olsson, S. Asnake, C. Modig, Orebro University / The Life Science Center Endocrine disrupting chemicals present in the environment can have differences in efficacy and potency when interacting with steroid hormone receptors from different species. This is due to both species variations in sequence and endogenous hormone utilization. There are a number of brominated flame retardants (BFRs) for which there are no information on their interactions with biological systems. We have been characterising BFRs using a combination of in silico molecular modelling, in vitro assays and in vivo studies. In an in silico analysis of 10 model BFRs we identified one of the test compounds, 2,4,6-tribromophenyl ether (TBECH) as a partial androgen receptor (AR) agonist. TBECH is an interesting compound as it has 4 different diasteromers (α, β, γ, δ) each with its own enantiomer. In a follow up study we compared the efficacy and potency of the TBECH diasteromers and discovered that TBECH-γδ exhibited the same efficacy and potency as the endogenous androgen DHT. However, 40% of these samples, e.g. AR knocked-out on TV or a null activity. Continued screening using in silico methodology has resulted in the discovery of yet another group of BFRs that interact with the human AR. These are 2,4,6-trimethylphenyl ether (ATE) and 2,3-dibromopropyl 2,4,6-trimethylphenyl ether (DPTE) and the metabolite 2-bromooctyl 2,4,6-trimethylphenyl ether (BATE) that we recently showed to be potent AR antagonists. As different species use different androgens we then focused on determining the ability of these novel BFRs to interact and regulate the activity of AR in different species as well as to determine if they interact with other steroid hormone receptors in a species-specific manner. TBECH was found to activate both the zebrafish AR and the chicken. However, the activation potency was lower than that observed for the human AR. TBECH-γδ showed a 40% increase in chicken and only 1.5% in chicken. TBECH-γδ, that showed about 30% induction in human AR showed minimal induction of zebrafish and chicken AR at the maximum TBECH concentration. ATE, BATE and DPTE were equally potent at inhibiting AR signalling in human, zebrafish and chicken. Following the identification of these BFRs as AR agonists/antagonists we also used in silico modelling and analysis of transcriptional regulation and observed that these compounds also regulate other steroid and hormone receptors, e.g. the oestrogen receptor and thyroid receptor signalling pathways.

126 Determination of the eco-toxicological risk of eluates of reactive flame retardants
Flame retardants are increasingly used in consumer products as e.g. electronics and furniture as well as in construction. Along with the increased usage, concerns about negative effects of flame retardants on human health and the environment have been raised. Concerning construction products one application for flame retardants is the furnishing of steel structures with reactive flameproof coatings. These coatings consist of a complex mixture of different chemicals, additives and solvents. For the evaluation of the risk possibly posed by their application to the environment, this whole mixture should be tested. Two different leaching tests were applied according to DIN/TS 16637-2 (sample permanently submerged in water) and DIN EN 16105 (immersion and drying cycles), respectively, which can be used for plate-like products. Eluates were divided into subsamples for the measurement of different, e.g. toxicity, persistence, biodegradability, bioaccumulation, and ecotoxicity. GC-MS chromatograms were recorded in the SCAN mode and mass spectra of the peaks were compared with spectra stored in the NIST library. Eluates of leaching test according to DIN CEN TS 16637-2 showed low electrical conductivity (20 to 150 μS/cm) and low release of cation and anions often near the LOQ. Differences could be observed for plates treated with the reactive coating at both sides and plates coated only at one side, while the other side is only furnished by the corrosive protection layer. Release of TOC and Zn was approx twice or 10 fold higher in the case of plates coated on one side. Furthermore, the comparison of the results for both types of plates showed that a large portion of the released compounds originate rather from the corrosion protection layer than from the reactive coating itself. Leaching tests according to DIN EN 16105 have not been finished yet, but first results show that the conductivity is much lower (1-4 pS/cm) than for the tests according to DIN/TS 16637-2 leading to the assumption that the release of compounds is lower. By comparison of the mass spectra with library data, mainly organic solvents as xylene, 1-methoxy-2-propanol acetate and n-butyl carbatate have been tentatively identified so far in the first test fraction done by DIN CEN TS 16637-2.

Microplastics in the environment: Sources, Fate and Effects (III)

127 Interactions of non-polar organic compounds with polyethylene and polystyrene micro particles
T. Hüffer, T. Hofmann, University of Vienna / Department of Environmental Geosciences
Concerns about the presence of micro scale polymers (i.e., microplastics) in the environment system are rapidly growing. Understanding the interactions between microplastics and other contaminants is thus essential for evaluating the materials’ fate. Additionally, sorption of organic compounds by microplastics is one of the major processes as it may affect the compound distribution in sediments and aqueous phases. However, there is currently little data on the sorption potential of diverse sets of hydrophobic organic compounds/microplastics systems that may occur in the environment. We applied a multi-phase sorption batch method to investigate the sorption of seven organic compounds by two most commonly found microplastics. Experimental sorption isotherm data by polyethylene (PE) and polystyrene (PS) measured over several orders of magnitude were fit to the non-linear Freundlich model to support mechanistic interpretations of the results. Correlation between sorption coefficients by microplastics and sorbates hydrophobicity indicates that hydrophobic interactions are of major importance. Linear isotherms by PE shows that uptake was driven by adsorption into the bulk polymer, while adsorption onto the surface seems to be the dominant sorption mode indicated by non-linear isotherms data for PS. However, the results emphasize that neither sorbate nor sorbent-specific parameters sufficiently explain the observed difference in sorption alone.

128 Chemical Adsorption of Hydrophobic Polycyclic Aromatic Hydrocarbons in the Marine Environment onto Microplastic Polymers and Subsequent SETAC Europe 26th Annual Meeting Abstract Book

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31
Desorption in a Simulated Gut

C.B. Crawford, University of the West of Scotland / Institute of Biomedical and Environmental Health Research; M. Russell, Marine Scotland Science / Marine Environmental Assessment; C. Ewins, B. Quinn, University of the West of Scotland / Institute of Biomedical and Environmental Health Research

With an apparent debasement. Most of the case of 450 years, plastic is one of the most durable and persistent environmental contaminants in the modern world. Production since the 1950s has increased prodigiously. In the marine environment, photo-oxygenative degradation and the abrasive action of waves progressively degrade larger pieces of plastic into tiny polymer particles less than 5 millimetres in diameter, termed microplastics. As a consequence, microplastics are inadvertently ingested by ocean dwelling biota, particularly susceptible filter feeders such as Mytilus edulis and many species of fish. This can result in detrimental effects such as inhibition of gastrointestinal function and feeding impairment. However, it has emerged fairly recently that microplastic polymers collected from the marine environment have been found to have Hydropathic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), adsorbed on their surface. Since marine organisms ingest microplastics, it can be proposed that microplastics have the potential to act as a transporter for the conveyance of hydrophobic persistent organic pollutants (POPs), such as PAHs, into marine biota. PAHs are formed from incomplete or inefficient combustion of organic material, diagenesis and biosynthesis. While there is a consistent background level from forest fires and volcanic activity, a significant fraction of PAHs present in the environment is due to anthropogenic activities, such as internal combustion engines. Consequently, PAHs reach the marine environment via sewage and industrial discharges, oil spillages and derelict ships. One particular characteristic of PAHs is their susceptibility to ultra-violet light. However, pericondensed PAH structures are more centrally condensed allowing them to withstand higher ultraviolet fluxes. This results in a decrease in susceptibility to photodecomposition by ultraviolet light and thus, resistance to degradation in the marine environment. Consequently, microplastics can then adsorb these contaminants. Since many of the susceptible organisms that ingest microplastics are located at the bottom of the food chain, chemical adsorption provides a potential mechanism for the bioaccumulation of contaminants in marine organisms and the potential trophic transfer of these contaminants up the food web to larger organisms, and possibly humans. Consequently, it is necessary to investigate this potential mechanism. 'n

Evaluation of the analysis of microplastics by mass spectrometry and assessment of their adsorption capacity for organic contaminants

m. Iloca, Institute of Environmental Assessment and Water Research; G.F. Schirrini; J. Sanchis, IDAEA-CSIC / Water and Soil Quality Research Group; D. Barcelo, IQAB-CSIC / Dept Environmental Chemistry; M. Farre, IDAEA-CSIC Plastic residues in the sediments have been identified as one the most important sources of human activity. The presence of these marine debris particles in the ocean represents not only an aesthetic problem since they also can imply harmful damages for the coastal areas and the socioeconomic activities linked to these regions[1,2]. The low degradability of these plastics and their size hamper the oxygen exchange and light which lead to eutrophisation processes[2]. Among these marine debris, microplastics are risky for primary producers and their impact has not been fully evaluated [1]. Nonetheless, different studies revealed their ubiquitous presence in the sea as well as its uptake by marine biota[3]. MPs can enter into marine food web and, finally, into human diet. In addition, these MPs can accumulate persistent organic contaminants and pathogens being also an environmental distributor of these ones [1,4,5]. The main objectives of this work are the evaluation of different ionization sources coupled to mass spectrometry analyzers for the analysis of MPs (High Density Polyethylene, polystyrene and functionalized polystyrene-COOH) in water columns and; the evaluation of adsorption mechanisms of organic compounds (i.e. polycyclic aromatic hydrocarbons (PAHs)) and emerging organic contaminants (i.e. perfluoroalkyl substances (PFAs)) onto MPs surface. The study of MPs by means of MALDI-TOF and APCI-QExactive allowed the chemical characterization as well as semi-quantitative analysis in the case of the last one. These technologies can be applied in the future for the analysis of environmental matrices such as water column or sediments or biological samples. The study of microplastics under controlled sea water conditions, the results evidenced that MPs can behave as carriers of the studied PAHs and PFASs in marine environments. The compounds are adsorbed onto MPs surface after short exposure time and these can act as carrier materials being an important contamination source of selected organic contaminants for marine flora and fauna. Key words: microplastics, mass spectrometry, carrier materials of organic contaminants [1] Cole M. et al. Marine Pollution Bulletin 62 (2011) 2588–2597. [2] Harrison J.P. et al. Marine Technology Society Journal 45 (2011) 12-20. [3] Cole M. et al. Environmental Science & Technology 47 (2013) 6646-55. [4] Iloca M. et al. Marine Pollution Bulletin 87 (2014) 286–291. [5] Riosa L.M. et al. Marine Pollution Bulletin 54 (2007) 1230–1237.

Dioxin-like activity of compounds sorbed to 4 different plastic polymers deployed in the San Diego Bay area

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In the early 1970s plastic particles were detected in the marine system for the first time and by the late 1990s over 180 species were reported to ingest plastic particles. The occurrence of plastic litter in the marine environment poses not only a physical threat to organisms via entanglement and ingestion, but also a toxicological threat to wildlife. In recent years it has been demonstrated that various anthropogenic environmental pollutants can be found on different types of marine plastic particles and that these are considered potential pollutants to humans and wildlife. It can act as mutagens, carcinogens and endocrine disruptors and exert adverse health effects to organisms. The toxicological potential of anthropogenic pollutants derived from plastic material is a yet little studied research area. In this study virgin plastic pellets of four different polymer types, polypropylene (PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), and low-density polyethylene (LDPE) were deployed for nine and twelve months in different sites of the San Diego Bay area. The plastic pellets were extracted using hexane and ultra-sonication. Extracts were analyzed for their dioxin-like activity in the H4IE luc assay, a mechanism specific bioassay based on the recombinant receptor/reporter vertebrate cell line, and chemical analysis of 23 PAHs including the 16 priority PAHs listed by the U.S. EPA. Biso-TEQ2000 and Chem-TEQ2000 values were calculated. Among the different polymer types LDPE demonstrated the highest dioxin-like activity in the samples followed by PP, PVC and PET. The calculated Chem-TEQ2000 values supported a higher toxicity potential for LDPE compared to the other polymers. The time dependency of sorption were hampered by too few sampling time points but a slightly higher PAH concentration after twelve months compared to nine months could be observed indicating continuing sorption capacity. In contrary, the biological activity decreased from nine to twelve months. This leads to the assumption that other compounds than the analyzed PAHs might show a desorption from the plastic between 9 and 12 months which results in a decrease in PAHs concentration. This study shows that virgin polymer pellets are able to sorb pollutants from ambient water that induces a dioxin-like effect and that LDPE has a higher sorption capacity for PAHs and other dioxin-like compounds that were not measured in this study compared to the other polymers.

Characterisation, Quantity, and Sortptive Properties of Microplastics Extracted From Cosmetics

L. Napper, A. Bakir, S.J. Reidwood, Plymouth University; R.C. Thompson, Plymouth University / School of Marine Science and Engineering

Cosmetic products, such as facial scrubs, have been identified as potentially important primary sources of microplastics to the marine environment. To better understand the source, we characterised, quantified and then investigated the sorptive properties of plastic microbeads that are used as exfoliants in cosmetics. Polyethylene microbeads were extracted from several products, and shown to have a wide size range (mean diameters between 164 to 327 μm). We estimated that between 4594 – 94500 microbeads could be released in a single use. To examine the potential for microbeads to accumulate and transport chemicals they were exposed to a binary mixture of 1H-phenanthrene and 13C-DDT in seawater. The potential for transport of sorbed chemicals by microbeads was broadly similar to that of polystyrene (PE) particles used in previous sorption studies. In conclusion, cosmetic exfoliants are a potentially important, yet preventable source of microplastic contamination in the marine environment.

Cost effective and ecological relevant approaches in environmental toxicology using invertebrate species

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Cost effective and ecological relevant approaches in environmental toxicology using invertebrate species

1. Is Multixenobiotic Resistance System in Daphnia magna a general stress response mechanism?

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Recent investigations show that the cellular multixenobiotic resistance (MXR) system represents a broad-scale defense mechanism protecting cells against environmental toxicants. The system is mediated by membrane transporter proteins which pump chemicals out of the cell, thus keeping their levels low. Organisms and cells with potent MXR defense are less sensitive to the toxic impact of compounds, because comparatively few of those molecules reach their site of toxic action. In a recent study this system was characterized in the crustacean Daphnia magna, sequencing four gene transporters that were constitutively expressed across life stages and induced by several chemical contaminants. Transporter activity of these proteins was also characterized showing that inhibition of MXR-like efflux activity by MXR-inhibitors co-administered with toxic substrates of MXR enhanced the toxicity of the latter.
compounds. However, model ABC transporter substrates, inducers and inhibitors used did not allow distinguishing between ABCB and ABCD transporters activity types. Here, we present results of experiments designed to clarify the role of these transporters and its specificity. We selected tolerant clones by exposing outbred populations to high concentrations of chemicals that are known to be substrates of ABC transporters. Analyzed tolerant clones were then used to characterize MXR mechanisms. The response of heat shock proteins (Hsp70) was also studied to distinguish between substrate specific responses from general stress. An ABCB knock-down gene clonal line was also developed taking advantage of the recent development of the CRISPR/Cas9 methodology in Daphnia. Results showed successful selection of tolerant clones relative to the lab clone by mitoxantrone, pentachlorophenol and ivemectin. Mitoxantrone and pentachlorophenol enhanced the transporter activity in their respective selected tolerant clones. However, high transcriptional levels of the abcb1 gene were only positively related with high transporter activity in the selected pentachlorophenol tolerant clone. All tolerant clones showed decreased levels of abcb1 protein, which means that the selection of clones with enhanced levels of MXR may be part of a general stress response. In conclusion tolerant genotypes having higher levels of general stress and MXR proteins exist in natural populations of D. magna and can be selected by toxic compounds that are substrates of ABC transporters.

133 Functional characterization of hemocyte populations of zebra mussel, Dreissena polymorpha and use in acute ex vivo toxicity tests

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The zebra mussel, Dreissena polymorpha, is an invasive freshwater bivalve mollusk native from Ponton-Caspian region that successfully colonized Western Europe and North-America. Because of its biological characteristics, transplanted or natural populations of zebra mussels were used as sentinel species in freshwater pollution monitoring and also in laboratory studies. Immune system constitutes a central point in organism homeostasis. This physiological process relies on hemocyte activities that are sensitive to chemical stress. Disturbance in immune response may affect mussels in their related populations. The first objective of this study was to characterize D. polymorpha hemocyte subpopulations under natural conditions. According to microscopy and flow cytometry analysis, three types of circulating hemocytes can be observed: haemocytes and blast-like cells for agranular hemocytes and one granulocyte population. Flow cytometry analysis of hemocytes functionalities indicated that blast-like cells possessed low activities and that haemocytes and granulocytes are fully equipped to perform innate immune response and exhibited close functionalities. The second objective of this study was to assess the cytotoxic effects of cadmium, a non-essential metal and persistent pollutant, on hemocyte subpopulations by investigating a multi-marker approach. Hemocytes were exposed ex vivo to concentrations of cadmium ranging from 10^{-7} to 10^{-5} M for 21 hours prior flow cytometric analysis of cellular markers. Measured parameters (viability, phagocytosis, oxidative activity, lysosomal content and mitochondrial activity) were affected in a dose-dependent manner. A different influence of cadmium was observed on cell-endpoint and cell-subtype. This work highlighted importance of hemocyte characterization for a better understanding of toxicological responses of invertebrates to xenobiotics.

134 Highly cost-efficient determination of efflux pump inhibition and chemosensitization using single embryos of the pond snail Lymnaea stagnalis

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Measurements of efflux pump activity in single embryos of Lymnaea stagnalis was shown to be a highly cost-efficient alternative method compared to cell cultures. Results for the inhibitory effects of a range of pharmaceuticals showed the same effects in the same concentration ranges as previously reported in cell culture experiments. Further, the system was shown to be able to distinguish different modes of inhibition as well as being able to conveniently show chemosensitizing effects of pharmaceuticals.

135 Time-course immunomarker assessments and life-history traits in the freshwater snail Lymnaea stagnalis in response to environmental contaminants

P. Drouaux, Irstea Lyon / MAEP Laboratory of Ecotoxicology; P. Noury, Irstea Lyon; H. Thomas, Université de La Rochelle / UMR LIENS laboratory; J. Garcia, Irstea Lyon / Ecotoxicological Laboratory

Animals are under constant multirstress pressures (e.g. chemicals and microbes). Hence, their immune system may be affected by the presence of chemicals (i.e. immunotoxicity). A variety of external defences are important to remove the microbiological burdens with possible consequences at the population level. In ecotoxicology, development and comprehension of biological tools that evaluate the effects of chemicals onto the immune system of invertebrate species are required (Galloway and Depledge 2001). Lymnaea stagnalis is a freshwater gastropod ecologically representative of lentic systems from holartic zones of the planet. This species has been promoted in OECD guidelines for reprotoxicity testing (Ducrot et al. 2001). The aim of the study was to extrac a sufficient quantity of hemolymph that allows an individual approach (i.e. not pooled) of immunomarkers assessment in an non invasive manner, which also means that a time-course evaluation can be realized without animal sacrifice. Plus, hemolymph can be collected easily only by using a micropipette. Gust et al. (2000) reported immunomarked contaminants in L. stagnalis. More investigation remains essential in order to understand the feasibility of using markers of immunotoxicity from L. stagnalis. Herein we investigated the time-course development of individual snails hemocyte biomarkers (i.e. density, viability, oxidative activity, phenol-oxidase activity and activity of phagocytosis) under co-administration of MXR. Simultaneously, an evaluation of life-history traits was carried out (i.e. survival, reproduction and feeding behavior). The pyrethroid Deltamethrin was used in a flow-through system. The Cadmium chloride (CiclC) was set up in semi-static conditions. Hemocyte biomarkers were evaluated by flow cytometry (densities, viabilities and activity of phagocytosis) and by spectrophotometry (phenol-oxidase and NADPH-oxidative activities). Reproduction was followed by collection of egg-masses and observation under binocular microscope. We observed that phenomena of immunotoxicity may be transient (i.e. biological recovery) and so the time-course tracking of individual parameters is important. The comprehension of hemocyte biomarker variability is mandatory : inter-populations, interpopulation (i.e. intra-individual) and intra-individual (i.e. time effect, physiological fluctuations). Thus, L. stagnalis deserves more attention as model organism in immunotoxicology.

136 Nickel toxicity to tropical marine biota

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Over two-thirds of the world’s nickel production is in tropical regions including Indonesia, the Philippines, Australia, New Caledonia, Brazil and Cuba. The mining, refining and smelting of lateritic ores can present many potential environmental risks, as with any such operations located in tropical regions of high ecological diversity. However, our current understanding of the toxicity of nickel to tropical biota is limited, largely due to the lack of ecotoxicity data with tropical species endemic to these regions. The Nickel Producers Environmental Research Association is currently leading a research program to develop and apply risk assessment tools to support the sustainable development of lateritic nickel deposits in the tropical region of South East Asia and Melanesia (SEAM). This research involves the compilation of exposure and effects assessment data for nickel in tropical marine waters, specifically in the SEAM region. Key gaps for ecologically important tropical taxa of the SEAM region include corals, crustaceans, gastropods, bivalves and fish. The aim of this study was to investigate the sensitivity of tropical marine biota to nickel, with a focus on those species relevant to the SEAM region. Data produced from this study will contribute to the development of a reliable and ecologically relevant water quality guideline value for nickel in tropical marine waters. Chronic nickel toxicity to two crustaceans (copepod and barnacle) and one gastropod (snail) native to the SEAM region was investigated. Toxicity tests were carried out at temperatures of 28–30 °C and a salinity of 35%. Endpoints measured included 72-h development (from egg to copepods), 96-h metamorphosis and 96-h growth for the copepod, barnacle and snail, respectively. Throughout all tests, water quality parameters were monitored and sub-samples were taken to measure total and dissolved (<0.45 µm) nickel. The copepod was the most sensitive to nickel, followed by the barnacle and snail, with IC10 (10% inhibition concentration) values of 15, 72 and 79 µg Ni/L. This study provides valuable toxicity data which can be used in the development of an ecologically relevant guideline value for nickel in tropical marine waters.

137 Effects of multiple stressors on freshwater macroinvertebrates in Iberian rivers


Aquatic ecosystems worldwide are impacted by a variety of stressors, including organic and inorganic pollution, excess input of nutrients, geomorphological alterations, land use changes, hydrological stress, invasive species and pathogens.
As a consequence, the biodiversity decline is one of the greatest ecological problems nowadays [2]. However, little is known beyond the described effects of single stressors on specific ecological endpoints [3] and our understanding of the main causes for the losses of biodiversity still remains vague. Besides the usual taxonomy-based approaches, trait-based approaches are being increasingly used as an alternative to existing ecological and biological toxicity models to discriminate the influence of individual anthropogenic stressors and natural influences. Traits may help to reveal the cause of impairment and give an indirect insight into which ecosystem functions may be affected by human disturbance [4]. The objectives of this study were following: a) to assess the risk/presence of multiple stressors in four Iberian rivers and b) to test if we can observe the adverse effects of stressors in the local benthic macroinvertebrate communities c) to determine the difference of taxonomical and functional (traits) diversity changes in relation to stressors present and finally d) to discriminate which stressor or stressors are influencing more than others on the macroinvertebrates. The presence of one or more potential stressors was evident during the sampling year. The highest risk was mainly presented at the lower parts of the rivers in the area surrounded by agricultural lands and urban zones where high concentrations of toxicants, in particularly insecticides, were found. Both organic micropollutants (particularly pesticides) and heavy metals significantly contribute to acute ecotoxicological risk. Besides, most of the sampling sites were characterized by combinations of two or more stressors, such as organic pollution, metals, high nutrient levels and conductivity.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (III)

A biophysicochemical approach for assessing the dynamics of metal biotakeup and toxicity E. Rotureau, CNRS UMR 7369 Laboratoire interdisciplinaire des environnements continentaux, LIEC CNRS UMR, P. Billiard, R. Présent, LIEC Université de Lorraine CNRS; N. Paquet, M. Lavoie, C. Fortin, INRS Centre Eau Terre Environnement; J.F. Duval, CNRS / Laboratoire interdisciplinaire des environnements continentaux LIEC CNRS UMR Understanding the toxic and essential trace compounds uptake by microorganisms under conditions relevant to natural environment is a major trigger of concern in environmental risk assessments. While toxicity assays are well documented in numerous studies where exposed ambient concentrations of metals are related to toxicity endpoints such as mortality or growth rate, predicting metal toxicity with clear account of the dynamic interplay between cell growth and metal biointerfacial partitioning is still very scarce. We recently proposed an integrative theory where metal transport, adsorption, exclusion, internalisation and depletion processes are rigorously accounted for. In addition, the effects of key cell biophysicochemical features (e.g. electrostatics or presence/absence of cell surface layer such as EPS) on metal uptake and toxicity were highlighted. In this presentation, the theory is briefly presented together with supporting experimental data collected on bacteria and algae suspensions exposed to Cd(II) solutions. In both situations, it is shown how a critical examination of these data with help of theory can be valuable in deciphering the mechanisms governing the partitioning of metal at biointerfaces over time and accompanied toxicity effects. The theoretical formalism detailed in this presentation clearly highlights the intimate relationships amongst metal partitioning dynamics at the cell–solution interphase, the biophysicochemical properties of that biointerphase, and the cell growth inhibition kinetics.

Toxicokinetic-toxicodynamic model to explain the decreased bioaccumulation but increased toxicity of Cu in an estuarine clam under higher salinities W. Chen, Xiamen University / College of the Environment Ecology; Q. Tan, Xiamen University - College of the Environment & Ecology / College of the Environment Ecology Although it is well recognized that salinity affects metal toxicity to estuarine organisms, it is difficult to quantitatively simulate and predict the effects of salinity is still a challenge. Salinity affects metal toxicity not only through water chemistry, but also by altering the physiology of organisms. The biotic ligand model (BLM), which can only consider the effects of water chemistry, is probably not applicable to estuarine waters. Indeed, in this study, we found that higher salinities led to lower Cu bioaccumulation in the euryhaline clam Potamocorbula amurensis; however, the associated toxicity was not lower but higher. The toxicokinetics of Cu were determined by using Cu-65 as a tracer at 7 salinities, i.e., 5, 8, 10, 15, 20, 25 and 30 psu. Cu toxicity tests were also conducted at these 7 salinities. A two-compartment toxicokinetic-toxicodynamic model was then developed to explain the divected effects of salinity on Cu bioaccumulation and toxicity. With the increase of salinity, the uptake rate constant of 65Cu decreased significantly from 0.546 L/hg to 0.213 L/g/h. The inhibitive effects of salinity on Cu bioaccumulation was consistent with the prediction of the BLM. Elevating salinity from 5 psu to 30 psu significantly raised the killing rate of Cu from 0.60 mg/g/h to 2.08 mg/g/h, while did not significantly affect the internal toxic threshold of Cu. This indicated that the excessively accumulated Cu manifested higher toxic effects to the clams at higher salinities, probably due to their higher osmoregulatory stress. Using the toxicokinetic-toxicodynamic model, we can quantitatively predict the effects of salinity on both Cu bioaccumulation and toxicity. This model can serve as a useful tool for assessing ecological risks of Cu in estuary waters. Key words: salinity, copper, toxicokinetic-toxicodynamic model.

Behavior and toxicity of silver nanoparticles and silver nitrate in aquatic indoor microcosms C. Ge, P. Schäfers, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Environmental Monitoring In this study effects of AgNPs and AgNO3 are investigated to assess toxic effects of AgNPs and Ag+ . Therefore the release of Ag+ from AgNPs and AgNO3 and the bioavailability of Ag+ in the water phase is researched in separated microcosms with a volume of 1 m3. The microcosms contain natural sediment (0.25 m3), surface water (0.75 m3), zooplankton and phytoplankton. AgNPs with a mean diameter of 20 nm and AgNO3 were applied into aquatic indoor microcosms at four different target concentrations, respectively. In addition four control microcosms were used. During a period of 100 days target concentrations were maintained by regular test item additions. The recovery was followed over additional 150 days. To allow observation of toxic effects, data of cell density were collected. The aim of this near-to-nature approach is to give an as precise as possible picture of nanoparticle behavior and toxicity on aquatic communities and to enable a direct comparison to effects caused by AgNO3. 78 days after start of the exposure, measurable concentration of Ag+ was already reached at the highest AgNP exposure. The application of lower AgNP concentrations (0.078, 0.313 and 1.25 µg/L) did not lead to changes of the amount of cells in the water phase. This may indicate that the zooplankton is not directly affected by exposure to AgNPs. Nevertheless, after stopping the test item additions similar amounts of cells could be found in the water phase of all treated microcosms and controls. Apparently similar communities were present in all microcosms and the recovery of the communities occurred in spite of the presence of remaining silver in the system.

Arsenic induces changes in gut microbiota associated to mucus in vitro models M. Calatayud, Laboratory of Microbial Ecology and Technology; W. Ossieur, Ghent University / Center for Microbial Ecology and Technology; T. Van de Wiele, Ghent University / Laboratory Microbial Ecology and Technology Arsenic (As) is an environmental contaminant widely distributed through the Earth crust. The main route of exposure to inorganic arsenic, the most toxic arsenical specie in contaminated water and food, is via oral. Arsenic absorption occurs mainly in the small intestine, however non-absorbed arsenic can reach distal gastrointestinal regions. Human trials showed that 44-60% of arsenic ingested after rice consumption was quantified throughout in five days of excretion, while the remaining amount of As could reach colon or be retained in the human body [1]. Therefore the colonic absorption of arsenic can contribute to As internal exposure and, moreover, arsenic could interact with distal gut microbial community. Gut microbiome, therefore, should be considered as an environmental factor affected and, also, influencing arsenic toxicity. This research evaluated in vitro the effect of environmentally relevant doses in mucus associated gut microbiome using batch assays and the simulator of the human intestinal microbial ecosystem (SHIME, including the mucus environment (mucus-SHIME, M-SHIME). Faecal samples from healthy individuals were inoculated in batch assays and M-SHIME reactor and exposed to arsenic (10 and 100 µg/L). After different time points, the metabolic activity of the gut microbiota was assessed by quantifying short chain fatty acids, branched short chain fatty acids and ammonium contents. Structural changes on mucosal microbial community was performed by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) of the 16S ribosomal RNA (rRNA) gene and quantification of 16S rRNA gene copy numbers by real-time PCR (qPCR). The results obtained show that arsenic exposure affected significantly the branched short chain fatty production in fed batch and M-SHIME experiments, indicating changes in the metabolic activity of gut microbiome related with the protein metabolism. The DGGE fingerprint of total bacteria in alginate mucus beads was significantly affected after arsenic exposure after processing gel bands with Bioinformatics software. The results presented in this work suggest that the gut microbiome is an environmental factor to consider in risk assessment of arsenic exposure. The health consequences of arsenic interaction with gut microbiota need further research. The mucus associated bacteria were suggested as a key element in the arsenic – host interplay.
Copper-induced changes in intracellular thiols in marine algae
D.F. Jolley, C. Smith, University of Wollongong / School of Chemistry; J. Stauber, CSIRO / CSIRO Land and Water
Toxicity of metals to aquatic organisms is dependent on both external factors, such as exposure concentration and water quality parameters, and intracellular pathways significantly varied metabolites were mainly responsible for osmotic models used to predict copper (Cu) toxicity in microalgae do not effectively consider the intracellular processes. This research examined the toxicity of Cu towards microalgae by investigating the intracellular Cu-binding ligands of phytochelatins and glutathione from four species of marine microalgae exposed to Cu. Their respective IC50 (concentration required to inhibit growth by 50%) at intervals over 72 h. IC50 values were chosen to represent equal amounts of cellular stress across the four species despite their differences in Cu tolerance. Despite similar Cu tolerances in Phaeodactylum tricornutum and Ceroxemia clustera, differences in internalised Cu, phytochelatin production and reduced glutathione were observed. P. tricornutum maintained reduced glutathione at 58-80% of total glutathione levels throughout, whereas in C. clustera reduced glutathione constituted <10% of total glutathione after 48 h of Cu exposure. P. tricornutum internalising significantly less Cu but produced more phytochelatins and of longer chain length than C. clustera. Twogreen algae, Dunaliella tertiolecta and Tetraselmis C. sp. had very different Cu tolerances, and also exhibited differences in internalised Cu, phytochelatin production and reduced glutathione concentrations. Tetraselmis sp. internalised three times more Cu than D. tertiolecta and had significantly more intracellular thiols. In both green species a decrease in the reduced/oxidised glutathione ratio was observed following Cu exposure. Phytochelatin production was markedly different between acute (<24 h) and chronic exposures (24-72 h), with shorter chain lengths dominating for chronic exposures. Reduced glutathione increased slightly at longer exposures in both species, potentially indicating a decrease in intracellular oxidative stress despite a consistent increase in intracellular Cu. This work has shown that the intracellular reaction to Cu is species specific, and responses were not necessarily related to their respective tolerance differences, or the amount of intracellular Cu. This suggests that there may be multiple modes of Cu toxicity and that detoxification processes vary between species.

143 Time-course metabolomics responses of transplanted oysters Crassostrea hongkongensis to estuarine metal pollution
C. CAO, Hong Kong University Sci. Technol.; W. Wang, Hong Kong University Sci. Technol. / DIVISION OF LIFE SCIENCE
In a dynamic and fluctuated estuarine environment, heavy metal toxicity is greatly induced in estuarine organisms while various environmental stressors may reduce the difficulty of accurately predicting potential biological/ecotoxicological effects simply based on indoor laboratory experiments. In this research, metabolomics responses of oysters Crassostrea hongkongensis were characterized under metal contamination in an estuary. Oysters were transplanted from a clean site to multiple-metal-contaminated sites in an estuary for six months. Both metal concentration and metabolomics responses in oysters’ digestive glands were investigated over different periods of exposure (0 week, two weeks, four weeks, and six months). Following transplantation, all metals (Ni, Cr, Pb, Cd, Cu and Zn) in digestive gland tissues showed an elevated trend over time. Cu, Zn and Cd were dominant polluted metals. Using 1H NMR metabolite approach, we demonstrated that significantly varied metal-binding sites and detoxification. Current regulation, energy metabolism, nucleotides and glycerophospholipid metabolism. Within initial four weeks of exposure, the amount of amino acids and osmotolys was increased for osmotic regulation. Additionally, energy demand was decreased to accumulate glucose and glycogen for adaptation. However, oysters mobilized early accumulated amino acids and energy storage compounds to produce ATP and maintain a normal physiological function over a longer period of exposure. The metabolomics study demonstrates that oysters adopt different strategies to maintain living status in a heavily-metal-contaminated environment.

Modelling of pesticides and biocides fate and exposure in a regulatory context

144 Impact of updated modelling methodologies on pesticide E-fate risk assessments
A. Boivin, ANSES
Regulation 1107/2009 states that active substances used in plant protection products are only approved in the EU if it may be expected that their use will not have any harmful effects on human and animal health or the environment. In practice, risk assessment is performed for the active substance (at European level) and for the product containing the active substance by EU member states (in the context of the Zonal procedure). While regulation may provide indications on the data requirement and the protection goals, procedure on how to derive predicted environmental concentrations in the different compartments is made in guidance documents publicly available. Such guidance are valuable in order to improve the risk assessment methodologies. It’s also noticeable that guidelines are also updated or complete with aim to achieve a high scientific quality in the risk assessment. In addition they may help to harmonize the risk assessment carried out for pesticides at EU and zonal level. In the environmental fate and behaviour area, at least 9 guidances are available covering the various compartments, process and crop type. Anses proposes some feedbacks of guidance implementation and the practical or formal consequences of the guidance. It includes worked examples to identify sensitive topics highlighted during risk assessment performed by member states. Consequences for the environmental fate and behaviour (and ecotoxicology) sections and the associated risk assessment are discussed. Benefits and drawbacks are discussed.

145 Can the Danish regulatory modelling approach assess the leaching risk of pesticides and their metabolites as monitored via the Danish Pesticide Leaching Assessment Programme?
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The regulation of pesticide use in Denmark is based on the European Union (EU) registration processand guidance documents for the Northern Zone and Denmark. As described in these guidance documents, the Danish assessment of the risk of leaching of pesticides to groundwater is based on modelling with the FOCUS Hamburg model scenario (PELMO) or the two national model scenarios Karup and Langvad (MACRO). The approach taken by Denmark also differs from that of the EU with respect to consideration of relevancy of metabolites, selection of degradation rates and the influence of environmental concentrations in groundwater. In Denmark, if modelling indicates leaching below, but close to the threshold of 0.1 µg L1, the compound’s leachability may be evaluated in the Pesticide Leaching Assessment Program (PLAP). PLAP is an intensive monitoring programme evaluating the risk of leaching of pesticides and/or their metabolites under field conditions. PLAP today encompasses five fields representing various types of soils and near surface geology where the pesticides are used at a realistic worst case dose and according to good agricultural practice. The PLAP-fields have been thoroughly characterized (e.g. pedology, hydrogeology) and monitored as regard to the water balance. Modelling was carried out according to the Danish and EU approach for 27 pesticides and 19 metabolites that have been tested in PLAP. The modelling results were compared to the findings in the groundwater wells in PLAP. As expected there are differences between the Danish and the standard EU approach with regard to the modelling results. Due to the more conservative input and more conservative approach for interpreting output, the results for the Danish approach generally predicts a greater risk of leaching. Comparison with the results for PLAP reveal that in some cases the models over-estimate the leaching risk. The potential causes for this discrepancy between simulated and observed leaching scenarios will be explored.
are needed to test the improved models and as well as on elaborating the type of measurements needed to test these models in the field.

147 Options to consider during accumulation calculations for predicted environmental concentrations in sediments

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Predicted environmental concentration in sediment (PECsed) for regulatory purposes is currently calculated according to the FOCUS surface water model. The standard time frame of a FOCUS surface water simulation (12-16 months) could potentially limit the estimation of chemical accumulation in sediment. TOXSWA, however, is not currently parameterised to run over longer periods within current the regulatory framework. In this work we aim to identify the main processes which drive accumulation in the sediment and to discuss options to consider during accumulation calculations for regulatory exposure assessment. We considered a slowly decaying sorbative compound applied annually to winter wheat. The FOCUS surface water run-off scenarios were used to calculate the PECsed for one year and for 20 years. A second analysis was performed to examine sediment burial within run-off scenarios for annual application to winter wheat. Soil mass input and sediment chemical mass over 20 years were generated using SWASH. A simple, conservative assumption was made that 100% of the soil adsorbed chemical and eroded soil mass is deposited to the sediment along with partitioning to and from the water column. Applying the stratigraphic principle of superposition a stratigraphic column spanning 20 years was constructed. Both the stream and pond scenarios demonstrate that dissipation in the sediment is dominated by desorption/back-diffusion. Neglecting dissipation by desorption and back-diffusion leads to overestimation of PECsed by a factor of 2.5 to 4. In the second analysis the constructed stratigraphic column demonstrates that in some cases >20cm of sediment would be deposited during a 20 year simulation. Furthermore, chemical mass would be distributed heterogeneously throughout this deposited thickness and not solely within an upper 5 cm or 1 cm compartment. Neglecting the processes described here leads to overestimation of the potential accumulation in the sediment. Desorption and back-diffusion as a major sediment dissipation process is already considered in the current exposure assessment scheme over a 12-16 month time period. It could and should be used to quantify accumulation over longer time periods more realistically. Additionally, when calculating PECsed over a longer period the option of simulating burial of sediment should be available as a more realistic, higher-tier option for calculating sediment PECs within a regulatory context.

148 Effect of long-term sorption kinetics of pesticides on their simulated leaching to groundwater

J. Boesten, Alterra / ERA team

Assessment of leaching to groundwater is an important aspect of the risk assessment of pesticides within the EU. The lower tiers of this assessment are based on calculations with simulation models. The first tier is commonly based on the assumption of equilibrium sorption based on batch adsorption studies with a typical equilibrium time of 24h. In the past decade, interest has increased to include long-term sorption kinetics (LTSK) as a higher tier. It is therefore interesting to assess the likely magnitude of the decrease in leaching concentrations due to inclusion of LTSK. Calculations were made with FOCUS_PEARL_v4.4.4 for the FOCUS Okehampton groundwater scenario with an annual application of 1 kg/ha in winter wheat one day before emergence. This was done for a range of substances using regular grid of Kow and DegT50 values; the Kow ranged from 15 to 210 L/kg and the DegT50 from 5 to 70 d. The desorption rate coefficient k_d was 0.0025 d^{-1} and the ratio of the non-equilibrium Freundlich coefficient divided by the equilibrium Freundlich coefficient (f_{eq}) was 0.5. The Freundlich exponent N was set at either 0.7 or 0.9. There is a complication with respect to the parameterisation of the DegT50 because the overall degradation rate is a function of k_d and f_{eq}. So if the effect of k_d and f_{eq} on leaching is studied while keeping the same DegT50, this would lead to comparing leaching of substances with different overall degradation rates which is not meaningful. This problem was overcome by simulating for each substance the decline of the mass of substance in a hypothetical laboratory degradation rate study with the Okehampton top soil at field capacity and 20°C and by deriving the first-order overall degradation half-life of this substance from a fit to these simulated data. Both for N = 0.7 and N = 0.9, for the vast majority of the DegT50-Kow combinations the effect of including LTSK was closely related to the level of the leaching concentration: the lower the leaching concentration of the substance, the higher the effect of including LTSK. The effect of including LTSK was found to be considerably larger for N = 0.7 than for N = 0.9; for N = 0.9, a leaching time frame of a FOCUS US (with LTSK simulation 12-16 month simulation of about 1 µg/L without LTSK whereas for N = 0.7, 0.1 µg/L with LTSK corresponded with 3-8 µg/L without LTSK.)

149 The EFSA PPR Statement on the FERA Proposal for Aged Sorption Guidance

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Aged sorption (also referred to as non-equilibrium sorption) refers to the increase of sorption of a substance over time. Although aged sorption is a generally accepted scientific phenomenon, the procedures to derive parameters of the aged sorption process are uncertain. The interest in aged sorption led to the inclusion of recommendations regarding the use of aged sorption parameters in the revised FOCUS groundwater report. However, the extent of investigation into this subject was limited and a general recommendation was made that default parameters for aged sorption could be adopted at higher risk assessment tiers for any substance. Separate experiences with the difficulties in considering aged sorption data in regulatory risk assessment triggered the UK Chemicals Regulation Directorate (CRD) to charge FERA (the Food and Environment Research Agency) with the development of guidance on study conduct and generation of aged sorption parameters suitable for use in regulatory risk assessment. The scientific Panel on Plant Protection Products and their residues (PPR Panel) was asked to prepare a scientific opinion on the FERA guidance proposal. The experimental and modelling approaches in the proposed guidance are reasonable compromises between the required effort and what is desirable from a theoretical point of view. The Panel has, however, concerns about the interpretation of the experiments and how results of the experiments should be used in the leaching assessment. The Panel investigated options for improvement. Evaluation will be finalised after the guidance document has been revisited by the authors.

Prospective and retrospective soil risk assessment of chemical stressor

150 Utility of a site specific soil microbial health assessment test suite to assess the impact of single chemicals on soil microorganisms for risk assessment

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Soil microorganisms are an integral part of the terrestrial ecosystem playing a significant role in nutrient cycling and organic matter decomposition. As such, impairment of the soil microbial function and diversity, due to contamination, can lead to impacts at higher trophic levels over a period of time. To assess the impacts of soil contaminants on soil microbial function and diversity, Environment Canada (EC) developed a site specific soil microbial test suite. The test suite evaluates soil microbial biomass, activity, and community structure using the following tests: heterotrophic plate count, fumigation-extraction, nitrification, organic matter decomposition, substrate-induced respiration, basal respiration, bait lamina, enzyme assays, community level physiological profiling, and next generation DNA sequencing. Initially, the test suite was used to determine the impacts of site specific boreal forest contamination events (polycyclic aromatic hydrocarbon, metals) on the soil microbial community; however, until recently, the utility of the test suite has been limited for laboratory risk assessment research. Data will be presented on the use of the microbial test suite to study a healthy sandy loam reference soil spiked with different concentrations of chemicals. In contrast to the site specific results where EC50 values are not possible to calculate, risk assessment EC50 results will be presented to inform risk assessors of the impact of the chemicals on the soil microbial community.

151 Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015): problem definition and conceptual model

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Multidisciplinary and international collaboration is essential for developing a science-based methodology for risk assessment of chemicals released to or reaching soil. Topical Scientific Workshop on Soil Risk Assessment brought over 200 experts representing industry, academia, regulators and other stakeholders from all over Europe and across the world in order to discuss the state of the art in assessing the impact of industrial chemicals, biocides and plant protection products in soil. The overall aim was to identify the most critical improvement needs for soil risk assessment, and to identify in which cases the harmonisation of different regulatory approaches would bring an added value. Three main topics were assessed during the workshop: Potential Playbook for conceptual model for soil risk assessment, Environmental exposure and fate assessment, and Effect assessment. Several areas where identified harmonisation of the approaches in scientific assessments for pesticides, biocides and industrial chemicals under REACH and international cooperation would bring added value for the soil risk assessment. This presentation will focus on the conclusions reached in discussion on the problem definition and conceptual model for soil risk assessment.
assessment and describe identified developments needs within regulatory framework. Following conclusions and development needs will be elaborated; It was seen as beneficial if technical development of specific protection goals (SPG’s) could be harmonised across the industries and regions, if and where applicable, with clear definition of land/soil use, product use, exposure scenario and time scale. Ecotoxicological approach was considered as useful, enabling communication tool in defining what do we want to protect. Considering bioavailability in soil risk assessment and defining concept of biodiversity (including functional biodiversity) in regulatory context. Combining exposure assessment – how the chemicals enter into and behave in the soil – with effect assessment – how the chemicals then affect different organisms. Additionally, other identified regulatory science-oriented R&D needs such as the application of the equilibrium partitioning method, a screening level for soil risk assessment, using information from aquatic organisms, and a calibration based on information on higher-tier studies will be discussed in more detail.

152 Translation of Ecosystem Services based Protection Goals into an appropriately calibrated tiered Collembola risk assessment of plant protection products - case study: lindane

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Soils provide relevant Ecosystem Services (ES) e.g. by regulating water and nutrient cycles which have a direct impact on the EsS food production. In agricultural landscapes the biodiversity and the fertility of soils have to be protected from an unacceptable impact from PPP. However, landscapes are heterogeneous and different ecosystem services are provided by different parts of the landscape. Thus, a spatial differentiation needs to be taken into account when setting protection goals. Due to conflicting needs of different ES it is not possible to protect biodiversity in all areas and all the time. Therefore, biodiversity should be protected on landscape level. Thus, the risk assessment should differentiate between 1) an off-field risk assessment where the focus is on protection of biodiversity and 2) an in-field risk assessment which should focus on protection of those soil functions which are directly related to the most relevant EsS for agricultural areas (i.e. food production). In order to improve the current toolbox for soil functional test systems that could be used in an in-field soil risk assessment a project was initiated by the European Crop Protection Association in 2014. Different functional test systems (litterbag test, minicontainer test, bait lamina test) are tested on their sensitivity to different insecticides in a field trial and evaluated with regard to their relevance for the EsS provided by soils, their degree and potential for standardization, and their suitability for being used in the risk assessment. The case study is focusing on one specific group of soil organisms (Collembola) and the ecosystem services they provide by examining different structural and functional tests in a tiered risk assessment approach. Laboratory tier 1 test will be compared with an intermediate tiered two-generation test, and higher tier field effect data on both a structural and functional level. We want to discuss how protection goals derived from the Ecosystem Services approach translate into an appropriately calibrated tiered risk assessment approach for collembola.

153 Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015): environmental fate and exposure assessment

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The main principles and elements of exposure assessment of chemicals in the soil are known and used across various legislations. However some of the properties and processes should gather more consideration in the exposure assessment than is currently the case. For example, sorption should be considered even for possessing specific structures (e.g. structures that earmark a substance as PBT) or physico-chemical properties. The lack of realism in the fate testing requested under current regulatory frameworks, leads to the difficulties when the testing data are employed for the simulation of processes under real environmental conditions. In the workshop it was also discussed that the link between fate and exposure assessment on the one hand and hazard assessment on the other hand should be further improved. It was concluded that the bioavailability of the chemicals should be further understood and implemented in the regulatory framework. The possible harmonisation of the approaches and models used today for the soil exposure assessment was also elaborated. The workshop was an attempt to bring together different tools and methods to improve the use of different modelling tools for the exposure assessment and risk assessment in general has a great potential for further development and practical use, but the limitations in the use of available/newly developed tools needs to be well understood. The sharing of experiences and harmonisation of approaches for the fate and exposure assessments within different legislations was seen as a key possibility for further improvement of the relevance and reliability of the soil risk assessments. Discussions on NER formation and bioavailability resulted in the conclusion that the topic has potential for the implementation into regulatory exposure assessment schemes. However, some concerns around the topic were raised. It was concluded that higher realism of the fate/exposure assessment (regardless whether it is based on laboratory/field measured or modelled data) and its linking with results of hazard assessment is necessary. There were specific proposals for initiating actions for the development of a holistic approach and methods for soil exposure assessment taking into account additional parameters (physico-chemical) of substances and of the medium (soil) as well as integrating into the assessment methodologies other processes (e.g. desorption) and conditions relevant for the real environment. Research needs and the directions for development of the regulatory science were identified.

154 Validation of a New Environment Canada Test Method for Measuring Contaminant Effects on Survival and Reproduction of Soil Mites using Oppia nitens

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In 1995, Environment Canada (EC) initiated a multi-year program to develop biological test methods that could be used to assess the toxicity of contaminants in soils using terrestrial organisms. The goal was to develop test methods that were applicable to Canadian soil types using species that were representative of Canadian soil ecosystems. The creation of a new Environment Canada test method for measuring survival and reproduction effects in contaminated soil using an orbicatid mite is the latest soil testing methodology to be developed into a national standardized method. The proposed protocol is a definitive 28-day test measuring chronic toxicity using adult survival and number of live juveniles as endpoints. The mite species recommended as the test organism of choice is Oppia nitens (C.L. Koch, 1839), a species found in surface soils across Canada. This toxicity test is based on EC research conducted by staff of the Soil Toxicology Laboratory. An inter-laboratory validation study will be initiated in January 2016 as part of the EC method standardization process. Eleven ecotoxicology laboratories from across Canada and Europe have volunteered to participate in this method ring testing effort. Participating laboratories have been establishing their O. nitens cultures for the past three months following standard operating procedures developed by EC. Three rounds of testing are planned for this method validation project which is schedule to begin in late January 2016. As a parallel effort, Environment Canada has begun formal method writing with the goal of having a 1st draft of the official test method completed before completion of the ring testing program. An overview of the test method and early results of the inter-laboratory testing program will be presented.

155 Outcome of the Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015): state-of-the-art and new developments in soil effect testing

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The primary objective of this workshop was to review the state of the art regarding soil risk assessment in view of developing updated scientifically-sound principles and approaches for assessing ecological risks of chemical substances, which are released to or reach the soil. The workshop also provided a platform for academia, regulators and other stakeholders to address how the main long-term challenges from the regulatory perspective can be reflected and employed in the current and future research topics on soil risk assessment. The discussions were reinforced by information on the recent scientific developments and on risk assessment methodologies applied in chemicals management both within and outside the European Union. There was a common understanding that the Ecosystem Services (ES) approach can be a suitable conceptual framework to define specific protection goals in order to ensure the protection of the environment including biodiversity. This process encompasses the identification of the services provided by the soil ecosystem and associated species or groups (SPUs). It is responsible for the provision of these services (SPUs) through a dialogue with risk managers. Afterwards, a process for setting reference tiers (allowing direct comparison with the protection goals) and to calibrate down for intermediate and lower tiers, is needed. Particularly for the lower tiers, it has to be checked whether standard test methods used today or alternative relevant species or groups (SPUs) are available. If not, alternative test species and test methods have to be identified. Very few standard higher-tier tests are available. Finally, a list of recommendations was prepared by the workshop participants, providing ideas on how to proceed regarding soil effect test methodology in the light of the new risk assessment approach. During the workshop already several topics were identified where harmonisation of the approaches and international collaboration would bring added value for the soil risk assessment such as, just to mention a few, defining specific protection goals,
concluding bioavailability in soil risk assessment and defining the concept of soil biodiversity in the regulatory context. In addition, already concrete proposals were made about launching activities on defining boundaries for applicability of the Equilibrium Partition Method approach, considering non-extractable residues, application of Species Sensitivity Distributions (SSDs) and revisiting assessment factors (AFs).

Life Cycle Data and Modeling Developments - From Data Collection to Usage (III)

156  A model and tool to calculate life cycle inventories of chemicals discharged down the drain
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A new model and tool to calculate life cycle inventories (LCIs) of chemicals discharged down the drain is presented. Its main innovation is the fact that it attributes the exchanges with the technosphere and the environment taking into account the expected behavior of individual chemicals in the wastewater treatment plant (WWTP) and the environment, thus contributing to better decision making and data availability in the context of the life cycle of chemicals. The model covers treatment of organic and inorganic chemicals and input variables by the user include chemical-specific data (composition, phys-chem properties, half-lives, and fate factors in the WWTP) as well as scenario variables (% portion disposed [to WWTP, landfill, incineration, agriculture], sludge disposal scenario including landfill, incineration and agriculture). The WWTP is modelled with a mass and energy balance taking into account the partitioning of the air to sludge, treated effluent, and depending on its degradability, the transformation by microorganisms to CO2 and new biomass (excess sludge). Sludge is treated by anaerobic digestion and the fate of any fraction of the chemical released to the environment (e.g. treated effluent, direct discharges) is assessed with a previously developed model, which calculates greenhouse-gas (GHG) emissions following degradation in environmental compartments. Besides GHG emissions, emissions of nutrients (N, P) are also considered. Concerning sludge disposal by incineration and landfilling, this is assessed by means of the models developed for the ecoinvent database, whereas reuse of sludge in agriculture is accounted for by means of tier 1 emission factors from the IPCC. The model is programmed in an Excel spreadsheet, the WW LCI tool, which accommodates simultaneous calculations for 30 chemicals, either individually or as a mixture. The output of the WW LCI tool is a comprehensive LCI linked to ecoinvent v3 data sets, that can be imported to LCA software in order to complement a life cycle assessment study of a particular chemical or a chemical mixture associated to a product or service. This model is the first one to address a chemical-specific and comprehensive LCI of chemicals discharged down the drain, including all the described processes. It constitutes an advance over previous models using generic descriptors like BOD, COD, etc. and constitutes an advance in how the end-of-life stage of chemicals is addressed in LCA.

157  Typology of mixtures for world-leading production inventory for CLCA
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Throughout the past two decades, conventional LCA (CLCA) has emerged as a modelling approach to assess consequences of either direct or indirect changes. The development of CLCA has received a lot of interest from practitioners and requires specific inventory data. These data, often based on economic models are long to set and require a good understanding of processes. Moreover, no robust generalised technical databases for consequential LCA about indirect changes have been provided yet, especially in the case of electricity production, which may lead to uncertain LCA results. In that context, the development of more generalised LCA data represents a great challenge for the LCA application. A first step to address this issue is to better understand electricity production mix worldwide. So, the aim of this work is to set a typology of electricity production mixes. The proposed typology is based on the analysis of two factors: the former involves the composition of electricity production mixes of 94 countries producing more than 10 TWh in 2012 and the latter is their calculated GHG (gCO2eq/kWh) assessment from LCA using IPCC 2013 LCIA method2. The analysis of these two characteristics leads to the determination of a 4-group typology: very low (0-37), low (37-300), mean (300-600) and high (>600) emitting countries, each sharing general characteristics about electricity power generation, i.e., hydro, wind, nuclear, biomass, and gas, gas, and coal/ fuel. The next step of our work is to study the dynamic evolution of some countries selected from each group of the proposed typology, using historical data. The first case study will be the France energy system. The comparison of historical and contemporary observed production data, as previously carried out in the literature3 will help us to identify the uncertainty remaining in evaluating electricity production processes, and so the consequences of integrating such data in consequential LCI. The Shift Project, 2012. TSP Data Portal. tsp-data-portal.org. Accessed 03/16/2015. 2IPCC, 2013: Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, United Kingdom and New York, 1535 pp 3Mathiesen et al., 2008. Uncertainties related to the identification of the marginal energy technology in consequential life cycle assessment. J. Clean. Prod., vol. 17, n°15, p. 1331-1338, oct. 2009

158  Multiple recycling loops in attributional LCA - Misconceptions and solutions
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Modelling of recycling in LCA has always been an important topic for discussion. It is often argued that the modelling method should reflect the benefits of multiple recycling loops. Some guidance on this area is given in ISO/TR 14049: the avoided burden method is proposed if the material is recycled without loss of intrinsic properties, and the recycling method is proposed if the material is recycled with loss of intrinsic properties. However, it is questionable whether this guidance is suitable for attributional LCA. In the attributional database of ecoinvent 3, the life cycle inventory is partitioned over the co-products of multifunctional processes by “allocation at the point of substitution”. We show that this method is equally applicable to materials that are recycled at the end of life. We propose a new formula that calculates the LCI of one life cycle of a material, considering the consumption and production of co-products and recycled materials in that life cycle. If the consumed recycled material has been recycled several times before, the formula can be solved to calculate the inventory after a definite or infinite number of recycling loops. The formula in this case can be applied if the material is recycled via a material pool where the inventory regarding the primary production, recycling activities and waste treatment are similar in other product systems, and the average recycled content and the end-of-life recycling rate in the material market can be determined. The LCI after a large number (>5) of recycling loops corresponds to the avoided burden approach as described by ISO/TR 14049, only when the recycled content and the end-of-life recycling rate are equal. This is not a realistic condition for most materials. Therefore, it is important to distinguish these rates, which is not done in the current application of the avoided burden method. Other methods that consider multiple recycling loops often refer to downstream recycling activities. We show that only multiple recycling loops in the past are relevant in an attributional LCA. Whether the produced recycled material at the end of life can be recycled again in the future, is outside the scope of an attributional LCA that focuses on one product life cycle.

159  Methodological Guidelines for the Life Cycle Inventory of Agricultural Products
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In 2012, the sustainability consultancy Quantis[1] and Agroscope[2], the Swiss Federal research institution for agriculture, joined their forces to create a unique international consortium of nine major, global, private companies from the agro-food value-chain[3] and two governmental agencies[4] into what would become the World Food Life Cycle Assessment (WFLCA) project. In 2012, 400 datasets for crops, animal products and food products in 40 countries were delivered to the project partners and submitted to the ecoinvent Centre for publication in 2016 and 2017. Thereby, the entirety of the project developments are made available to a broad public of LCA and sustainability practitioners. A major outcome of WFLDB was the development of the Methodological Guidelines for the Life Cycle Inventory of Agricultural Products[5], a detailed and comprehensive guidance for inventory modelling of agricultural and food systems. To date, these are among the very few – if not the only – peer reviewed documents providing detailed and operational guidance applying to a wide variety of agricultural systems and countries, from farm to plate. The Guidelines are based on existing scientific emission models and are compliant with the most recognised standards (ISO, ILCD, ecoinvent, IPCC). They are also to a large extent aligned with draft Product Environmental Footprint Category Rules (PEFCR) for several sectors. All key methodological issues relevant to agro-food systems are addressed[6], such as the development of software boundaries to co-products allocation, inventory modelling for fertiliser and pesticides, emission flows, water modelling and land use change. A unique set of data collection principles combining statistical data from FAO and agronomic extrapolations was applied to generate consistent LCI data. Innovative approaches were developed where no global consensus is yet available for modelling decisions. The Guidelines were first made public in August 2014 and then updated in July 2015. The current version (v3.0) aims to serves as an open reference for LCA practitioners and LCI database developers. The second phase of the WFLDB project, starting in 2016, will further work on improving datasets and expanding these guidelines in cooperation with all stakeholders. Thereby we consider the latest methodological developments and global consensus on key topics such as pesticides emissions modelling, land use change or carbon sequestration in

38 SETAC Europe 26th Annual Meeting Abstract Book
characterization of archetypical weather days has shown the appropriate level of temporal differentiation in the LCI to assess the impacts from unmanaged pollutant loads from UWS during intense storm events. With such significant contributions of pollutant loads at the LCIA level, further research is required to include temporally-differentiated emissions in the methodological framework of the aquatic categories of eutrophication and ecotoxicity, to better understand how the performance of an UWS system affects the receiving environment for given local weather conditions.

Innovative techniques for monitoring chemicals in the environment

162

Analysis of 42 pesticides in fresh fish muscle by dispersive solid phase extraction with QuEChERS followed by LC/MS/MS analysis

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Multi-residue analytical methods for determination of pesticides in biota samples are scant in the literature. The present study describes a high sensitivity and selectivity method based on dispersive solid phase extraction with QuEChERS followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis for determination of a range of different polarity pesticides in fresh fish muscle and its subsequent application to various real samples in order to, first, prove its suitability, and, secondly, have a first picture of the occurrence of the compounds investigated. The method was under study at the ELSA providers belonging to the classes of organophosphorus, pyrethroids, carbamates, triazines, amines and chloroacetamides, among others, were initially considered for analysis. Extraction of the pesticides from the biota samples (10 g) was performed with QuEChERS kits using acetonitrile (1:1 v/v), 4 mg MgSO₄, 1 g NaCl, 1 g NaCitrate and 0.5 g disodium citrate sesquihydrate, and subsequent clean-up with 400 mg PSA, 400 mg C18 and 1200 mg MgSO₄. LC was performed with a Purospher STAR RP-18e column (125 x 2 mm, 5 μm). MS/MS detection was carried out in the selected reaction monitoring (SRM) mode with a triple-quadrupole mass spectrometer equipped with an electrospray (ESI) interface working in both positive and negative ion modes along the analytical run. Quantification was performed by the isotope dilution method, based on the peak areas obtained for the analytes and their deuterated analogues. The performance of the analytical method was evaluated in terms of linearity, sensitivity, accuracy, matrix effects and precision, according to the guidelines set for pesticide residues analysis in food and feed (SANCO/12945/2011), with satisfactory results for 42 out of the 49 initially considered compounds. As regards sensitivity, most of the target compounds could be determined at levels below 10 ng/g. Application of the method to a set of real samples showed the presence of some of the target compounds in fish muscles at relatively low levels (in the ng/g range). Main advantages of the method are, in addition to high sensitivity and selectivity, simplicity, rapidity and relatively low cost. Acknowledgement - The authors thank the financial support of the EU (FP7 projects GLOBAQUA (265264) and SOLUTIONS (603437)) and the Generalitat de Catalunya (Consolidated Research Groups “2014 SGR 418 - Water and Soil Quality Unit” and 2014 SGR 291 - ICRA).

163

Development of new analytical techniques for monitoring of POPs and emerging environmental contaminants

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Reliable and accurate analytical techniques are essential in order to study the occurrence, behavior, and fate of known and emerging environmental contaminants. The aim of this work was to develop a fast, simple, high-throughput analytical method for simultaneous determination of diverse persistent organic pollutants (POPs): polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), other flame retardants (FRs) along with diverse pesticides in a single analysis to improve the monitoring of these contaminants. Sample preparation for biological samples including fish (catfish, salmon, wreckfish) and shrimp was based on quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction with accelerative followed by clean-up using dispersive solid phase extraction (d-SPE). The sample preparation is fast and inexpensive, where one analyst can prepare a batch of 10 pre-homogenized samples in one hour, using approximately S3 of materials per sample. The sample analysis entails fast low-pressure (LP) gas chromatography (GC) tandem triple quadrupole mass spectrometry (MS/MS) allowing separation of over 200 compounds and internal standards in 10 min. Satisfactory recoveries (70-120%) were achieved for the majority of analytes with relative standard deviations under 20% even at 1-5 ng/g spiking levels. To minimize sample size, and reduce amounts of organic solvents and internal standards added before the extraction, we evaluated variables affecting QuEChERS-based extraction yields of incurred contaminants in fish samples. Our results demonstrated that 2 g subsamples were adequate for analysis of the...
incurred contaminants. Smaller test portion size often translates into faster, easier, and less wasteful methods, provided that the subsample adequately represents the original sample. To further improve identification of contaminants and to achieve lower detection limits, we are currently evaluating a new Agilent 7010 high sensitivity MS/MS. Also, novel lipid-extraction materials and new sorbents were researched for extraction of pollutants from salmon. The monitoring data on the occurrence of these contaminants may advance our understanding of their potential risk and aid in future risk assessment for the better protection of human health and the environment.

164

Two-dimensional algal arrays as innovative biosensing tool

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Because of their rapidity and sensitivity, microalgal biosensors can be used as early warning systems to detect early effects on organisms exposed to chemical in environmental samples. Among all the biological endpoints explored in whole-cell biosensors, chlorophyll a fluorescence is known as a routine technique for the determination of the stress effect and reactive oxygen species (ROS) production is becoming a promising key marker to evaluate chemicals toxicity in the environment. However reproducible immobilization of the living cells in facile array format for biosensing is still challenging and their viability is difficult to maintain. In this context, alternative current (AC) dielectrophoresis (DEP) allows a rapid and reversible control over the live cell for our study. An alternative current (AC) dielectrophoretic lab-on-chip setup was evaluated as a rapid tool to collect and assemble cells of microalg Chlamydomonas reinhardtii in two-dimensional (2D) arrays. Combined with fluorescence microscopy detection, the capability of using the 2D whole cell arrays to follow the algal sub-cellular response to several environments, including mercury, methylmercury, copper, copper oxide nanoparticles (CuO-NPs), and diuron was explored. The results showed significant increase of the cellular ROS when C. reinhardtii was exposed to increasing concentrations of methylmercury, CuO-NPs and 10⁻⁷ M Cu, as revealed by enhancement of the CellROX® stain cells. Less matrices of small size requires a good sensitivity. Therefore, an original coupling was developed. The TIMFIE sampler is easily assembled from inexpensive laboratory consumables and requires no electricity or other supporting equipment. It consists of a simple syringe pump connected to a flow restrictor (PEEK capillary) that enables a low microliter per minute water flow through an in-line solid phase extraction (SPE) cartridge. The force that slowly fills the syringe is accomplished by attaching a rubber band or a weight to the piston, or through applying under pressure in the syringe by pulling and locking the piston prior to sampling. Target compounds are continuously extracted and trapped on the SPE material during the sampling period. The extracted water ends up in the syringe barrel and after finished sampling the sample volume is determined and the SPE cartridge is sent to the analyzer. Here it is handled in the standard protocols, typically followed by gas or liquid chromatography coupled to mass spectrometric detection. Method development and validation is similar to standard SPE procedures and the large number of SPE packing materials available together with the possibility of stacking cartridges in series offers flexibility. The flowrate, and in the end the sample water volume, is modulately dependent on the force applied, the diameter of the syringe and the diameter and length of the restrictor, but also on e.g. temperature since this affects water viscosity. By altering the controllable variables of the TIMFIE sampler the flow can be adjusted to enable sampling periods from hours up to weeks. The final volume collected during a certain time period will differ between sampling occasions, but the sampled/extracted water volume will always be determined with good accuracy, thus enabling quantitative analysis. The TIMFIE sampler offers: 'n - Time-averaged sampling of water. 'n - Quantitative determination of compound concentrations by well-defined procedures. 'n - Low price, easy assembling and high flexibility. 'n - Minimized costs for shipping and storage of samples. Experiences and results from lab and field experiments looking at pesticides in surface water will be presented.

165

Quantification of emerging micropollutants in an amphipod crustacean by nanoliquid chromatography coupled with mass spectrometry in MRM3 mode

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Over the last decades, human activities (industries, agriculture, transport, consumption, urbanization…) and their impacts on the environment have not ceased to increase. Many molecules related to these activities are released to the environment and their occurrences within aquatic ecosystem become an important issue. In particular, the dose-response relationships of emerging pollutants on organisms at lower trophic levels remain little-known. In order to conduct biomonitoring studies, it is necessary to acquire knowledge about the kinetics of bioaccumulation and depuration of these molecules within species, taking into account the inter-individual variability. In this context, an innovative analytical method was developed to quantify, in an amphipod crustacean (Gammarus fossarum), three emerging micropollutants known as anthropic pollution markers: carbamazepine, oxazepam and testosterone which are respectively an anti-convulsant, an anti-inflammatory and an endogenous hormone. A salting out liquid-liquid extraction known as QUENCHER (Quick, Easy, Cheap, and Rugged) was miniaturized and optimized. The miniaturization of the method made it possible to adapt to the low mass sample (approximately 5 mg dry weight) and to perform the analysis at the scale of a single organism. Beside an efficient sample preparation, ultra-trace analysis of compounds in such matrices of small size requires a good sensitivity. Therefore, an original coupling was developed. It consisted of a separation by nanoliquid chromatography (nanolC) coupled to tandem mass spectrometry using multiple reaction monitoring cubed mode (MRM³). For the nanolC separation, a preconcentration system by off-column trapping was optimized to increase the injection volume. In order to keep both sensitivity and selectivity, detection is usually performed by multiple reaction monitoring mode (MRM). Nevertheless, proteomic studies showed that sensitivity and selectivity could be improved using MRM³ mode. This technic was developed in this project and compared to the classic MRM. In the best of our knowledge, this was the first time that MRM³ was used for small molecule (The analytical strategy developed allowed to obtain limits of quantification lower than 1 ng g⁻¹ (wet weight) and to establish the kinetics of bioconcentration of contaminants within G. fossarum.

166

Time Integrating, Micro Flow, In-line Extraction (TIMFIE) sampler for determination of pesticide concentrations in water - a new quantitative tool in pollution monitoring

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There is a gap between the need for adequate concentration measurement of pesticides and other organic pollutants in water and the usefulness of the field sampling procedures available. In response to this a new device for quantitative, time-integrated whole water sampling of pesticides and field extraction has been developed. The TIMFIE sampler is easily assembled from inexpensive laboratory consumables and requires no electricity or other supporting equipment. It consists of a simple syringe pump connected to a flow restrictor (PEEK capillary) that enables a low microliter per minute water flow through an in-line solid phase extraction (SPE) cartridge. The force that slowly fills the syringe is accomplished by attaching a rubber band or a weight to the piston, or through applying under pressure in the syringe by pulling and locking the piston prior to sampling. Target compounds are continuously extracted and trapped on the SPE material during the sampling period. The extracted water ends up in the syringe barrel and after finished sampling the sample volume is determined and the SPE cartridge is sent to the analyzer. Here it is handled in the standard protocols, typically followed by gas or liquid chromatography coupled to mass spectrometric detection. Method development and validation is similar to standard SPE procedures and the large number of SPE packing materials available together with the possibility of stacking cartridges in series offers flexibility. The flowrate, and in the end the sample water volume, is modulately dependent on the force applied, the diameter of the syringe and the diameter and length of the restrictor, but also on e.g. temperature since this affects water viscosity. By altering the controllable variables of the TIMFIE sampler the flow can be adjusted to enable sampling periods from hours up to weeks. The final volume collected during a certain time period will differ between sampling occasions, but the sampled/extracted water volume will always be determined with good accuracy, thus enabling quantitative analysis. The TIMFIE sampler offers: ‘n - Time-averaged sampling of water. ‘n - Quantitative determination of compound concentrations by well-defined procedures. ‘n - Low price, easy assembling and high flexibility. ‘n - Minimized costs for shipping and storage of samples. Experiences and results from lab and field experiments looking at pesticides in surface water will be presented.

167

High throughput microarray for complex matrix assessment

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Ecotoxicological assessment using different bioassays for environmental sample analysis has been used this last years for comparing them recombinant bioluminescent or fluorescent bioassays have been developed to determine the presence and the toxic effect of pollutants, because even if chemical analysis are sensitive and specific, they failed in the determination of the bioaccessibility and biodispollutability of the pollutant. All the recombinant bacteria carried out fusion between reporters genes (lux CDABE for bioluminescence and gfp for fluorescence) and the promoter of genes involved in metal resistance mechanisms (for metal detection) or genes involved in degradation of organic compounds. These bioassays are generally not specific for one compound, in particular those dedicated for Metal trace element (MTE), leading to a difficult application in complex media. In order to overcome this problem, we used an array of bioluminescent and fluorescent recombinant bacteria with different cross-sensitivity to MTE and organic pollutant. We combined the bacterial response of all the bacteria to obtained a database. In a frist approche we used a set of bioluminescent recombinant bacteria sensitive to MTE, we build a database with the results of the induction of the bacteria exposed to 15 metal and metal mixture. Four specific decision trees based on CHAID algorithm were designed and tested on waste water. The result showed that the concentrations of MTE detected by our model match the chemical measurements. In a second approche we completed our panel of bioluminescent bacteria to improve our model by using a database of more than 1930 flask samples. Preliminary results obtained on complexe environmental samples (wood-waste leachates) showed variation of bacterial response according to the contamination allowing an interesting method of screening. We propose a mixture of targeted and non targeted highthroughput arrays of bacteria to describe more realistically the toxicity of very unusual complex matrix combined with data mining interpretation.

Biological effects of emerging micro pollutants at realistic
and accumulation of Gd in bivalves in realistic conditions of exposure to Gd-CA. To further, we will implement bioaccumulation and toxicity assays with other aquatic organisms and effects along a short trophic chain (algae-daphnids-fish).

170 The marine mussel Mytilus galloprovincialis bioconcentrate and metabolize antidepressant pharmaceutical venlafaxine

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A recent investment in research has been conducted to define exposures and potential hazards of pharmaceuticals in freshwater and terrestrial ecosystems. In contrast, comparatively few empirical studies have been conducted for pharmaceuticals that are likely to enter coastal and marine ecosystems[1,2]. The antidepressant venlafaxine (VLF) and some of its metabolites were recently found in mussels caged in a coastal site receiving treated wastewater[3]. In those marine organisms, very scarce data is available on the accumulation and/or metabolism of pharmaceuticals. Consequently, it appears hazardous to conclude on the origin of those metabolites in mussels, which could include a bioaccumulation through direct exposure as well as a metabolism of VLF in mussels. The aim of the present work was to quantify the accumulation of VLF in the marine mussel Mytilus galloprovincialis and to evaluate the possible metabolism in laboratory controlled experiments. The accumulation of VLF was evaluated in the whole mussel tissues after 1, 3 and 7 days of semi-static exposure by water (100 µg L-1-day-1) followed by 1, 3 and 7 days of depuration. Under those conditions, VLF attained an average tissue concentration of (n=5) 2146.3 ± 156.0 ng g-1 dry weight (d.w.) in seven days. A kinetic bioaccumulation factor (BCF) was 265 ± 19 L.g-1.d.w. Seven days of depuration allowed a decrease of tissue concentration to 21 ± 1.0 g·ng-1 d.w. Four VLF metabolites were quantified in mussel tissues and excreted in water. The kinetics of those metabolites in water confirmed the metabolism of VLF by mussels. Complementary experiment conducted at 1, 10 and 100 µL-1 nominal concentration clearly confirmed that M. galloprovincialis metabolize VLF, with the quantification of studied metabolites excepted the NNO-VLF. These results gave a first approach on the ability of mussel to metabolize pharmaceuticals. Together with bioaccumulation information, this study provided a first approach on the pharmacokinetics of a pharmaceutical in a wild marine species, underlining the need of further experiments to better understand how venlafaxine modulate the receptors in mussels and how exposure to this antidepressant affects physiological functions of invertebrates. [1] Arpin-Pont, L., ESPR. DOI: 10.1007/s11356-014-3617-x. [2] Gaw, S., Philos Trans R Soc Lond B Biol Sci. 2014; 369(1656): 20130572. [3] Martinez Bueno, M.J., Anal Bioanal Chem 2014; 406:601–610.

171 Mechanism study for cardiac negative effect of propranolol to Daphnia magna by utilizing acute toxicity syndrome

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Acute toxicity syndrome is a set of biological responses in behavioral, physiological parameters resulted from distinguishable toxic mechanism. In aquatic toxicology field, monitoring study for the syndromes has been conducted to fish and has proven able to categorize chemical agents based on their mechanism of action because different molecular stimulation result in different visible outcomes. In this study, we tried to distinguish mode of action of propranolol, a human pharmaceutical ingredient, which is usually considered as a baseline toxic chemical to aquatic non-targeted species based on lethal concentration from QSAR. Daphnia magna was employed as a model organism and not only phenotypic outcomes but also metabolomics profiling were monitored for more stereoscopic insight in mechanism characterization. Narcotic agents (4-chloroanilin, ethylacetate), β-adrenergic receptor antagonist and 5-hydroxytryptamine receptor antagonists (nadolol, yohimbine, cyproheptadine) were treated with propranol to Daphnia magna and their syndromes were compared to categorized potential mechanism of propranolol. Syndromes include swimming behavior, heart beat and metabolism profiling. In the time series monitoring during 24h of swimming behavior, Daphnia magna tend to be deactivated at high concentration of propranolol. Likewise, nadolol and yohimbine showed hypo activity as time goes by in effective concentrations. In opposite, exposure of cyproheptadine resulted in hyper activity and narcotic agents showed reduced or swimming away behavior. These findings are unique in the field of metabolomics and Activity-concentration relationship was compared between swimming behavior and heart beat at 6h. Although propranolol exposure affect to both swimming behavior and heartbeat, heartbeat was decreased at concentrations where no effect of swimming behavior was observed. Most similar pattern was observed from nadolol exposure and yohimbine also showed similar relationship with higher effects on swimming activity. Treatment with 4-chloroanilin and ethylacetate, Laboratory swimming behavior was so sensitive that several concentrations showed deactivation without
significant heartbeats. NMR Analysis is now being conducted for metabolite profiling and the result will be combined and be presented. We expect that metabolite profiling provide information how metabolism balance changed with chemical exposure and stricter categorization tool by integration with visible outcomes described above.

172 Environmentally relevant concentrations of antidepressants alter behaviour and gene expression in amphipods

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The effects of antidepressants on wildlife are currently raising some concern due to an increased number of publications indicating biological effects at environmentally relevant concentrations. Depression antidepressant concentrations had a significant effect (> 0.01) on swimming speed in the presence of a mirror. Activity of sertraline, fluoxetine and duloxetine was tested in a concentration range of 0.1-100 mg L\(^{-1}\).

173 Eco-interactions of engineered nanoparticles in sea water media and implications for toxicity

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The physico-chemical properties of engineered nanoparticles (NPs) such as specific surface charges might play a crucial role in their behavior in complex natural media as sea water, with implications on cellular uptake and toxicity in exposed organisms. In this study, we investigate the ecotoxicity of nano-TiO\(_2\) (25 nm), PS-COOH (40 nm) and nano-SiO\(_2\) (100 nm) as model of negatively charged NPs in sea water. The animals were exposed to these five drugs at environmentally relevant concentrations from 0.001 to 1 \(\mu\)g L\(^{-1}\) during short-term (1 hour and 1 day) and medium-term (8 days) experiments. The movement of the amphipods was tracked using the behavioral analysis software during 12 min alternating dark/light conditions. Antidepressant concentrations had a significant effect (p < 0.01) on swimming speed in the presence of a mirror. Activity of sertraline, fluoxetine and duloxetine was tested in a concentration range of 0.1-100 mg L\(^{-1}\) for both NPs and PS-COOH. The results showed a common pattern of no toxicity for all species and the perceived slowness to therapeutic effects recorded in humans and other vertebrates. Since their mode of action is thought to be by modulation of the neurotransmitters serotonin, dopamine, and norepinephrine, aquatic invertebrates that possess transporters and receptors sensitive to activation by these pharmaceuticals are potentially affected by them. We highlight studies on the effects of antidepressants, on specifically crustacean groups showing they are susceptible to a wide variety of neuroendocrine disruption at environmentally relevant concentrations (< 100 ng L\(^{-1}\)). Behavioural and transcriptional changes in this crustacean were studied when exposed to the most prescribed SSRIs (citalopram, sertraline, fluoxetine), SARI (trazodone) and SNRI (duloxetine). The animals were exposed to these five drugs at environmentally relevant concentrations from 0.001 to 1 \(\mu\)g L\(^{-1}\) during short-term (1 hour and 1 day) and medium-term (8 days) experiments. The movement of the amphipods was tracked using the behavioral analysis software during 12 min alternating dark/light conditions. Antidepressant concentrations had a significant effect (p < 0.01) on velocity for Duloxetine (1hr, 1day & 8days); Sertraline (1hr & 1day) and Fluoxetine (1day) but not trazodone or citalopram (p > 0.05). We have also applied high-throughput sequencing technology to animals exposed to 100 ng L\(^{-1}\) concentrations of sertraline, fluoxetine and duloxetine to reveal the broad transcriptional responses to these compounds. Preliminary analysis of gene expression profiles indicates a broad range of potential neurological pathways confirming that these drugs must have multiple targets. In the light of new studies indicating effects on the human brain with just of dose of SSRIs using MRI scans, confirming that these drugs have multiple targets. In the light of new studies indicating effects on the human brain with just of dose of SSRIs using MRI scans, confirming that these drugs have multiple targets.

174 Are trophic interactions between aquatic invertebrates affected by AgNPs exposure route?

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Silver nanoparticles (AgNPs) have been increasingly used over the last decade, resulting in their growing release into freshwater ecosystems, where AgNPs and Ag \(^{+}\) derived from NPs can have toxic effects on aquatic species and compromise important processes such as organic matter use and decomposition. However, little is known about how species interactions in multi-trophic systems will affect such ecosystem processes when exposure routes of AgNPs vary. The goal of this study was to assess the impacts of low doses of AgNPs and Ag \(^{+}\) on trophic interactions between aquatic invertebrates and consequent ecosystem effects. Specifically, we assessed the importance of both direct (via water) and indirect (via food) exposure routes by exposing a simplified detritus food web comprising leaf litter, microalgae, a shredder species and collector species to: (i) water contaminated with AgNPs (0.1, 1.00 \(\mu\)g L\(^{-1}\)) or AgNO\(_3\) (0.1, 0.1 \(\mu\)g L\(^{-1}\)), and (ii) leaves contaminated for 6 days with AgNPs and AgNO\(_3\) at the same concentrations. Shoeddies (Gammarus pulex, Gammaridae, Amphipoda) and ciliates (Habropoditi sp., Leptophriidae, Euplomerophora) were placed in laboratory feeding containers where collectors had access to fine particulate organic matter (FPOM) produced by P. pulex, but were separated by a mesh screen. Leaf consumption and FPOM production by P. pulex as well as fungal biomass and decomposition activity were determined after 15 days. The stress induced by AgNPs and Ag \(^{+}\) in both invertebrate species was assessed by measuring the activity of antioxidant and neuronal enzymes. Microbial decomposition was lower by direct exposure to AgNPs and Ag \(^{+}\), whereas leaf consumption by P. pulex only decreased when leaves were contaminated with the lowest concentration of AgNPs. There were no effects on FPOM production. Changes in the activity of a key antioxidant enzyme, catalase, indicated stress caused by AgNPs and Ag \(^{+}\) in both invertebrate species, mainly in response to direct exposure. Overall our results demonstrate that ecological effects on different functional groups of stream invertebrates vary with exposure route of AgNPs at environmentally realistic concentrations. Thus the pathway by which stream food webs are exposed to AgNPs will influence the impacts of AgNPs on food-web components and the processes in which they are involved.
Ecology, L. Guluzada, University of Tuebingen / Center for Applied Geosciences; L. Luo, University of Tuebingen / Institute of Inorganic Chemistry; J. Wicht, University of Tuebingen / Institute of Physical and Theoretical Chemistry; E. Früh, S. Dietz, University of Tuebingen / Institute of Evolution and Ecology JD Botany; C. Hahn, University of Tuebingen / Institute of Physical and Theoretical Chemistry; Y. Liang, University of Tuebingen / Institute of Inorganic Chemistry; S. Haderlein, University of Tuebingen / Center of Applied Geoscience; H.- Köhler, University of Tuebingen / Institute of Evolution and Ecology Animal Physiological Ecology

The increasing number of publications in the last years show that nanoparticles became more and more a high concern in ecotoxicology. This is, on one hand, due to increasing usage of nanomaterials while, on the other hand, little is known about their fate and interactions in the environment. Hence, we wanted to examine, if and how Al2O3 nanoparticles interact with the neomycinoid insecticide thiacloprid. To test this, we measured how toxicity of thiacloprid is altered when Al2O3-NPs are present, based on the non-biting crustacean *Chironomus riparius*, a well-established study organism in ecotoxicology. The conducted acute toxicity test with fourth instar larvae of *C. riparius* revealed that Al2O3-NPs as single substance had no effect on the mortality rate. However, larvae which were exposed to a mixture of thiacloprid and nanoparticles showed a decreased mortality rate, in relation to larvae that were exposed to thiacloprid solely. This protective effect of the Al2O3 nanoparticles was inversely correlated with the applied amount of nanoparticles, with higher concentrations of Al2O3 nanoparticles resulting in lower mortality. The underlying mechanisms of these interesting phenomena are still under investigation. Yet, solely sorption of thiacloprid onto nanoparticles (pristine and aged) can be excluded as being underlying mechanism. Further research which focuses on chemical analyses, histopathology as well as an extended exposure regime of the larvae, which is based on the OECD guideline 218.

**178 Anthracene sorption to TiO2 nanoparticles and bioavailability of UV-activated anthracene by-products in larval zebrafish.**

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Engineered nanoparticles (NPs) can be released into aquatic environments and present a risk of toxicity for aquatic organisms. Among the most commonly used NPs are TiO2-NPs, and, although these NPs appear to be of minimal toxicity, their potential for photo-activity and sorption to toxic substances are persisting environmental concerns. Several studies have shown sorption of organic compounds to TiO2-NPs and enhanced toxicity of the co-contaminants. The toxicity of polycyclic aromatic hydrocarbons (PAHs) has been extensively studied and there is information on the toxicity of UV-activated PAHs but the interaction between TiO2-NPs, PAH sorption, and UV-activation is unknown. The objective of this study was to evaluate sorption of anthracene (to TiO2-NPs by 1) analysis of the preparations with and without UVA irradiance by fluorescence spectroscopy and 2) assessment of changes in expression of target biomarker genes including cytochrome P450 1A (cyp1A), DNA repair (ddh2), superoxide dismutase (sod1) and aydril hydrocarbon receptor 2 (ahr2) genes, in larval zebrafish. Zebrafish larvae (72 hpf) were exposed (24 h) to anthracene (0-30 µL/L) in freshwater (IC50 = 71 µL/L according to OECD no. 203). Preparations were exposed to 80 kJ/m2 UVA at the end of the 24 h exposure, and larvae were sampled at 3 h after UVA exposure. For experiments in which sorption of anthracene to TiO2-NPs (4-8 nm diameter) was investigated, TiO2-NPs (2 mg/L) were added to UVA preparations and stored for 24 h prior start of exposure. Anthracene was not acutely toxic to zebrafish larvae at the concentrations tested. After the UVA exposure, zebrafish mortality was 28.4% (±SD, n=3) at the highest anthracene concentration (30 µL/L). When fish were exposed to anthracene and UVA, a 45-fold increase in cyp1A expression and a 1.7 fold-induction of sod1 were observed at 15 µg/L anthracene. The presence of 2 mg/L TiO2-NPs with anthracene resulted in a significant decrease of either cyp1A or sod1 after UVA exposure compared to unexposed controls. For fish exposed to TiO2 (2mg/L) and UVA without anthracene there was no induction of either cyto1A or sod1, which is in accordance with other studies that have also not observed photo-induced toxicity at relatively low TiO2-NP concentrations. Future work will investigate the association between TiO2-NPs and PAHs and the products of photo-induction.

**179 Influence of multiwalled carbon nanotubes on the toxicity and bioaccumulation of tribocarban in aquatic organisms of different trophic levels.**

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Although the acute toxicity of MWCNTs is rather low, this nanomaterial was shown to be taken up by different organisms and could therefore influence the bioavailability of environmental pollutants due to its high sorptive capacity. We investigated the sorption of the antibacterial and preservative agent used in personal care products, tribocarban (TCC), to MWCNTs, and the influence of this interaction on the bioavailability of TCC for organisms related to one another in the food chain than the green alga *Desmodesmus subspicatus*, the water flea *Daphnia magna*, the brine shrimp *Artemia salina*, and the zebrafish *Danio rerio*. Next to unaltered MWCNTs, the released material of plastic composites, in which MWCNTs were embedded, was tested. It was shown that the presence of MWCNTs protects the polymer matrix from degradation due to simulated solar radiation. However, up to 1% of the embedded CNTs were released from irradiated samples that were transferred to different media, whereas release from non-irradiated control samples was much lower. Both single nanotubes as well as nano- and microscale CNT-polymer fragments were detected and visualized. Both *D. magna* and *A. salina* were able to take up this material. TCC sorption to MWCNTs was quite high with sorption coefficients (LogKow) of about 7.5 in all media. This resulted in lower toxicity of TCC to all test organisms when MWCNTs were present in the exposure media. Similarly, higher bioaccumulation factors (BAFs) were obtained when MWCNTs were absent in the media. These results show that MWCNTs lower the bioavailability of TCC to freshwater and saltwater species although this nanomaterial is known to be taken up by these organisms to a relatively large extent. From the elimination experiments with *D. magna* and *A. salina*, it can be derived that excetration of TCC was faster when MWCNTs were present in the media during the uptake phase. Hence, it seems that TCC bound to MWCNTs is hindered from passing the gut epithelium. Currently, biomagnification experiments are performed in order to evaluate the importance of nanoparticle transfer through the food web. TCC, and other contaminants, will be a focus of future research. Moreover, it will be determined whether the organic compound and the nanoparticle are distributed to different organisms and tissues of fish after dietary exposure.

**Microplastics in the environment: Sources, Fate and Effects (IV)**

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Microplastics (MPs) contamination is a major concern in eco-toxicological studies. To better assess the impact of MPs on marine environment, scientists need to take ecological and environmental concerns into account. Recent estimations of small size MPs concentrations at the surface layer of the oceans were lower than model-predicted estimations based on larger plastic wastes. Different sinks were suggested to explain this mismatch including physical mechanisms and interactions with marine organisms. Among marine organisms, phytoplankton may have a role in MPs distribution. In this experiment, we studied the possibility of phytoplankton aggregates to bind and incorporate MPs through their sedimentation. At the same time we investigated the respective impacts of MPs on aggregate parameters and the impacts of aggregate composition on MPs. Three types of phytoplankton aggregates (Chaetoceros neogracilis aggregates, Rhodomonas salina aggregates and a mix of the two species) were produced in a roller-tank. These aggregates were exposed to a flow (680 mL per hour) of 2 µm microbeads of polysulphryne (yellow-green fluorescent, 1.050 kg L⁻¹, 10^6 beads mL⁻¹) in a roller tank that mimic aggregate sinking. After exposure, aggregate parameters were assessed like size, permeability, excess density, sinking rates and MPs content. Diatom aggregates were on average bigger, stronger, stickier and sank faster than the aggregates made from *R. salina* or from the mix of microalgae. After MPs exposure, all laboratory-made aggregates incorporated the MPs similarly and more than aggregates from *R. salina* incorporated more MPs than *C. neogracilis* and mix aggregates. When incorporated into aggregates, sinking rates of MPs decreased from tens to several hundred meters per day. Simultaneously aggregates sinking rates was impacted. Sinking rates of *C. neogracilis* aggregates strongly decreased while sinking rates of *R. salina* increased. This study demonstrated that MPs can be incorporated through aggregates during their settling in the water column, and that the efficiency depends on the composition of marine aggregates. A new pathway was highlighted for MPs sink that may partially explain the lack of MPs in surface layer. Even though further studies are needed with different laboratory-made aggregates or field sampled aggregates to better assess the potential for marine snow to aggregate MPs. In parallel, in-situ quantification of MPs in marine snow from should be done to address the relevance of this process in MPs cycle.

**180 Aquatic ecotoxicity testing of nanoparticles - lessons learned from nanotoxicology**

SETAC Europe 26th Annual Meeting Abstract Book
Analyses and metabolization of microplastic-associated POPs.

Micron-sized particles have so far been the main research focus in regard to aquatic plastic particle pollution. Due to mesh netting sizes, microplastics are not detected in commonly used methods for plastic pollution surveys. Nonetheless, nanoplastics are likely to be pervasive in the aquatic environment. Here, as for microplastics, nanoplastics may cause direct ecotoxic effects and carrier-effects through adsorption of co-contaminants and the smaller particle size could be an influential factor. However, actual knowledge is currently limited to a few scientific studies. Based on literature information, combined with our own previous work on nano- and microplastics as well as test method development and adaptation for ecotoxicity testing of engineered nanomaterials, we here present an overview of lessons learned from nanocotoxicology. We offer suggestions on how these can be transferred into recommendations for eco-toxicologically testing of nanomaterials. In addition we suggest how to implement some of these recommendations in algae growth inhibition tests, studies of carrier-potential of nanoplastics and uptake in aquatic invertebrates and fish.

181 Effects of ingested PVC micro particles with and without sorbed benzo(a)pyrene on the cellular and sub cellular processes of the macrobenthonic organism Hediste diversicolor

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Along with impacts such as global warming, ocean acidification and habitat destruction, pollution can cause undesirable changes in marine ecosystems. In the last decade, plastic pollution has been a matter of increasing scientific and public concern. Marine organisms are exposed primarily through ingestion of plastic polymers and it is considered a multiple stressor in aquatic habitats as a consequence of the large mixture of chemical contaminants potentially adsorbed from the aquatic environment and/or associated with it in the polymer production. Polymers with densities higher than seawater (i.e., PVC) tend to sink and continuously accumulate in sediments. Animals from sedimentary habitats are therefore vulnerable as microplastic particles may adsorb organic pollutants from the aquatic environment and be largely accumulated in sediments. In laboratory conditions, microplastics have been shown to be ingested by amphipods, barnacles, mussels and lugworms [1]. However, the available data concerning the effects of microplastic particles is limited and even less is known concerning the “Trojan horse” effect caused by microplastics contaminated with ubiquitous and highly toxic environmental contaminants such as PAHs. Thus, the current study focused on the acute effects of the combined exposure to microplastics and benzo(a)pyrene sorbed particles on the macrobenthonic model organisms Hediste diversicolor, a species with a relevant role in trophic webs.

182 A trophic food web experiment with Artemia nauplii and zebralish (Danio rerio) for the transfer of microplastics and associated POPs

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In the present study, a simple artificial food chain with Artemia spec. nauplii and zebralish (Danio rerio) was established to analyze the transfer of microplastic particles and associated persistent organic pollutants (POPs) between different trophic levels. The uptake of microplastic particles and the transfer of potential harmful substances along with microplastics has been studied in a variety of organisms, especially invertebrates. However, the potential accumulation of very small microplastic particles along food webs ending with vertebrate models has not been studied so far. Artemia nauplii and zebralish were exposed to very small (1 - 20 μm) microplastic particles, which accumulated within nauplii and were subsequently transferred to fish. For the analyses of the transfer of POPs via microplastics, the polycyclic aromatic hydrocarbon (PAH) benzo(a)pyrene (BaP) was used since (a) it is a common POP also found on environmental microplastics and (b) it shows autofluorescence at respective wavelengths. Virgin particles not loaded with BaP did not cause any observable physical harm in the intestinal tracts of zebralish. The visual fluorescence tracking of BaP was strong enough to detect the transfer and accumulation of BaP both in Artemia nauplii and zebralish intestines. This indicates that food-borne microplastic-associated POPs do desorb in the intestine of fish and are thus transferred to the intestinal epithelium and liver of higher organisms. However, further research is needed on different spectra analyses and metabolism of microplastic-associated POPs.

183 Does microplastic in recycled organic resources pose a risk to terrestrial environments?

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Problems related to plastic litter pollution have been recognised since early 1970’s, and in recent years microplastic (MP) contamination of particularly the marine environment has been subject to increased focus. Although a number of publications in the last couple of years have pinpointed that also freshwater systems may be at risk from MPs, mainly due to release of particles from waste-water (WW) treatment processes, MP contamination of terrestrial environments has up until now rarely been studied. Due to processes taking place in WW treatment plants, a large proportion of particles in WW is likely to settle out and sludge, or biologically degrade with mineralization. This reduces the amount of MPs in the effluent water and consequently contaminates of aquatic environments. However, when sludge resulting from WW treatment processes is used for soil amendment, in order to recycle phosphate for agricultural purposes, deposited plastics are added to the terrestrial environment. Because plastic is slowly broken down, it may accumulate in soil, and assess potential risk of MPs to terrestrial environments it is necessary to increase knowledge on type and amount of MPs in organic resources as well as in recipient soils. At the same time studies of potential effects of MP to soil organisms is largely absent from the scientific literature. In this presentation we give an introduction to what we currently know about potential exposure in terrestrial environments, including some recent studies from our lab on type and amount of MPs in Danish WW sludge and soils fertilised with different organic resources. These studies confirm the presence of MPs in both sludge and soils fertilised with sludge or composted household waste. We also include preliminary information on hazard of MPs in terrestrial environments, based on recent experiments on epigeic earthworms, studying the effects of MPs along with the combination with relevant contaminants. The first studies showed limited effects of MPs on earthworms, which however may be due to the employed exposure scenario. Results will be compared to on-going studies with a modified exposure scenario. Although studies so far suggest that MPs will end up in the terrestrial environment, knowledge on hazard of MPs for terrestrial organisms is currently limited as are more ecologically relevant exposure predictions. Therefore more knowledge is required to realistically assess the risk of MPs in terrestrial environments.

184 Be positive or negative? Long-term toxicity of polystyrene nanoparticles to marine planktonic species

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Micro as well as nanoplastics have been identified by the international community as one of the greatest challenges for marine ecosystems worldwide. Concerning the nano-sized debris (<1 μm), their occurrence in the environment has not been quantified yet, due to current standardized methods for sampling as well as identification by analytical techniques. Polystyrene nanoparticles (PS NPs) have been recently adopted as model for nanoplastics in short-term ecotoxicological studies, but data concerning long-term toxicity on marine organisms are still scarce. The present study addressed the long-term effects of polystyrene nanoparticles (PS NPs) on marine planktonic species as green microalgae Dunaliella tertiolecta and brine shrimp Artemia franciscana, exposed to anionic carboxylated (PS-COOH) and cationic amino (PS-NH2) PS NPs. Since aggregation and surface charges can be key factors driving the eco-interactions of nanoparticles in the marine environment, their behaviour was assessed by DLS in both natural sea water (NSW) and algal medium (AM) as well as the formation of an eco-corona investigated. In terms of ecotoxicity, PS-COOH did not affect the growth of marine algae (72 h) and brine shrimp (14 d) up to 25 μg/ml and 10 μg/ml respectively but high retention of PS-COOH aggregates was observed in the digestive tract of brine shrimps at 14 days. It may oppose, F in soils. To assess mortality of brine shrimps (LC50 0.83 μg/ml, 14 days) as well as inhibition of algal growth and alterations (at 5 μg/ml) in the microalgae plasma membrane respect to the control group. These results represent a first insight into nanoplastics long-term toxicity to marine planktonic species underlining the role of nanoparticles surface charge in agreement with previous findings on other organisms and marine species. This study emphasizes that long-term exposure constitutes a valid tool to assess the ecotoxicity of emerging contaminants including nanoplastics.

State of the science on poly- and perfluoroalkyl substances (PFASs) in the environment and humans (I)

185 Characterizing spatiotemporal pattern of global PFAS releases using a dynamic flow analysis model

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This work developed a dynamic substance flow analysis (DSFA) model to imitate the temporal evolution and spatial shift of global environmental releases of homologous fluorotelomer and perfluorocarboxylic acids from both lifecycle and end-of-life stages. Major findings include: (i) The temporal pattern is illustrated by the evolution of environmental releases of PFOA and 8:2FTOH in China. Our DSFA results show that the major processes for global PFOA releases, therefore the scheduled phase-out of PFOA is anticipated to nearly eliminate future PFOA releases, while industrial processes and end-of-life disposal have almost equal contributions to 8:2FTOH releases. The time course of environmental releases of 8:2FTOH exhibit two sequential peaks a 10-year gap, which indicates that emissions from waste stocks will be noticeable in the future. (ii) The spatial pattern is demonstrated using snapshots of geographical distribution of global PFOA and 8:2FTOH emission hotspots for 1995, 2010 and 2020. The emission hotspot of PFOA shifted from Europe and North America in 1995 to China in 2010. The evolution of 8:2FTOH emissions in China and Japan is one of the largest increases, corresponding with a 10-year delay. This delay means that the Western Pacific would become a remaining dominant source region in 2020 if no appropriate end-of-life management are implemented. Our case highlights the implication and importance of the DSFA in future risk management of PFASs. Based on our DSFA-derived emission estimates, scientists are able to extrapolate the performances of global end-of-life management options and assess the impacts of the geographical shift of emissions on environment and human.

186 Assessing the temporal shift of concentrations of perfluorooctane sulfonic acid (PFOS) in the environment following industrial transition

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Along with impacts such as global warming, ocean acidification and habitat loss, plastic pollution is becoming one of the greatest challenges for marine ecosystems worldwide. Concerning the aquatic environment and be largely accumulated in sediments. In laboratory experiments, PS-COOH aggregates was observed in the digestive tract of brine shrimps at 14 days post-exposure to anionic carboxylated (PS-COOH) and cationic amino (PS-COOH) nanoparticles. The long-term toxicity of PS-COOH nanoparticles to freshwater invertebrates and fish is largely absent from the scientific knowledge. However, previous studies on other invertebrate marine species. This study emphasizes that the food chain is a major route for the transfer of POPs to higher trophic levels and indicates that POPs desorb from the fish diet to the water environment, this would subsequently transfer to fish. For the analyses of the transfer of POPs from sediment to fish, zebrafish transport model is used.

187 Levels of perfluoralkyl substances in global air from 3 years of sampling under the Global Atmospheric Passive Sampling (GAPS) Network

C. Raurer, Environment Canada / Air Quality Processes Research Section; L. Andren, Swedish Environment Research Institute / Chemical and Bioengineering; J. Chang, University of Michigan / Department of Atmospheric Sciences and Assessment; J. Schuster; M.E. Shoeb, Environment Canada / Atmospheric Science and Technology Directorate; T. Harner, Environment Canada / Atmospheric Science Technology Directorate. Poly- and perfluoralkyl substances (PFASs) have been used globally with industrial and commercial applications that include oil and water repellants, and fire-fighting foaming foams. Perfluorooctane sulfonic acid (PFOS) and its precursors are of particular interest as it is a priority chemical under the United Nations Environment Program (UNEP) Stockholm Convention for Persistent Organic Pollutants (POPs). The Global Atmospheric Passive Sampling (GAPS) Network monitors priority chemicals at over 50 global sites, and is the only global-scale air monitoring network. GAPS sites are equipped with passive air samplers (PAS) during three sampling years, to determine spatial and temporal trends of PFASs in global air. The data presented here shows the first global air comparison of PFASs, using SIP disk PAS samplers. Perfluorobutanoic acid (PFBa) had the highest concentrations in all samples (3) and was detected at almost all site sampled during the 3 campaigns. Temporal trends were not evident for any of the perfluorosulfonic acids. However, PFBa showed evidence of reduced levels at Asia and African sampling sites as compared to levels seen in Europe and Central/North America. PFOA had the highest concentrations of the sulfonic acids (3) and a reduction in global air concentrations of this chemical due to the phase out of PFOS in 2009 is not yet in evidence.

188 Effects of weathering on PFASs used in durable water repellence of textiles

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Per- and polyfluoralkyl substances (PFASs) are used in textiles for their oil and water repellent properties. Because PFASs with long perfluorinated chains have shown to be persistent in the environment, bioaccumulative and (eco)toxic, the textile industry is phasing out the long-chain PFASs and is replacing those compounds with alternative chemistries to deliver the desired durable water and soil repellent (DWR) effect. Those alternative chemistries can be divided in three main groups: fluorocarbon-based, silicon-based and hydrocarbon-based polymers. In 2017 SUPFES (Substitution in practice of prioritised fluorinated compounds for textile applications) project the alternative DWRs are assessed to (i) their structural properties and connected performance, (ii) loss and degradation processes resulting in diffuse environmental emissions, and (iii) hazard profile for the emitted substances. As part of SUPFES weathering experiments are performed with textiles from outdoor clothes which are exposed to elevated UV radiation, humidity, and temperature in an aging device for 300 h, which is equivalent to the life time of the outdoor clothing. Before and after aging of the samples, the textiles were analysed for their perfluoralkyl acid (PFAA) content. Results showed that weather conditions, like sunlight, high temperature, or humidity have an effect on PFASs used in DWR of outdoor clothing. Concentration of PFASs increased, and PFASs not present in the original textiles were formed during exposure to weather conditions. More research is needed to clarify the origin of the PFASs and to determine the transformation route.

189 Investigation on the presence and behaviour of precursors to perfluorinated compounds in the environment

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Wastewater treatment plants (WWTPs) have been identified as a significant pathway for the introduction perfluorooctanoic acid (PFOS) to natural waters. In several studies it was observed that the concentration of certain PFASs were higher in the WWTP effluent compared to the corresponding influent. One reason for this might be the biotransformation of precursors substances, which are converted into known and analysed PFASs. The objective of the present study was the identification of potential precursor substances of persistent PFASs in WWTPs. Following the development of robust and validated analytical methods 65 PFASs, which included persistent PFASs and their potential precursors, sampling campaigns in six WWTPs (industrial and municipal, located in Europe) over a period of four weeks each were realised. The influent water and the effluent water, the air above the influent and the sludge of the WWTPs were sampled and analysed. Several transformation products of fluorotelomer alcohols (FTOHs) e.g. the unsaturated fluorotelomer carboxylic acids (FTUCAs), fluorotelomer carboxylic acids (FTCAs) and the x3-carboxylic acids could be observed in the influent and, particular, in the effluent of the WWTPs. The further biotransformation of these intermediates led to an increased concentration of perfluoralkyl carboxylic acids (PFACAs) in the effluent of WWTPs. Based on the frequency of detection and concentration of FTOHs, biotransformation intermediates (e.g. FTUCAs and FTCA) and persistent biotransformation products, fluorotelomer alcohols / olefins and precursors were identified as most relevant precursors of PFACAs. Data for several corresponding WWTP influent, air and effluent samples suggests that FTOHs could be present as a residual synthetic intermediate of non-targeted PFASs, such as fluorinated polymers or other unknown low molecular weight fluorotelomer-based chemicals, which might be a further reason for the high frequency of detection. This should be investigated in future studies.

190 Perfluorinated alkyl substances (PFASs) in household dust in Central Europe and North America

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SETAC Europe 26th Annual Meeting Abstract Book
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In everyday life we come into contact with various chemical substances contained in consumer products. Perfluorinated alkyl compounds (PFCAs) are one of the largest groups of such chemicals. PFCAs have been used in a variety of applications such as surfactants, lubricants, in paper and textile coatings, polishes, food packaging, and fire-fighting foams for more than 60 years. They are added to consumer products to make them resistant to water, oil, stains and even fire. They are persistent, bioaccumulative and they have become ubiquitous. Several exposure pathways for PFCAs have been identified: food and drinking water are the most important ones, followed by dust ingestion and indoor air. Humans spend almost 90% of their time indoors and most children play on the floor and hand-to-mouth activity is more significant than for adults. This can lead to higher exposure to PFAS than for adults. This work is part of a larger study within the Czech-American Scientific Cooperation Program aimed at identifying levels of contamination in residential indoor environments from three countries (Czech Republic, Canada and USA). The aim of the present study was to investigate and compare indoor concentrations of twenty PFASs in house dust samples collected in living rooms and bedrooms in 2013 from more than 30 homes of volunteers in these three countries. We also looked at differences between countries and compound patterns. Several PFASs were found in dust samples from more than 50 homes from three countries. Concentrations are of the same order of magnitude as in previous studies. Slightly significant differences were observed between samples from North America and Czech Republic. The dominant contaminant in all countries was PFOS but eight out of 20 target PFASs were detected in more than 50 percent of the samples. Among the carboxylic acids, PFHxA is a major contaminant in Czech samples while in North America PFOS and PFOSA are the dominant contaminant. Correlation analyses revealed good correlations between PFOS and shorter-chain carboxylic acids, suggesting that short-chain PFCAs are in use but still do not replace PFOA.

Interpreting Biological Effects of Metals and Their Mixtures

191

Metals in the aquatic environment - interactions and implications for the speciation and bioavailability
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In most case scenarios, individual metals exist as components in mixtures with organic and inorganic substances and/or particulate matter. While the concepts encompassing mixture toxicity and modeling have been around for decades, only recently have new approaches (dynamic speciation techniques and fate and bioavailability models) been expanded to consider metal mixture scenarios. For example, the kinetic features of humic substances and inorganic colloids on the complexation of metal ions are generally considered. Although current environmental regulations rarely require the assessment of metal mixtures, research on these mixtures in the environment is essential for future regulatory demands and it is vital for ensuring adequate environmental protection. Interpretation of speciation and bioavailability data from metal mixtures can be very complex and demanding, due to the existence of kinetic physicochemical transformations of the dynamic components. The mixture effect largely affects metals' dynamic speciation, culminating in different transformed metal-containing products with different contributions for the metal uptake by a consuming interface. The environmental fate of metal mixtures, which determines how the mixture is biogeochemically processed and which receptors are most exposed (organisms and exposure route), with a special focus on their dynamic speciation, including a critical evaluation of the current challenges and available dynamic speciation techniques as well as computer codes and models, will be discussed.

192

Chronic metal mixture toxicity to Ceriodaphnia dubia can be predicted using an independent action based bioavailability model
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Currently, several bioavailability-based models have been shown to predict acute metal mixture toxicity with reasonable accuracy. However, the application of such models to chronic mixture toxicity is less well established. In this study, we developed a chronic metal mixture bioavailability model (MMBM) by combining the extrapolation of their independent bioavailability models for the individual metals with the independent action model. The MMBM assumed that each metal binds to its own biotic ligand (BL) site and that metal (mixture) toxicity is related to the concentration of free metal ion binding to its metal-specific BL. Additionally, based on previous toxicity testing with Ni, Zn, and Pb mixtures, we assumed that Ni<sup>n+</sup>, Zn<sup><b>2+</b></sup> and Pb<sup>2+</sup> do not compete for binding at the biotic ligand sites. To evaluate the predictive capacity of the MMBM, we investigated the chronic toxicity of a Ni-Zn-Pb mixture to Ceriodaphnia dubia in 6 (modified) natural waters differing in pH, Ca, and/or dissolved organic carbon (DOC) (pH range: 7.8-8.2, Ca range: 1.2 mM, DOC range: 5-12 mg/L). The toxicity of the Ni-Zn-Pb mixture was assessed concurrently with that of the individual metals. The predictive capacities of the chronic MMBM for mixtures was evaluated relative to that of the MMBM for the individual metals. Dissolved metal toxicity in the individual metal treatments, expressed as 7d-EC50, varied 4.3-fold, 3.3-fold, and 2.7-fold for Zn, Ni, and Pb depending on the water chemistry, respectively. The MMBM predicted the relative reproduction of 85% of the mixture treatments within 20% error, while the relative reproduction in the individual Zn, Ni, and Pb exposures were predicted within 20% error for 73, 83, and 70, respectively. This indicates that the MMBM predicted chronic toxicity of the ternary Ni-Zn-Pb mixture at least equally well as the observed chronic toxicity to the individual metal treatments. Based on our study chronic MMBMs are a promising tool to account for the effects of water chemistry on metal mixtures during chronic exposure and could be used in metal risk assessment frameworks.

193

Uptake and toxicity of copper and cadmium in single and mixture exposures in the zebrafish, Danio rerio
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The aquatic environment receives a wide range of pollutants from a number of sources and is highly impacted by pollution. Due to the simultaneous presence of numerous pollutants it is important to investigate the effects of co-contamination. While the concepts of metal mixture toxicity with reasonable accuracy. However, the application of such models to chronic mixture toxicity is less well established. In this study, we developed a chronic metal mixture bioavailability model (MMBM) by combining the extrapolation of their independent bioavailability models for the individual metals with the independent action model. The MMBM assumed that each metal binds to its own biotic ligand (BL) site and that metal (mixture) toxicity is related to the concentration of free metal ion binding to its metal-specific BL. Additionally, based on previous toxicity testing with Ni, Zn, and Pb mixtures, we assumed that Ni<sup>n+</sup>, Zn<sup><b>2+</b></sup> and Pb<sup>2+</sup> do not compete for binding at the biotic ligand sites. To evaluate the predictive capacity of the MMBM, we investigated the chronic toxicity of a Ni-Zn-Pb mixture to Ceriodaphnia dubia in 6 (modified) natural waters differing in pH, Ca, and/or dissolved organic carbon (DOC) (pH range: 7.8-8.2, Ca range: 1.2 mM, DOC range: 5-12 mg/L). The toxicity of the Ni-Zn-Pb mixture was assessed concurrently with that of the individual metals. The predictive capacities of the chronic MMBM for mixtures was evaluated relative to that of the MMBM for the individual metals. Dissolved metal toxicity in the individual metal treatments, expressed as 7d-EC50, varied 4.3-fold, 3.3-fold, and 2.7-fold for Zn, Ni, and Pb depending on the water chemistry, respectively. The MMBM predicted the relative reproduction of 85% of the mixture treatments within 20% error, while the relative reproduction in the individual Zn, Ni, and Pb exposures were predicted within 20% error for 73, 83, and 70, respectively. This indicates that the MMBM predicted chronic toxicity of the ternary Ni-Zn-Pb mixture at least equally well as the observed chronic toxicity to the individual metal treatments. Based on our study chronic MMBMs are a promising tool to account for the effects of water chemistry on metal mixtures during chronic exposure and could be used in metal risk assessment frameworks.

194

Departures from additivity in a wastewater sample spiked with binary mixtures of heavy metals
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Due to the well-known toxicity of heavy metals, monitoring and regulation of their concentration in the environment has been a very important issue in the past years. There are many methods for heavy metals detection based on physical and chemical techniques, however, such methods are not able to distinguish between-available and non-available fractions of metals existing in the environment. In contrast to chemical methods, whole-cell bioreporters measure the bioavailable fraction of these analytes. Recently, we have mathematically developed a novel additivity framework for mixture research in the context of whole cell inducible biosensors [1]. This new method is based on Concentration Addition (CA) model, also called Loewe Additivity. Our new approach proposes a multivariate extension of the effective dose (ED)<sub>50</sub> to take into account the occurrence of differential maximal effects presented by the mixture components, and also, an extension of Loewe Additivity that enables its direct application in a biphasic dose-response framework. Moreover, this method proposes a two-dimensional formulation of Loewe additivity computed for the two components of ED<sub>50</sub> dose (D) and effect (E). Analyses of departures from additivity are based on Concentration Index (CI) [2] for the D and E dimensions, where, CI<sub>D</sub>, CI<sub>E</sub> or CI<sub>CI</sub>=1 indicates...
additivity effect and Cl₂ or Cl₂>1 indicates antagonism. For global assessment of departures from additivity, a simplification of the two-dimensional information can be obtained by calculating a weighted index CIₚ for any fractional effect p: CIₚ=Clₓ-Clₓ, where Clₓ>1 indicates overall antagonism, CI₁= an overall additive effect, and CI₁ [1 Martin K et al. (2015) Scientific Reports 5:17220 [2] Rodkiewicz et al. (2010) Water Research 44:427-438 [3] Martin K et al. (2015) Frontiers in Microbiology 6:186 Acknowledgement – This research was supported by MINECO grant CTM2013-45775-C2-2-R

195 Effect of temperature on Ni bioaccumulation and chronic toxicity to Daphnia magna
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The mechanisms involved in nickel toxicity are not fully understood. No information is available about the effect of temperature on chronic nickel toxicity to Daphnia. Therefore, our first objective was to investigate the effect of temperature on chronic nickel (Ni) toxicity and if a different effect is observed among four Daphnia magna clones. The second objective was to understand how temperature affects Ni uptake and elimination in four D. magna clones. At 15, 20 and 25°C 21-day life table experiments with Ni were performed with four D. magna clones taken from the same natural population. A linear model was built to predict the Ni effect on the reproduction per individual female (R₀) as function of temperature (T) and cliff effect concentration (C Cliff). The relationship response curves were calculated for R₀ of all clones (to mimick the effects on a population). Effect concentrations of the individual clones were also calculated. Uptake experiment was performed with the four D. magna clones exposed to 70 μg Ni L⁻¹ at 15, 20 and 25°C. Organisms were exposed to the stable isotope ⁶⁵Ni during 48h. The linear model built indicated that the effect of nickel on reproduction per individual female (R₀) was significantly affected by temperature, and that this effect depended on the clone. The 21 d EC₅₀ based on R₀ of all clones (to mimick a population) tested at 15, 20 and 25°C were 59.2, 74.3 and 121.2 μg Ni L⁻¹, respectively. This represents a two-fold variation on the 21 d EC₅₀, significant interactions were detected between temperature, nickel and clones. After 48h of exposure nickel concentrations in Daphnids were lower at 25°C than at 15 or 20°C. No significant correlation was observed between 21 d EC₀ and nickel accumulation after 48h of exposure. This suggests that it is not only the internal body concentration determines the effect of temperature on Ni toxicity but that effect of temperature on the internal sequestration or toxicodynamics processes may also play a role in nickel toxicity. The results of the present study are in contrast with previous acute studies which indicate that acute metal toxicity increases with increasing temperatures. Additionally, on average chronic nickel toxicity decreased at higher temperatures. The present study showed that chronic nickel toxicity was significantly affected by temperature and this effect was different among the four D. magna clones tested.

196 Fishing for mixture effects: impacts of diclofenac and cadmium on oxidative stress in a threatened indigenous fish species
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Pharmaceuticals and trace metals are increasingly prevalent in the aquatic environment. Significant interactions pressures such as population increases. Although present at low concentrations, environmental persistence and high bioavailability of these chemicals can result in toxicological impacts on non-target biota. There is however, very little understanding of how emerging contaminants, such as pharmaceuticals interact with other toxicants. This study aimed to investigate the toxic impacts that occur when a native New Zealand fish, inanga (Galaxias maculatus), is exposed to mixtures of an emerging pharmaceutical contaminant, diclofenac (DCF), and a trace metal, cadmium (Cd). Inanga inhabits near-coastal freshwaters, and migrate through estuaries. As result they are potentially exposed to water bodies where toxicants are most concentrated. In laboratory experiments, inanga were exposed to single Cd or DCF or their mixtures (DCF, 1000 μg L⁻¹; Cd, 10 μg L⁻¹) for 96 h. The sublethal effects on hepatic oxidative stress were examined by measuring oxidative damage (lipid peroxidation) and antioxidant defence (catalase). There was a significant increase in hepatic catalase activity in fish exposed to Cd (10 μg L⁻¹) alone. However the increase in this important anti-oxidant defence enzyme was not sufficient to prevent an increase in lipid peroxidation, which also increased significantly relative to the unexposed control. Fish exposed to the Cd (10 μg L⁻¹) and DCF (1000 μg L⁻¹) mixture exhibited a different response whereby lipid peroxidation decreased compared to the individual Cd exposure. These results indicate that DCF is protective against metal-induced oxidative damage. These are the first such data to investigate the net effects of DCF and metal mixtures in fish. Understanding the impacts of toxicant mixtures, and the mechanisms by which they modify toxicity, is a critical need for the development of realistic environmental regulations.

Interactive effects of climate change and contaminants: environmental risks and human health implications
197 The impacts of pharmaceutical drugs under ocean acidification: new data on single and combined chronic effects of Carbamazepine on Scrobicularia plana
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Ocean acidification (due to increased oceanic CO₂ uptake from atmosphere) and increasing discharges of pharmaceutical drugs, such as carbamazepine (CBZ), an antiepileptic, into the aquatic environment can exert toxic effects on marine organisms, including bivalves. However, the impacts of pH and CBZ when combined are poorly studied. Thus, the present study evaluated the chronic effects (28d) of an environmental concentration of CBZ (3 μg L⁻¹) and the decrease of pH (to 7.1) alone and in combination on the marine clam Scrobicularia plana. CBZ quantification based on ELISA, protein content (PROT), electron transport activity (ET), lipid peroxidation (LPO) levels, glutathione S-transferase (GSTs), superoxide dismutase (SOD) and catalase (CAT) activities were determined. The mortality rates were 23% and 28% for the exposure to pH (7.1) led to lower mortality (22%) than the exposure to single CBZ or the combination of CBZ and pH (33% for each) and that in individuals exposed to low pH mortality was recorded up to the first 96 h of exposure. This indicates an adaptive response of clams to the low pH (7.1), possibly due to changes in acid-base homeostasis, through an increase in the H⁺ concentration. The exposure to CBZ led to an increase in Ni⁺ concentration, affecting the cellular acid-base homeostasis and resulting in higher mortality in clams exposed to CBZ and CBZ + pH. PROT increased after the exposure to CBZ and pH, single or combined, possibly indicating a higher rate of enzyme synthesis to prevent oxidative damage. The increase of GST's activity under all exposure concentrations may be a consequence of that. ET decreased after all exposure conditions, revealing a lower metabolic rate as a defense mechanism to prevent the damage induced by these stressors. LPO results indicated higher levels in clams exposed to CBZ and pH, especially when combined. The activities of SOD and CAT were maintained or significantly decreased in comparison with the control, respectively, justifying higher LPO levels observed after exposure to CBZ, pH 7.1 and their combination. In conclusion, S. plana avoided oxidative damage under low pH for a long period (28 days), showing higher survival when exposed to this stressor compared to CBZ or CBZ combined with pH 7.1. The toxicity was higher when clams were simultaneously exposed to both stressors (CBZ + pH 7.1) since individuals' survival was reduced and oxidative stress increased when compared to single exposures.

198 Coping with pharmaceuticals and personal care products (PPCP) in a changing ocean: bioaccumulation and ecophysiological implications in seabass (Dicentrarchus labrax) exposed to diclofenac
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Anthropogenic activities have led to great environmental challenges: remarkable chemical contamination and dramatic climate change. Both factors strongly affect marine ecosystems and are expected to worsen in the future, threatening marine species’ welfare and survival. It is unknown how marine organisms will cope with the exposure to chemical contaminants under climate change. Given the lack of data and regulation for the the presence of the emerging the pharmaceutical and personal care products (PPCP), monitoring of these compounds in the aquatic environment becomes crucial. In this way, assessing climate change effects is even more imperative. Hence, the present work aimed to assess the synergistic effects between climate change and the exposure to the widely and massively used PPCP diclofenac, by analysing its bioaccumulation and elimination process in the European seabass (Dicentrarchus labrax), as well as the physiological and biochemical implications to this fish species. Results suggested that temperature and pH strongly influences the bioaccumulation and elimination of diclofenac, particularly it’s partitioning in the different fish organs. Widespread impairments in fish enzymatic machinery, and a positive correlation between diclofenac...
accumulation and fish biochemical responses were observed. Hence, the deleterious synergistic effects of ocean warming and acidification combined with dolofenac exposure observed in the present work evidenced great biological challenges to marine vertebrate populations in the NE Atlantic coastal ecosystems in the future.

199 Global warming causes conflicting effects on pesticide sensitivity: integrating multiple effects across latitudes

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Freshwater biodiversity is particularly vulnerable to global warming and pesticides. Both stressors are directly linked because most pesticides are assumed to be more toxic at higher temperatures. In addition, habitat loss because of the net effect of pesticides in nature when their degradation is more rapid at higher temperatures. Understanding how both stressors interact is therefore crucial to correctly estimate the ecological impact of pesticides in a warming world.

Therefore, we investigated in larvae of the damselfly Ischnura elegans multiple effects of the pesticide chlorpyrifos (CPF) under a simulated global warming scenario on life history, thermal tolerance and physiology. By studying both low- and high-latitude populations we applied a space-for-time substitution to assess the role of thermal adaptation in shaping the vulnerability to CPF under global warming. CPF exposure negatively affected survival, growth rate, thermal tolerance, fat content and AcH activity and increased lactate dehydrogenase (LDH) activity. Under simulated warming CPF caused lower mortality, a less strong growth reduction (in high-latitude larvae) and less oxidative damage than at 20°C. This could be explained by the lower degradation of CPF at 20°C leading to a higher accumulation after four pulses compared to the treatment at 24°C. This indicates that testing effects of global warming on pesticide sensitivity may be misleading in experiments that keep pesti cide concentrations artificially constant. Furthermore, this temperature effect on mortality was fine-tuned by local thermal adaptation with at a given temperature mortality being lowest in larvae tested at their local mean summer water temperature. This pattern suggests that the lower impact of a temperature increase of 4°C on the toxicity of CPF can be further strengthened if gradual thermal adaptation takes place in the high-latitude larvae. Notably, CPF exposure reduced the thermal tolerance and therefore the ability to deal with heat waves. This generates the complex scenario where exposure to CPF under a realistic field application scenario may cause lower direct negative effects under global warming, yet may cause higher negative effects in case of heat waves which are expected to increase in strength, duration and frequency under global warming.

200 Exposure to a heat wave under food limitation makes an agricultural insecticide lethal

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Synergistic combinations of anthropogenic stressors are of major concern for biodiversity loss. While extreme temperatures and exposure to agricultural pesticides are becoming more frequent and intense under global change, their joint effects have been poorly studied. The potential for delayed interactions, and their modulation by the often co-occurring food limitation may be especially problematic, yet have been ignored. We tested for the effects of a transient heat wave combined with food limitation, and subsequent exposure to a widespread agricultural pesticide (chlorpyrifos) in Coenogonium puelha damselfly larvae. The direct effects of the heat wave included a 3% increase in mortality and reductions in immune function (measured as activity of phenoloxidase, PO) and in metabolic rate (measured as activity of the electron transport system, ETS), which were not magnified by starvation. Starvation had both direct and delayed negative effects on growth rate, Hsp70 levels, total fat content, and activity levels of PO and ETS. Exposure to chlorpyrifos negatively affected all response variables, yet only in larvae that were previously exposed to both the heat wave and starvation. This was partly explained by the inhibition of acetylcholinesterase (AChE) and the cumulative metabolic depression caused by each of these stressors. Furthermore, the delayed negative effects of the transient heat wave and starvation, combined with the direct negative effect of chlorpyrifos, considerably reduced larval growth rate. The here identified delayed synergism provides a novel explanation for the poorly understood delayed effects of heat waves and of sublethal pesticide concentrations to cause mass mortality.

201 Multi-biomarker responses between two dreissinid species in a global change context

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Under actual climate warming, further stressors such as contaminants might impact species distribution and ecosystems functioning. The ability of ectotherms to handle multiple stresses (food limitation and temperature change) will depend on their acclimation or adaptation capacities. Current literature raises the importance of understanding how organisms will respond and adapt to these changes in abiotic factors. Biomarkers can be used as very informative tools to evaluate exposure and effects of stressors on organisms. The freshwater bivalve Dreissena polymorpha is currently used in bio-monitoring because it is a widespread species, tolerant to a wide range of environmental conditions and contaminants. It presents a sedentary lifestyle and is a filter feeder, often used to evaluate the accumulation of contaminants in its tissues and their subsequent effects on biological processes. However, D. polymorpha populations are in general contaminated with non-exposed organisms. Even if the two species do not respond to multiple stresses the same way. We first showed that temperature do not affect biomarker responses four days after heat stress. However, we evidenced great differences between the two species, since biomarker responses were generally higher for D. polymorpha than for D. bugensis, particularly in non-exposed organisms. Even if the two species assimilated both metals the same way, the effects of contamination on biomarker responses were more marked in D. polymorpha, especially under nickel exposure.

Higher tier tests in the risk assessment of plant protection products (I)

202 Aquatic mesocosm studies - use in risk assessment

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Aquatic mesocosm studies are a useful tool in aquatic risk assessment, as the highest experimentallier in aquatic effects assessment. These studies are the only experimental studies looking at populations and communities potential effects and recovery and as such aligned with the specific protection goals for aquatic invertebrates and primary producers. Furthermore they are the reference tiers against which lower tier approaches can be calibrated. Despite this, the conduct of such studies is reducing because they are becoming less accepted in regulatory risk assessment, as the interpretation of such studies and the assessments become ever more precautionary. The background to mesocosm studies and information on their design, conduct and interpretation can be found in the EFSA Aquatic Guidance. New validity criteria have recently been developed with respect to the number of sensitive and vulnerable taxa which should be present in the test systems and meet statistical criteria (appropriate minimal detectable difference – MDD) with the aim of ensuring that the effects could be detected and recovery observed. Strict adherence to these criteria can lead to studies being declared invalid and consequently ignored in the risk assessment. Despite not meeting the new criteria studies can still contain very useful information and should not be ignored in the assessment. Mesocosm studies can be bespoke to answer specific questions posed by the formulation problem and they should be evaluated similarly. Illustrative examples, such as how mesocosm studies can contribute to and inform the risk assessment, whilst potentially not fulfilling all validity criteria.

203 How to deal with multiple micro-/mesocosm studies for the ETO and ERO-RAC derivation

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In the tiered effect assessment scheme developed for the EFSA Aquatic Guidance Document (2013), in principle, all tiers are able to address the ETO (Ecological Threshold Option). However, only the model ecosystem approach (micro-/mesocosm experiments) allows to address the ERO (Ecological Recovery Option) when addressing risk to algae, vascular plants or invertebrates. In order to address the reliability of the micro-/mesocosm experiments for the ETO and ERO-RAC derivation the AGD establish some requirements to be fulfilled (e.g. at least 8 potentially sensitive populations present in sufficient number with an acceptable MDD value). In this study we selected an insecticide as a case study
for which more than 6 micro-/mesocosm studies performed in different parts of the world and/or under different experimental condition to to assess if the power of the statistical test of these studies was sufficient to demonstrate treatment-related responses. From the 6 studies evaluated 4 of them comply with the criteria (at least 8 taxa of potentially sensitive taxonomic groups with MDDabu values < 100%) proposed by Brock et al. (2015). Two studies don’t fulfil the criteria proposed as there were less than 8 potentially sensitive taxonomic groups belonging to Category 1 taxa. This can be partially explained by the fact that those studies were focused only on zooplankton and the number of taxa evaluated was lower than in the other studies. The results obtained after the re-evaluation of the data are similar to those reported previously in published reviews and scientific papers and in the new EFSA Aquatic Guidance Document with an effect class 2 concentration of 0.1 μg/L. This threshold level has been derived irrespectively of climatic test conditions (temperate, Mediterranean and also tropical as is the case of Thailand) and also even irrespectively of the exposure pattern (single application simulating spray drift and repeated application simulating run-off events). This can be explained by the fact that the most sensitive taxa (crustaceans and insects) were well represented in the cosmos evaluated.

204 Stream mesocosms in the context of insecticide risk assessment

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In the risk assessment of plant protection products (PPP) aquatic mesocosm experiments are one of the most realistic tools in ecosystem context under more realistic conditions than in single species tests. Therefore, mesocosm experiments should simulate realistic exposure scenarios and should feature a representative biocenosis including potentially sensitive species. Usually these experiments are conducted in lentic mesocosms (artificial ponds or enclosures) for regulatory purposes. But in natural cases lotic mesocosms (artificial streams) may be the more appropriate choice to describe the ecotoxicological risk of a PPP in the aquatic environment. Many different factors have to be considered to decide between lentic or lotic mesocosms: The environmental behaviour of the test substance, exposition scenarios, intrinsic sensitivity of freshwater species and their vulnerability derived from their ecology. In this presentation we want to discuss these factors and bring some light in the discussion by presenting results of an experiment with four stream mesocosms in comparison with simultaneously performed pond mesocosm studies.

In addition we compare the artificial stream data with the data of two local natural streams representative for a landscape with moderate agricultural use. Finally we present the results of a pilot stream mesocosm study with the insecticide carbaryl. The comparison study shows that the macroinvertebrate community of the stream mesocosms corresponds well with the reference streams regarding diversity, feeding types, ecological traits and number and abundance of taxa, which are potentially sensitive and vulnerable.

Abundance and number of taxa with a high intrinsic sensitivity against carbamates, organophosphates and neonicotinoids was higher in the artificial streams than in the pond mesocosms. On the other hand, for pyrethroids the lentic mesocosms seem to be more meaningful caused by the occurrence of the focal species Chaoborus. First results of the carbaryl study shows, that the most sensitive macroinvertebrate taxa are Plecoptera, Gammarids, the mayfly Ephemera danica and the caddisfly Polycentropus flavomaculatus, all taxa preferring or restricted to lotic water bodies.

205 Tier IV Landau stream mesocosm facility

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In the current tier-3 risk assessment for plant protection products (PPPs) in aquatic systems, tier IV studies are mainly based on pond-like mesocosm approaches. However, transient and dynamic PPP exposure scenarios as observed in lotic systems are hardly achievable in pond-like mesocosm approaches. The present compilation of studies performed at the Landau stream mesocosm facility provides knowledge on dynamic PPP exposure scenarios at different time scales (i.e. peak, hour- and day-scale) under flow-through and recirculating conditions. To address the effects of a pesticides on aquatic plants under a day-scale exposure, the submerged macrophyte species Elodea canadensis and Myriophyllum spicatum were exposed to a sulfonlurea herbicide for 24 hours (in recirculating flow mode) followed by a subsequent PPP-free flushing period to enable a recovery period of 12 to 18 days. In addition on invertebrates having adverse short-term effects on shoot growth and yield, but also a subsequent recovery at the end of the experiment. Preliminary to the effect assessment of insecticides on hour- or day-scale the setup of stream mesocosms with macrophytes and aquatic organisms was considered in general. Abundance and robustness of invertebrates was evaluated including sensitive and/or vulnerable species. Non-dosed streams were used to evaluate the effects of different macrophyte species and the duration of the pre-experimental period on the establishment success of macroinvertebrates. To address the stream-internal longitudinal changes of the concentrations and the residence time of PPPs a location-dependent invertebrate sampling scheme was used within the scope of a 6-hour application of the insecticide etofenprox (0.05, 0.5 and 5 μg/L; n=4). Short-term effects were observed on population and on community level. The threshold endpoint drift was revealed as most sensitive as significant effects were visible occasionally at 0.05 μg/L for Simulid and at 0.5 μg/L for potentially sensitive species as Polycentropodidae. An integrated stream mesocosms test design capable of identifying inter-ecosystem boundary effects was established using the widely distributed web-building spider Tetragnatha extensa as a representative riparian species. On different scales, this approach may be used to evaluate PPP transfer due to emerging insects, bioaccumulation of PPPs in riparian spiders and alteration of trophic structures in riparian ecosystems.

206 Effects of chronic exposure to thiamethoxam on a summer generation mayfly population in an outdoor mesocosm

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The effect of neonicotinoids on mayfly nymphs has been investigated in recent literature, demonstrating they are amongst the most sensitive aquatic insect taxa to these insecticides. A publication by van den Brink et al (2015) reported the chronic effects of a continuous thiamethoxam exposure (28d) under laboratory conditions on over-wintering generation mayfly larvae (Cloeon dipterus). 28d EC50 = 0.43 μg a.s./L. A GLP outdoor mesocosm study was performed to investigate chronic effects in an outdoor mesocosm on a summer generation mayfly (Cloeon dipterus) population. Twenty stainless steel enclosures within one large pond enclosure were used for the study; five untreated controls and five treatment levels with three replicates each. The treatment levels were: 0.1, 0.3, 1.0, 3.0, 10 μg a.s./L. Concentrations of thiamethoxam were maintained with twice-weekly applications to the surface water of the summer generation mayfly (Cloeon dipterus) population. The time weighted average concentrations ranged from 93 to 108% of nominal, with a mean of 101% of nominal. Mayfly abundance was assessed with sweep-netting and substrate sampling (two baskets with aquatic macrophytes and stones). Adult emergence was also sampled. Mayfly sampling occurred weekly for the duration of the study. The % minimum detectable difference (MDD) values for larval abundance ranged from 37 to 49% up until day 27 of the study, indicating that small effects could be detected. On day 34, the MDD value was 87%, due to the natural population decline. The no-observed-effect-concentration (NOEC) for larval abundance was determined to be 0.5 µg a.s./L and the lowest-observed-effect-concentration (LOEC) was determined to be 1.0 µg a.s./L. The results for adult emergence support the observed effects on the larval population. The results of the study indicate that under conditions of continuous exposure, there is no apparent difference in sensitivity to thiamethoxam between summer generation mayfly larvae tested under field conditions and over-wintering generation mayfly larvae that were tested under laboratory conditions.

This study adds to the growing debate on the effects of neonicotinoids on sensitive aquatic insects by determining a no effect level for mayfly populations in outdoor mesocosms continuously exposed to thiamethoxam, representing worst-case conditions with respect to FOCUS modelling drainage scenarios.

207 Experiences from a suite of higher-tier laboratory tests on aquatic larvae of four potentially-sensitive insects, and an amphibip, with the aim of comparing sensitivity to an insecticide

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The EFSA Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013) makes recommendations for sensitivities to insecticides as the taxon melolontha (Melolontha melolontha), commonly known as indicator species for water quality, are particularly sensitive to insecticides. Therefore, actually testing their relative sensitivity compared with taxa which are found in microcosm studies, could minimise uncertainty (and therefore the ‘uncertainty factor’ when implementing microcosm results in the risk assessment. A set of laboratory experiments on invertebrate species is undertaken in the Cambridge Environmental Assessments (CEA) to assess relative sensitivity of larvae of Plecoptera (stonefly), Megaloptera (aldersfly) Trichoptera (caddisfly), and to ‘benchmark’ this against tests on Chaoborus and Cranogonyx – both of the latter two being present and sensitive to this insecticide in previous microcosm experiments. To achieve this, a novel laboratory test design using microcosm water and sediment was developed. Plecoptera and Megaloptera species of different aquatic macroinvertebrates were sourced from CEA microcosms. SETAC Europe 26th Annual Meeting Abstract Book 49
Separate tests were conducted for each taxon using 3-litre beakers containing sediment, microcosm water, alder leaves as a source of detritus, with gentle aeration, and pebbles to simulate the natural environment of stonfly larvae. After addition of the insecticide, observations were made during 96 hours, and final counts of survival were recorded. The results were used to determine the relative sensitivity of the species. Practical experiences will be shared on selecting test organisms based on life history and abundance, and conducting and assessing the sensitivity of these non-standard species in laboratory.

**Development, standardization and implementation of LCA and integration with economics for transportation infrastructure and operations**

208 A multi-objective optimization-based pavement management decision-support system for enhancing pavement sustainability

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In a society where the public awareness of environmental protection is increasing remarkably and the availability of resources and funding is limited, it is more vital than ever that departments of transportation (DOTs) and decision-makers (DMs) seek new tools that enable them to make the best and most rational use of these resources, taking into account environmental and social factors, along with economic and technical considerations. However, the practice adopted by highway agencies with regards to pavement management, has mostly consisted of employing life cycle costing analysis (LCCA) systems to evaluate the overall long-term economic efficiency of competing pavement design and maintenance and rehabilitation (M&R) activities alternatives. This way of supporting the decision-making process as it relates to pavement management, in which little or no importance is given to environmental considerations, does not seem to be effective in advancing sustainability in pavement systems. In view of this, it is clear there is an urgent need for pavement management decision-support systems (DSS), which, by integrating multi-disciplinary and complementary pavement life modelling approaches, enable the DMs to properly account for, and consider the cumulative and long-term impacts of their decisions and practices regarding sustainability goals and targets. This only can be achieved by employing techniques that are objective and wide-spectrumed, and facilitate cradle-to-grave capacity of analysis. To address this multifaceted problem, this paper presents a comprehensive and modular multi-objective optimization (MOO)-based pavement management DSS which comprises three main components: (1) a MOO module; (2) a comprehensive and integrated pavement life cycle costs - life cycle assessment (LCC-LCA) module that covers the whole life cycle of the pavement; and (3) a decision-support module. The potential of the proposed DSS is illustrated with a case study consisting of determining the optimal M&R strategy for an one-way flexible pavement section of a typical Interstate highway in Virginia, USA, which yields the best tradeoff between the following objectives: (1) minimizing the present value (PV) of the total life cycle highway agency costs (LCHAC); (2) minimization of the PV of the life cycle road user costs (LCRUC); and (3) minimization of the life cycle greenhouse gas emissions.

209 Aggregation Errors in Life-Cycle Assessment of Freight Trucks

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Life-cycle emission factors for heavy-duty trucks found in the literature and life-cycle assessment (LCA) databases typically reflect a generic or industry-average perspective in global or regional context. As a result, emission factors often lack the specificity to accurately characterize the performance of the vehicles, which could lead to misdirected strategies and actions. We believe that by collapsing over many individual vehicles to establish a formal emission factor, this aggregation process restricts the explanatory power of LCA for individual cases by removing information that may be useful for policy-making. This information includes knowledge of specific trip attributes (location, road type, time of day, etc.), vehicle attributes (gross weight vehicle rating and payload), driving conditions (levels of congestion), and characteristics of their respective supporting infrastructure systems (location of critical infrastructure and route topology). We assess the accuracy of greenhouse gas (GHG) emission factors of heavy-duty trucks by accounting for how they are utilized in real life and the sensitivity of GHG emission factors to vehicle loading factors (payloads) and commodity types transported. Case studies suggest that emission factors based on average payload always underestimate emissions. We provide examples of how existing emissions inventories change in response to infrastructure, vehicle, and payload characteristics. This research advances the scientific community's understanding of how GHG emissions from heavy-duty trucks can be allocated on more resolved spatial and temporal scales, thereby improving decision-making on a case-by-case basis.

210 Prospective Fleet LCA of Swiss Air Transport

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This work presents a Life Cycle Assessment (LCA) of passenger aircraft travel at both the individual aircraft and Swiss fleet level with projections until 2050. In the first stage of this work we perform LCA of three different classes of aircraft with construction years ranging from 1970 to 2050. Parameters such as aircraft material composition, weight, lifetime distance travelled, fuel consumption, operating emissions, and seat load factor are modelled as variables dependant on the size of the fleet, the construction year, and flight distance. Future fuel consumption and operating emissions are modelled for two scenarios reflecting either a continuation of historic improvements (BAU), or an optimistic acceleration of technology improvements (OPT). Future Swiss air transport demand is modelled to increase significantly at 2.75% per year. Flight length distributions, Fleet composition, and aircraft type parameters are based on the Swiss and European statistical data. Results are presented for ReCiPe midpoint for indicators: climate change, terrestrial acidification, photochemical oxidant formation, and particulate matter formation. Results show that environmental impacts are strongly related to fuel consumption. For climate change and acidification this is due to combustion in the cruise phase, while the fuel production phase is most important for other indicators, though impacts from landing and take-off cycles were also significant. Results for all impact categories varied by more than 50% depending on aircraft size and construction year. Flight distance is found to have a strong impact on per passenger kilometer (pkm) environmental impacts for flights below 1000 km, which make up approximately 20% of all pkm. Fleet average environmental impacts per pkm have decreased over 40% since 1990, and are found to decrease by another 40% to 60% for BAU and OPT technology development scenarios, respectively. The total pkm performance of the fleet is forecast to increase by over 2.5 times by 2050 compared to 2015. These large increases in future demand are expected to leave Swiss fleet level environmental impacts at levels similar to today’s, even with greatly accelerated technology development. Thus, reductions in fleet impacts from Swiss passenger air travel are only likely in the future with growth rates much slower than those currently predicted. This work was completed within the SCCER Mobility (www.sccer-mobility.ch).

211 Using life-cycle assessment to guide the development of bio-based fuels, lubricants, rubber, and plastics

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Environmental Impacts

Decarbonizing the transportation sector is critical to achieving global climate change mitigation. Although biofuels will play an important role in conventional gasoline and diesel applications, reducing societal reliance on oil and gas cannot be eliminated unless the full suite of products can be replaced. In this study, we explore the climate implications of producing bio-based fuels, lubricants, and precursors for rubber and plastics. We show that, guided by life cycle assessment combined with linear programming, integrated sugarcane biorefineries for producing jet fuels and lubricants could be built to minimize the overall GHG impact or maximize total energy output through novel combinations of furan and fermentation pathways.

212 Using LCA for Comprehensive Sustainability Assessments of Road Pavements

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A review of sustainability assessment tools for transportation systems shows that sustainability is often evaluated based on the goal of improving the environmental state (Brodie et al. 2013); however, comprehensive sustainability must also account for improvements to the socio-economic state of society. In pavement rating tools applied at the project level, such as BESt-in-Highways and GreenPave, environmental impacts are evaluated using LCA, but tools are needed in addition to LCA to support decision making for comprehensive sustainable outcomes. The LCA methodology has been expanded to apply to economic aspects through LCCA and attempts have been made to measure social aspects similarly (SLCA). This paper reviews several LCA methods as environmental calculators for use in sustainability assessments of road pavements and explores LCCA and SLCA methodologies. The results highlight the uses and limitations of LCA, LCCA, and SLCA for comprehensive sustainability evaluation and provide insight into approaches to address limitations and to improve sustainable decision making at the project level.

**Biological effects of emerging micro pollutants at realistic environmental concentrations (II)**
213 Effect of environmentally realistic doses of pesticides on tritrophic interaction in grass strips

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In agricultural agro-ecosystems, weeds and pests are of prime concern, and in order to protect water quality are regularly subjected to residual pesticide contaminations mainly resulting from runoff. Grass strips are also reservoirs of biodiversity for plants and insects that are thus potentially exposed to these compounds. Within plant-insect tritrophic chains, interactions between trophic levels are governed by exchanges of resources (nutrients, water) as well as chemical signals. As a consequence, each trophic level can regulate lower (top-down control) and higher (bottom-up control) trophic levels. This work aimed at studying the effects of environmentally realistic low doses of pesticides on the interactions between Lolium perenne (ryegrass), an aphid pest Rhopalosiphum padi and its parasitoid Euphisis platitor. To simulate a runoff-resulting pesticide exposure of the trophic chain in a context of grass strip, the plants were exposed via their root system to an herbicide (isoproturon) and an insecticide (Thiamethoxam), applied alone or in combination. Insect exposure was thus related to pesticide transfer along the trophic chain. We found that the three trophic levels were affected by pesticides, the combination of both compounds resulting in synergistic, additive or antagonistic effects depending on pesticide dose and traits measured. Moreover the observed effects were found not to be always linked to deterioration in the quality and/or quantity of resources produced by lower trophic levels, suggesting actions of pesticides on non-target organisms as chemical stressors and/or potentially alteration of plant-insect chemical signals. However, pesticides effects can be offset by fecundity of the sexual population levels. Thus, in agricultural landscapes, the interactions between plants, herbivores and their natural enemies are subject to change with pesticide exposure, even at low doses typically found in grass strips.

214 Signalling and regulation pathways involved in cryptic effects induced by residual levels of pesticide degradation products on higher plants

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Pesticides are pollutants of high concern due to environmental ubiquity resulting from extensive use in modern agriculture and persistence in soil and water. Study of plant behaviour towards such molecules and their breakdown products in situation of residual pollution is important to evaluate toxicity at low concentration in the context of environmental risk assessment. Studies at various spatial scales have highlighted frequent occurrences of major herbicide breakdown products in surface and ground water. In order to understand mechanisms underlying the impact of such molecules, the model plant Arabidopsis thaliana was confronted with low levels of breakdown products of widely used herbicides (aminomethylphosphonic acid for glyphosate, desethylatrazine and hydroxatrazine for atrazine), quantified in soils of field margins in an agriculturally-intensive area. Integrative analysis of physiological, biochemical and molecular responses showed that these residual xenobiotics rapidly led to cryptic effects with major reorientations of carbon and nitrogen metabolism and gene expression changes. The extent of such changes, in absence of significant action on primary targets, was consistent with involvement of early regulatory processes and signalling mechanisms. In order to identify such xenobiotic-related signalling, Arabidopsis seedlings were subjected to low levels of structurally-related xenobiotics (atrazine, hydroxatrazine, desethylatrazine) under conditions of No Observable Adverse Effects, and a genome-wide transcriptomic analysis (CATMAv7 microarray) was carried out. Functional analysis of differentially expressed genes and of their promoters revealed that signalling pathways related to plant hormones (cytokinins, ABA), low energy sensing (M2H2), environmental stresses (light, drought, heat, oxidative stress, hypoxia) and biotic interactions (WRKY40) were involved in xenobiotic signal transduction. Further studies will use hormone analogues and inhibitors, or mutants related to hormone signalling, in order to analyse xenobiotic-hormone interactions. Characterisation of xenobiotic signal transduction pathways should be of interest for design of detoxification systems for protection of wild plant communities and ecosystem functioning. Identification of crosstalk processes between xenobiotic, abiotic and biotic stress signalling may give novel insights into the interplay between chemical pollution and climate change stressors.

215 The effect of marine litter at cell level: cytotoxicity evaluation of common nanomaterials and emerging contaminants

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During the past decade, new technologies were based on the use of efficient and cheap alternative materials, according to industrial interest. Therefore, a lot of common commercial products are currently made of nanomaterials or synthetic materials, such as plastic. Their input to the environment, years after years, contributes to increase the number of waste in coastal waters, estuaries and oceans. This is also known as marine litter. Therefore, the aim of recent several researches is to study the toxic effects of these compounds on the marine life and their potential to cause deterioration and human health effects caused by them. The scope of this study was to understand the toxicity of common NMs and emerging contaminants at cell level in terms of oxidative stress and cell viability and to compare the cytotoxic response of two different cell lines exposed to the same conditions. In addition, with regards to carbon based nanomaterials, such as fullerenes (C60, C70…), due to their sorptive properties, they may act as carriers for other contaminants, providing rapid and long-range transport or immobilizing them. The change in the bioavailability of other contaminants is known as Trojan Horse Effect. In the present work, the cytotoxicity of nanomaterials (including fullerene soot, graphene nanosheets, and several other of metal and metal oxide nanoparticles) has been evaluated in vitro using two different cellular lines: T98g, cerebral human cells, and HeLa. The cells were exposed during 24-48 h to different level of contaminants from 10 ng/ml to 60 µg/ml. Furthermore, cells were also exposed to increasing concentration of different binary mixtures containing fullerene soot, in order to evaluate Trojan Horse Effect. The cell viability and cell’s oxidative stress were measured via High Content Analysis. Cells were selected as objects in two channels with two different wavelength (λ=485 nm and λ=549 nm) close to the Hoechst and DHE emission maximum, respectively. The obtained results contribute to the current knowledge of the cytotoxicity of both nanomaterials and microplastics. According to them, oxidative stress is one of the mechanisms that explains the toxicity of this type of contaminants. The obtained data has been observed for both cell lines. In addition, carbon nanomaterials have the potential to interact in a complex way with the co-existing contaminants, changing the bioavailability and, therefore, their resulting toxicity in either a synergistic or an antagonistic manner.

216 Seabream macrophages response to combinations of stressors: gene expression changes

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Marine macrophages are among the different classes of contaminants of emerging concern (Buchberger 2007, Sauge and Desrosiers 2014). NPs are increasingly used in several areas of human activity, despite the lack of knowledge regarding their impact in ecosystems. Pharmaceuticals have a bioactive nature and their consumption is increasing worldwide. Further, their removal by wastewater treatments is not sufficiently efficient, and in some cases more toxic or slowly degradable transformation products are formed (Oliveira et al. 2014). This concern may be emphasized taking into account that, in the environment, such contaminants may occur simultaneously and their interaction may become more harmful. Few studies have focused on the combined effects of these contaminants on coastal and estuarine ecosystems. This study aimed to test the in vitro effects of gold nanoparticles (AuNPs), with different coatings, and the human pharmaceutical gemfibrozil (GEM) in gilthead seabream (Sparus aurata) macrophages. Macrophage gene expression after exposure to these contaminants (individual or combined exposures, and combined with bacterial lipopolysaccharide, LPS) was assessed focusing on different pathways (e.g. apoptosis, inflammatory responses, antibacterial responses, oxidative stress or calcium signalling). The present study revealed different patterns of response to AuNPs with different coatings. Citrate coated AuNPs induced expression of genes such as caspase 1 (CASP1), tumour necrosis factor P (TNFA) or extracellular calcium sensing receptor precursor (GPCR). It is also noteworthy that, with the exception of IL10, all the other genes showed downregulation for the ternary mixture treatment AuNPs+GEM. In the combined environment AuNPs+GEM, genes related to similar pathways (CASP1 / TNFA or NCF4 / GPx4b) presented opposite patterns of expression. Gilthead seabream macrophages showed to be a useful in vitro model to investigate combined effects of these emerging contaminants.

217 Biological-based profiling of wastewaters from sources to the discharge into the environment

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Aquatic ecosystems are widely contaminated by trace micropollutants that may represent a risk for human health and wildlife. Even if some compounds are well removed by wastewater treatments, the discharge of effluents remains one of the major entry of such xenobiotics in the environment. It is particularly worrying for the compounds that are endocrine disruptors and/or that can induce genotoxicity. New approach using quantitative biological activities has to be applied along the water line, especially to monitor the pollution of wastewater.

The objective of this study was to apply a panel of in vitro bioassays to determine water contamination profiles from the wastewater network to the outlet of the WWTP. A panel of bioassays were applied on various samples collected in six sites on the network, along the WWTP in order to evaluate the impact of biological treatment and tertiary ozonation. Selected in vitro bioassays, applied on water SPE extract, enabled detection of active chemicals responsible of endocrine disruption (estrogens, progesterone, androgens, phloridzin, 3α-androstene-17β-diol). If these results were highly variable, induction of genotoxicity was evaluated by using the SOS Chromotest. In all sites along the wastewater network, ER, AR, MR activities and genotoxicity were detected. GR activity was measured at only three points of the network and was particularly important in the WWTP influent. Endocrine disruptor activities as well as genotoxicity were detected in raw wastewater (WWTP influent). Biological treatment highly reduced ED activities and genotoxicity. Nevertheless residual activities were still detected in the tertiary effluent. Results of the 1st sampling campaign allowed the identification of three notable points of interest on the network and at the WWTP influent. Results of two next sampling campaigns will help to confirm these “hot-spot” sites in order to apply effect-directed analysis to perform chemical identification of the compounds responsible of endocrine disruption activities and/or genotoxicity.

218

Effects of magnetic microparticles used for phosphorus removal in eutrophic waters on plankton community

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Eutrophication is a worldwide concern causing environmental degradation and compromising water resources use. Magnetic particles have been recently proposed as a promising method to adsorb and remove phosphate from lake water and sediment. However, before this method is applied into the whole-lake experiment, ecotoxicological studies must be performed to assess the effect of such microparticles on the aquatic community. In previous studies, EC50 for standard species showed a decrease from 42 μg L-1 to 14.4 μg L-1. Here we present a microcosms study to further explore the effects of these particles on the whole community. Our specific hypotheses were: a) the addition of MPs will cause transient effect on the structure of plankton community allowing its recovery by the end of the experiment and b) a top-MPs spike will provoke more changes on plankton community than a bottom addition because of a longer exposition time. In order to test these hypotheses, a microcosms experiment with three different treatments of MPs (i.e., control, bottom_addition and top_addition) was set-up. Bottom addition simulates an addition of the particles directly into the sediment; and, top addition if it would be sprinkled at the surface of the water column. The amount of amended MPs was the same for bottom and top addition treatments and it was based on the calculation of how much MPs are necessary for trapping all sediment mobile P pool (P_diss). However, and based on previous studies, we decided to double the value of that in order to counteract chemical interferences. This experiment has two goals: first, to assess the effects of magnetic particles on the plankton community; and, second, to estimate the efficiency of phosphorus removal by the MPs. The experiment was performed in outdoor microcosms (40 L volume) consisting of a stabilization period of 10 days, particles addition and retrieval after 24 hours simulating real field application, 79-days assessment under aerobic conditions and 5-days (stating on day 80) under anoxic conditions. Plankton abundance, species composition and richness are to be determined. The whole experiment will employ high-throughput sequencing statistical differences along the experiment between control and treatments. It may mean no changes of phytoplankton abundance or zooplankton grazing pressure but may hide community structural and richness changes. Based on chemical analysis, phosphorus was successfully removed by the MPs added with no detectable effect on the current biological parameter assessed.

Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (III)

219

Effects of engineered nanoparticle-contaminant mixture exposures to aquatic organisms

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Due to their high surface reactivity engineered nanomaterials (ENMs) can interact with other anthropogenic contaminants, potentially influencing their environmental distribution, bioavailability and toxicity. Suggested mechanisms behind those altered toxic effects include changes of contaminant bioavailability caused by contaminant adsorption to ENMs, or an altered toxicity due to interactions with toxic effects. Here we report the results from a range of experiments investigating the effects of several ENM-contaminant mixtures: carbon nanotubes (CNTs)-phenanthrene; titanium nanoparticles (TiO2-NP)-benzo(a)pyrene; silver nanoparticles (AgNP)-water soluble faction of crude oil (WSF); and AgNP-17β-ethinylestradiol. Studies included different experimental setups and exposure conditions (batch vs. continuous flow, short vs. long exposure times) and demonstrated that ENM-characteristics and their behaviour in the tested environmental matrices was determined. ENM-contaminant interactions, their uptake in organisms and effects on contaminant bioavailability were investigated. To determine potential changes in toxic effects, suitable endpoints or biomarkers were analysed for each test species. All tested nanomaterials were found to be taken up or to be associated to organisms’ surfaces; however interaction between ENM and contaminants and effects on bioavailability and toxicity varied. Adsorption of hydrophobic PAHs was observed to CNT and TiO2-NP. Undergoing aggregation, both CNTs and TiO2-NPs reduced dissolved PAH concentrations. However, both dissolved and ENM-associated appeared to remain bioavailable to varying extends. Similarly, both, single and mixture exposures of AgNP and the WSF of North Sea crude oil, caused activation of oxidative stress related biomarkers in calanoid copepods. The presence of AgNP did not significantly change 17β-ethinylestradiol bioavailability to juvenile turbot. Impacts on steroid hormone concentrations in mixture exposures followed 17β-ethinylestradiol single contaminant effects, however, impacts on some hormones were slightly increased in mixed exposures. ENM-contaminant mixture effects do not follow a general mechanism. Their interaction and mixture toxicity depends on multiple factors, including ENM material and contaminant properties. Further research is needed in order to provide sound data for ENM environmental risk assessment, which needs to include risks from ENM-contaminant mixtures.

220

Long term toxicity of Ag-NM on soil nitrifying bacteria after bioratransformation in WWTPs


Silver nanomaterials (Ag-NMs) are used in many cosmetics and textiles because of their antimicrobial properties. Several studies have shown that silver is released from textiles during washing and reach wastewater treatment plants (WWTPs), where significant amounts adsorb to sewage sludge. As sewage sludge is used as fertilizer in agriculture, adsorbed NMs enter the soil resulting in a negative impact on soil microorganisms. The aim of our study was to investigate the influence of silver nanoparticles on soil microorganisms after treatment with 17β-ethinylestradiol and Ag-NPs over long exposure times. Soil nitrification activity and abundance of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), Nitrospira and Nitrobacter were measured in soil solution to identify the main drivers of TiO2-NPs toxicity in soil. The activity was reduced by Ag-NPs over time, but the improvement by treatment with 17β-ethinylestradiol and Ag-NPs was not significant. The activity was highly accumulated in the root epidermis and also formed spots of several micrometers in the intercellular space of the cortex. Speciation analysis indicated that Ag-NPs are strongly adsorbed to sewage sludge. As sewage sludge is used as fertilizer in agriculture, adsorbed Ag-NPs enter the soil resulting in a negative impact on soil microorganisms.

221

Toxicity of TiO2 nanoparticles on soil nitrification at environmentally relevant concentrations: Evidences for an absence of classical dose-response...
relationships
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Nitrogen is a crucial microbiological process for soil fertility depending on the combined activity of ammonia- and nitrite-oxidizers. This key step of the nitrogen cycle is known to be very sensitive to environmental stressors. However, the impact of emerging pollutants such as titanium-dioxide nanoparticles (TiO₂-NPs) which are increasingly released in agricultural soils, remains to be investigated, especially at very low concentrations (< 1 mg kg⁻¹ dry soil) (Sun et al. 2014; Simoin & Richaume 2015). We assessed the impact of eight TiO₂-NPs concentrations ranging from 0.05 up to 500 mg kg⁻¹ dry soil in soil microcosms on nitration activity and abundance of ammonia-oxidizing archaea (AOA), bacteria (AOB) and nitrite-oxidizers (Nitrobacter and Nitrospira) using quantitative PCR. In addition, aggregation and oxidative potential of TiO₂-NPs were measured in soil solution to identify the main drivers of TiO₂-NPs toxicity in soil. After 90 days of exposure, AOA appeared to be the most affected by TiO₂-NPs, while Nitrospira seemed insensitive to this contamination. AOB and Nitrobacter abundance exhibited similar responses. The activity was reduced by the lowest (0.05 mg kg⁻¹) and the highest (100 and 500 mg kg⁻¹) TiO₂-NPs concentrations. Interestingly, all indicators exhibited a non-classical dose–response relationship that did not enable to calculate effect concentrations. This results could be linked to the influence of NPs concentration on the aggregation and oxidative potential of TiO₂-NPs assessed in soil solution. In fact, TiO₂-NPs aggregation was increased with concentration, while oxidative potential was consequently decreased. Thereby, the TiO₂-NPs toxicity potential in soil solution at the lowest concentrations that may explain the negative effects found at very low concentration (< 1 mg kg⁻¹) on nitrification activity and nitrifiers abundances. Altogether these results pointed out the need to include very low concentrations of NPs in soil risk assessment studies. This study also emphasized that classical approaches for risk assessment based on dose–response, toxicological tests are not pertinent for NPs in soil and highlights the necessity to characterize NPs properties for each concentration used in conditions close to those encountered in nature.

222
There are more than species differences: challenging the immune system with nanoparticle’s sex-specific biological identity
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We have previously reported that, through formation of a protein corona, the same nanoparticles can acquire a quite different biological identity depending on the species-specificity of the proteome to which they are exposed, and that they likely differ profoundly under immune vigilance. Here we hypothesise that the protein repertoire diversity matters in general (not simply because of significant cross-species variations), sex differences in the proteome would translate into differential corona formation even within the same species. In this study, we show for the first time that the protein repertoire differences by sex determine nanoparticle’s sex-specific biological identity, and that immune cells in zebrafish interact differentially with those nanoparticles in vitro. For the formation of protein coronas around 70 nm silica nanoparticles, zebrafish blood plasma was collected separately from female and male adult zebrafish (Danio rerio) and the obtained blood plasma was referred to as DrBB-F and DrBB-M, respectively. As protein repertoire was first characterised by sex, we firstly characterised each type of nanoparticle-protein complexes as-is and under the exposure condition, and presumed that the main difference between the coronas formed of DrBB-F/M is the biological identity, rather than physicochemical parameters such as colloidal stability. Among the strongly-bound proteins in the corona, female-specific proteins were identified to be vitellogenin, whereas the lack of vitellogenin in the male counterparts that seemed to have resulted in preferential enrichment of fetoins. We then exposed primary haematopoietic cells harvested from adult zebrafish to the nanoparticles with a pre-formed corona of DrBB-F or DrBB-M. Interestingly, it was those with a pre-formed corona of DrBB-F that resulted in a higher degree of cellular aggregation in a dose-dependently way. What we find striking is that the local build-up of single endogenous proteins at nanoparticles can dictate enhanced immune recognition; in the case of vitellogenin the nanoparticles would otherwise travel along with those proteins bearing the possibility to disturb vitellogenesis. The local effect of sex differences in the corona proteins should therefore deserve further attention particularly for oviparous animals.

223
Accumulation, speciation and phytoxicity of pristine and aged silver nanoparticles to wheat

Silver nanoparticles (Ag-NPs) are used in a wide range of consumer products due to their biocidal action. However, they have been demonstrated to be very easily leached from these products leading to their inadvertent release into the environment. The application of nanosilicides, in which Ag-NPs leached to sewage systems are retained, as fertilizer in soils, together with the novel use of nanopesticides, makes plants to be significant biological receptors of Ag-NPs. The objective of this work was to compare the induced phytoxicity as well as Ag distribution and speciation in wheat roots exposed to pristine and aged (sulfidized) Ag-NPs in hydrosols, in which Ag-NPs leached to sewage systems are blocked to wheat growth. After 79-days assessment under aerobic conditions and 5-days (starting on day 80) under anaerobic conditions, we observed a different sulfate levels had no influence on the bioavailability of Ag-NPs in hydrosols. AgNO₃ treatment was used to identify the mechanisms exclusively due to Ag⁺. By the combination of X-ray Fluorescence (μXRF) and micro X-ray absorption spectroscopy (μXANES), Ag aggregates were identified associated to the cell walls in the epidermis, the cortex and the endodermis of roots exposed to Ag-NP. A process of partial oxidation/dissolution of nanoparticles followed by a progressive chelation to GSH in plants was found in these roots. In Ag-S-NPs treatment, Ag appeared colocalized with S forming aggregates mainly adhered to the root surface. We found evidence of Ag-S-NPs reduction to metallic Ag in the root surface. For the plants exposed to AgNO₃, Ag was highly accumulated in the root epidermis and also formed spots of several micrometers in the intercellular space of the cortex. Speciation analysis indicated a predominance of Ag dissolution in the epidermis followed by Ag chelation to thiols and formation of metallic Ag in the root cortex. Phytoxicity data (thiobarbituric acid production and quantitative real-time polymerase chain reaction) showed that, even if the three Ag treatments induced growth retardation in exposed plants, the toxicity mechanisms that induce this retardation are specific of each Ag source. Toxicity caused by the release of Ag⁺, represented by the AgNO₃ treatment, seemed to be mainly mediated by oxidative stress. Nanoparticles phytoxicity may be due to both the release of Ag⁺ and to their high surface reactivity. Overall, the results show the complex interplay between nanoparticulate and ionic form of Ag, and that exposure to Ag nanoparticles induce a complex response possibly due to the multiplicity of Ag species in the plant. Such knowledge on the fate and impact of nanomaterials for plants, is necessary to better evaluate the risk associated to the use of these products.

State of the science on poly- and perfluoralkyl substances (PFASs) in the environment and humans (II)

224
Factors influencing the water-suspended sediment partitioning and sediment levels of legacy and emerging fluorinated substances (PFASs) along the salinity/turbidity gradient of a macrotidal estuary
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Fluorinated surfactants (PFAS) are organofluorine compounds that have been produced and used since the 1950s in numerous applications. Since the global declaration of perfluorooctane sulfonate (PFOS) in wildlife was reported, PFOS and long-chain perfluoralkyl carboxylates (PFCCs) have been of particular concern due to their potential for persistence, bioaccumulation, and toxicity, prompting international efforts to phase-out their production or propose more environmentally-sustainable alternatives. Since 2001, the science community has gained a better knowledge as to how long-chain PFASs enter aquatic environments (sources) and partition between water, sediments and biota (fate). However, knowledge gaps still remain to be bridged as regards the environmental behavior of legacy (e.g., PFOS, long-chain PFCCs) and substitution PFAS, especially at the land-sea interface. The question whether PFASs may be scavenged along the salinity/turbidity gradient of a macrotidal estuary is indeed of critical importance to predict their land-sea transport through river discharge into coastal waters. The present work was conducted in the Gironde (SW France), targeting both legacy and substitution PFASs such as short-chain PFCCs, fluoroelutrole sulfonates (FTS(A)) and, polyfluoralkyl phosphate diesters (diPFAs). In this estuary, PFASs were ubiquitous albeit at low levels (ΣPFAS < 8.3 ng L⁻¹ in the dissolved phase and < 3.3 ng g⁻¹ in sediments). In the dissolved phase, ΣPFAS decreased near-linearly with salinity, being PFOS, perfluorhexane sulfonate (PFHxS) and perfluoroheaxoxane (PFHxA) the dominant congeners. In the maximum turbidity zone (suspended solids up to 2.6 g L⁻¹), the particle-associated fraction was almost consistently > 50 % for long-chain perfluoralkyl acids (PFCAs) and > 90 % for short-chain PFCCs. Models of Σₙ partitioning coefficients were derived by integrating both particle-concentration and salting-out effects, and could be used in the future to implement transport models integrating hydrodynamic parameters to estimate PFAS mass budgets at the land-sea interface. Multiple linear regressions taking into account non-detects (data < LOD) provided insights into the relative influence of factors controlling PFAS sediment levels. In that respect, the organic
carbon fraction (strongly correlated to sediment grain size) appeared as a more important controlling factor than distance from upstream sources for medium- to long-chain PFAS.

225 Perfluoroalkylated Substances in Oceanic Plankton

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Perfluoroalkylated substances (PFAS), including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSA), are ubiquitous in the environment, reaching remote oceans and causing diverse effects on terrestrial and aquatic organisms. PFAS bioaccumulate and biomagnify in aquatic food webs, and even though there are previous studies on the occurrence of PFCs in biota, few of them report concentrations on the first levels of the aquatic food webs (phytoplankton and zooplankton). The objective of this study was to investigate the global oceanic occurrence of PFCA and PFSA in plankton samples, to elucidate the factors driving their occurrence, and to evaluate their bioaccumulation factors. The analysed seawater and plankton samples were obtained during the Malaspina circumnavigation campaign sampling the Pacific, Indian and Atlantic Oceans, and additionally, water and plankton samples were also obtained in the coastal Southern Ocean at Livingston Island (Southern Shetlands). SPFA concentrations in ranged from 3 ng.g\textsuperscript{-1} to 60.9 ng.g\textsuperscript{-1}, with minimum concentrations in the Pacific and Southern Oceans, and maximum concentrations in the tropical south Atlantic. There was a significant linear correlation between concentrations of SPFA in plankton and water (R\textsuperscript{2} = 0.45, p-value < 0.01), which was driven by the two major analytes in this study (PFOS w/ R\textsuperscript{2} = 0.71, p-value < 0.01). On the contrary, higher occurrences of PFBS and PFHxS were found in plankton samples. PFOS total branched isomers (5\textsuperscript{b}-PFOS) in plankton samples were higher in the Northen hemisphere, while PFOS total branched isomers (5\textsuperscript{b}-PFOS) followed the opposite pattern. This first report of PFAS concentrations in plankton samples from the global oceans shows that PFASs are ubiquitously found in oceanic plankton, and provides evidence that the concentration of PFASs is closely coupled between seawater and plankton. In addition, we also provide a first assessment of isomer composition for PFOS and PFBS in plankton in the global oceans. The occurrence and bioaccumulation of PFAS is further discussed in terms of sources, transport and cycling.

226 Levels and maternal transfer of PFAS in Arctic hooded seal mother-pup pairs


Perfluoroalkyl substances (PFAS) are persistent contaminants that are present in the Arctic, and which have the capacity to bioaccumulate and biomagnify in food webs. There is a paucity of studies on the role of milk as a vector for maternal transfer of contaminants. Plasma and milk were collected from 15 mother-pup pairs from the north-eastern Barents sea. Mean PFAS levels were reported in similar studies on pinnipeds. Mean PFAS concentrations were 65 ng.g\textsuperscript{-1} w.w in pup plasma, 36 ng.g\textsuperscript{-1} w.w in maternal plasma and 3.2 ng.g\textsuperscript{-1} w.w in the milk. Higher PFAS levels in maternal plasma compared to the milk supports the concept that binding to plasma proteins limits their incorporation into milk. The individual PFAS observed in their transfer and metabolism are depending on their carbon chain lengths, with the lowest transfer efficiency for the intermediate chained PFAS and higher transfer of both higher and lower chained carboxylates. The current study was the first to document PFASs in pinniped milk and in hooded seal plasma. We have confirmed maternal transfer of PFASs from hooded seal mothers to pups, and that this transfer likely takes place both via milk and placental transfer; the latter is thought to be the dominant pathway.

227 New concern on old substances - endocrine disrupting effects of PFAS

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Concerns for long chain per- and polychlorinated alkyl substances (PFASs) have been identified due to their persistent, bioaccumulative and toxic properties. Successful regulatory activities are targeting the long chain PFAS due to these properties but also resulted in an increased use of short chain PFAS as “alternatives” by the industry. As a consequence, already today, short chain PFAS are found increasingly in different environmental media. Since they are less bioaccumulative but persistent, they might be spread out in the environment ubiquitously due to their mobility. Therefore, it is of importance to reveal concerns related to their presence in different environmental compartments in order to initiate regulatory measures. Recently, first studies on PFAS endocrine disruptive properties have been published in the scientific literature. Most of the studies using in silico or in vitro test systems. Only limited number of studies has been found in the public scientific literature using in vivo test systems. Hence, most information on endocrine effects could be attributed to the endocrine disrupting mode of action but not to the related adverse effects. While in vitro estrogen receptor binding studies are contradictory for PFASs, in vivo results give the first evidences on estrogen mode of action in fish for some selected perfluorooalkyl carboxylic acids and fluorotolermel alcohols. Several studies indicate interactions of PFAS with the thyroid hormone signaling. Effects assessed by far, are related to the binding of thyroid receptors, thyroid hormone transport proteins and alterations of genes related to thyroid hormone biosynthesis, transport and metabolism. Unfortunately, no adequate in vivo assays are lacking for the short chain PFAS but the ones available for the long chain members indicating interaction with the thyroid hormone signaling. Only limited number of studies gives first indication on interaction with the glucocorticoid pathway. Although information on endocrine disruption properties of PFAS are missing, and mostly provided by in vitro studies, the first evidences show that this aspect might require more attention, especially for the short chain PFAS that might be spread out in the environment due to their mobility. Since endocrine disruptors are considered as substances of equivalent concern under REACH, special attention should be given to these properties when assessing short chain PFAS.

228 Effects of perfluoroacetate sulfonate and alternatives on long-term potentiation in rats in vivo

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With limited but ongoing production and application of perfluorooctanoate sulfonate (PFOS), the toxicity of its alternatives is lack of evaluation. The present study evaluates the effects of PFOS and its alternatives on long-term potentiation, aiming to provide some evidence for their potential to affect cognitive ability. Targeted alternatives included perfluorohexane sulfonate (PFHxS), perfluorobutane sulfonate (PFBS), and chlorinated polyfluorinated ether sulfonate (F-53B). Different dosages of PFOS and alternative chemicals were given to rats via intracerebroventricular injection exposure. The input/output incerceptors (IO), paired-pulse facilitations (PPF), and long-term potentiation (LTP) of the field excitatory postsynaptic potential (EFPSP) amplitude \textit{in vivo} were recorded. PFOS and alternatives exposure did not significantly affected the normal synaptic transmission, but inhibited LTP in hippocampus CA1 region in a dose-dependent manner. In addition, PFHxS and F-53B exhibit comparable potential to PFOS in disturbing LTP. The results suggested that acute exposure to PFOS and its alternatives resulted in the impairment of the synaptic plasticity by a postsynaptic rather than presynaptic mechanism. Besides, the LTP amplitude of baseline was reduced by F-53B but not by other compounds, indicating that F-53B might possess a different action mode. Therefore, further study was conducted to evaluate the effects of the chronic PFOS exposure during developmental period on LTP. The results found that LTP was repressed later in life by early PFOS exposure. Providing some electrophysiological evidence and potential mechanism of the neurotoxic effects induced by PFOS and its alternatives, the present study suggests further evaluation of their safety.

Alternative approaches to animal testing for ecotoxicity assessments

229 Comparison of two fish cell lines for the evaluation of model contaminants and PAH complex mixtures toxicity

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Fish are currently used models for the toxicity assessment of chemicals. The WEACH Regulation and the European directive on the protection of animals used...
for scientific purposes wish the establishment of alternative assays to animal experiments. In this concern, fish cell lines are being developed to provide fast and reliable results on the toxic and ecotoxic properties of chemicals while respecting ethical concerns about animal testing. Rainbow trout gut cell line RTW-1L, which is one of the best characterized cell lines derived from fish for mucin and mucous secretions was employed in this study. RTW-1L and OLCAB-e3 were used to validate different toxicity assays (MTT, EROD and comet assay) on four model contaminants with different properties: B[a]P, PCB126, Cadmium and Methylene sulphonate. In addition, these cell lines were used to analyze the effects of four polycyclic aromatic hydrocarbon (PAH) complex mixtures: Arabian light crude, coal tar, refined coal tar and a combination of these laboratory prepared mixtures and the bioassays and contaminants tested, dose-dependent effects were observed but with marked differences of sensitivity between cell lines. For the cytotoxicity endpoint embryonic cell line, OLCAB-e3 is more sensitive than trout cell line, RTW-1L for the majority of compounds. On the contrary, for genotoxicity, the rainbow trout cell line is more sensitive than the medaka one. However, both cell lines give information about toxicity mechanisms of compounds. Our preliminary results confirmed the possibility of using cell lines to assess the toxicity of chemicals, including mixtures, especially in high throughput screening assays. Differences in sensitivity have been found between fish cell lines. It is therefore necessary to carefully select a cell line based on the mode of action and the nature of the studied toxic compound. Same compound will be tested on other alternative to animal experiment: fish embryotoxicity assay. Results will be compared with those obtained on cell lines.

230 Fish & Chips: Development of a novel in vitro system of the fish intestine C. Drieschner, Eawag, Aquatic Research / Department of Environmental Toxicology; P. Renaud, EPFL / LMIS; K. Schirmer, Eawag / Environmental Toxicology

Fish are important test organisms in chemical risk assessment. One promising strategy of reducing unnecessary, time consuming and ethically questionable animal experiments with fish is the use of continuous cell cultures from rainbow trout. Our approach of recreating the intestinal microenvironment of fish in vitro, to study physiological responses towards toxics, is based on the cell line RTG-2/GC and newly developed microchip technology. In vivo, the intestine is lined by a single layer of absorptive epithelial cells, which faces the intestinal lumen on one side and the inner side of the body on the other. Herein, the basement membrane represents an ultrathin, highly porous substrate for the epithelial cells. In vitro, this delicate membrane is often mimicked with transwell integrated plastic membranes. However, these membranes are relatively thick, show low porosity and visibility of cells using light microscopy is not possible. Using standard microfabrication technology we were able to engineer an ultrathin, highly porous membrane as new cell culture interface. The new membranes are unique in terms of their high porosity and equally distributed nanopores. A single cell seeded on the substrate is in contact with ~3000 pores, while a cell on commercial membranes only faces ~20 pores. A great advantage of ultrathin membranes is their transparency which allows to follow cell growth over time with light microscopy. In addition, confocal microscopy through the membrane results in high quality images. For long term cell culture over 21 days we observed cellular polarization with basolateral actin stress fibers and apical tight junction formation. Non-invasive impedance monitoring over the culture period of 28 days revealed a stepwise increase in resistance within the first 24 hours, resulting from cell attachment. Thereafter, resistance increased slowly and steadily due to cell proliferation. Impedance was also used to on-line monitor the response of epithelial cells to toxins (e.g. sodium dodecyl sulfate). A decrease in oocyte resistance was associated with a decrease in cell viability and disruption of barrier functionality. For ongoing studies we aim to establish a co-culture system of epithelial cells (RTG-2/GC) and fibroblasts (RTG-F1) on chip. Moreover, we will integrate the microchip in a microfluidic bioreactor to allow for realistic, biologically relevant exposure and transport phenomena of chemical pollutants found in the aquatic environment.


Chemical biotransformation represents the largest source of uncertainty in chemical bioaccumulation assessments. Models of chemical biocokinetics in fish may be greatly improved by including biotransformation rates, as measured in vitro. Substrate depletion assays with trout hepatocytes or rainbow trout liver microsomes (99) have been employed in this context. Regulatory use of these procedures, however, requires that they be evaluated to determine their reproducibility within and among laboratories. Building on previous work, a multi-laboratory ring trial was coordinated by the ILSI Health and Environmental Sciences Institute (HESI). The specific aims of the ring trial were to determine the inter-laboratory agreement in CLint,inter, the performance of substrate depletion assays using the two biological systems, and support the development of an OECD test guideline (OECD Project 3.13). Six laboratories (Procter & Gamble, Fraunhofer IME, Dow Chemical, DuPont, KJ Scientific/SC Johnson, Givaudan) conducted substrate depletion assays for 6 test chemicals (perfluorooctane sulfonate, 4-n-monoalkylphenol, fenthion, cyclohexyl salicylate, deethylthirameth, methoxychlor) using both trout liver S9 fraction and trout hepatocytes to determine in vitro intrinsic clearance (CLint, in vitro). For the different test chemicals, the inter-laboratory agreement in CLint, in vitro averaged 14-22 %CV for the hepatocyte assays and 5-32 %CV for the S9 assays. The inter-laboratory agreement was somewhat higher, ranging from 23-40 %CV for the hepatocyte assays and 11-42% for the S9 assays. Extrapolation of the in vitro intrinsic clearance rates for each test system to in vivo intrinsic values resulted in good agreement (within 2x) for all test chemicals except pyrene (~5x difference). Much of the variability in the in vivo clearance predictions was further reduced upon incorporating blood flow limitations and protein binding algorithms into hepatic clearance predictions. Across the various test systems inter-laboratory agreement was further reduced upon incorporation of in vitro rates into BCF prediction models. The results of this ring trial firmly establish the reliability of these methods and provide strong support for use of this information in modeled BCF assessments for fish.
Ecological Threshold for Toxicological Concern (eco-TTC) - Assessing the potential of a new tool for environmental hazard assessment


The ecological Threshold for Toxicological Concern (eco-TTC) is a new tool under development that summarizes the wealth of ecotoxicological information in the form of Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of statistical (probability) distributions. Eco-TTCs can be developed that allow prediction of untested chemicals based on structural attributes (categories), mode of action, or functional use. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, provide hazard perspective on chemicals that lack QSARs, can be used to guide priority setting and screening, and assist in category derisking. The proposed eco-TTC is the statistically determined fifth percentile value for the distribution of PNECs for an assessed group. The eco-TTC approach has the potential to reduce the need for animal testing (fish) in many situations. In this talk we summarize the process that has been developed to quantify eco-TTCs and then bring this to life by providing a few examples. Fundamental to the development of eco-TTCs is the formation of a rigorous ecotoxicological database (transparent, high quality studies, traceable to the point of origin). A particularly interesting aspect of this work is segmentation of the data by mode of action and chemical categories. Non-polar and polar narcotic compounds comprise the vast majority of the PNECs in commerce and provide the first chance to assess the congruence of eco-TTC predictions and patterns with QSARs as well as environmental risk assessments built on empirical data submitted to REACH and between season and contamination condition was significant, indicating that PERMANOVA test was use to test for significant differences in the antioxidant response among species, levels of contamination and seasons. The interaction between season and contamination condition was significant, indicating that biomarker variation responds to complex settings. In the lab experiment, significant differences in antioxidant enzymatic activity depended on the sampling time after the exposure event. Changes in the enzyme activity were fast, with differences occurring between sampling separated only by 24h, proving that the time in which these endpoints are to be measured after a putative oil spill is of major importance. The overall pattern in the enzymatic response was species specific, showing that none of the enzymes measured can be expected to show the same pattern of response (induction vs inhibition) for all species. A field validation study with chronic diesel oil exposure, to be conducted in early 2015, will include background variability as an informative moderator for oil exposure response at the cellular level. Data provided from this work will contribute significantly to the post-spill monitoring assessment tool-box in tropical waters.

235 The marine environment of Kuwait: an integrated monitoring programme to assess the impacts of pollutants

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Threats to the marine environments of Kuwait, and the wider Gulf, are both evident and increasing, and the status of many aspects of the region’s unique biodiversity are at record low levels. Like many of the other countries which comprise the Gulf Co-operative Council (GCC), Kuwait has undergone major economic, social and industrial development following the discovery of its oil reserves. This has led to a variety of contaminants being discharged directly to the marine environment, including petroleum hydrocarbons, metals, nutrients, and contaminated brine from desalination plants. Here we report the findings of a comprehensive marine monitoring programme, which along with collecting contemporary data, included an extensive review of a 30 year dataset (water mass, hydrographic, quality, metal pollution and nutrient inputs) to establish the historic baseline of pollutants within Kuwait. A present day assessment of the ‘health’ of the marine environment, in relation to chemical and biological contaminants, included the analysis of water, sediment and biota for a range of chemical contaminants including polycyclic aromatic hydrocarbons (PAHs), metals, polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs). In addition historic inputs of sewage contamination using sedimentary faecal sterol markers were assessed alongside conducting ecotoxicology screening of seawater samples and the assessment of biomarker response and histopathology in key sentinel species. The findings indicate that while Kuwait’s marine environment has been subject to a wide array of pollution events, the actual levels of chemical contamination remains relatively low. However, sediment contamination hotspots associated with point sources of industrial contamination were evident at a number of locations around the coast. The assessment of histopathology in fish species indicated that lesions associated with chemical contamination were low when compared with other studies conducted globally. Sewage contamination continues to be a serious issue, with a number of coastal sites heavily contaminated. Linked to this was the observation that seawater samples displayed both acute toxicity and endocrine disrupting potential when screened with bioassay tests. Overall the data allowed ‘health assessments’ to be conducted at a number of sites and the findings are currently being used by the competent authorities in Kuwait to refine and redesign their national monitoring programme.

236 Is alkali lable phosphate (ALP) a suitable biomarker for endocrine disruption in marine mussels?

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Alkali lable phosphate (ALP) is usually measured as a proxy for vitellogenin in fish and molluscs, the ALP method detects inorganic phosphate liberated from phosphorylated proteins like vitellogenin and others. In mussels, it has been measured either in gonad, hemolymph or digestive gland and used in both laboratory and field experiments as a biomarker of endocrine disruption. However, recent studies have shown that vitellogenin is only produced in the female gonad and that phosphorlated proteins measured by ALP in hemolymph correspond to extrapallial protein precursor and not to vitellogenin, that is absent in this fluid (Agnese et al. 2013; Oliveri et al. 2014). This work aims to demonstrate if ALP can still be used as a proxy for vitellogenin in the marine mussel Mytilus galloprovincialis when measured in the gonad, the primary site of vitellogenin production. The in vivo and in vitro induction of mussel gonad were followed during two seasons representing different maturation stages (early gametogenesis and fully matured), sex and maturation stage were determined by histological analysis and vitellogenin was measured by two techniques: ALP and LC-MS/MS after separation by SDS-PAGE electrophoresis. Protein analysis by LC-MS/MS evidenced the presence of vitellogenin in females, but not in male gonads disregarding their potential activity. On the contrary, ALP normalized both by protein or by tissue weight, was similar or even higher in males than in females, and did not change with maturation stage. Further protein analyses are currently being done in order to quantify and compare vitellogenin contents in mussel gonad of different maturation stages and after being exposed to the synthetic estrogen ethinyl estradiol.

56 SETAC Europe 26th Annual Meeting Abstract Book
In situ toxicity testing using a nerite gastropod

M. Bighi, Stockholm University / Environmental Science and Analytical Chemistry ACES; B. Eklund, Stockholm University / Department of Environmental Science and Analytical Chemistry; A. Eriksson-Wiklund, Stockholm University from the NTW with the Sustainable Chemistry and Analytical Chemistry ACES; Field studies are considered to be more relevant than laboratory tests, as they allow experimentation under natural conditions. Moreover, for some species, endpoints such as reproduction can only be successfully achieved in situ. Thus, the aim of this study was to observe the long-term effects of harbor water on a common snail in the Baltic Sea - *Theodoxus fluviatilis*. Harbors (including marinas) are well-known for accumulating pollutants due to poor water exchange. Antifouling paints are one of the main sources of pollution due to their continuous release of biocides into the harbor waters. Thus it is important to assess the subletal effects of these mixtures of biocides and other contaminants, as they can be involved in the signal for a risk at potential sites. During two consecutive boating seasons we exposed caged snails in 2 or 3 marinas (2014 and 2015, respectively). The cages were immersed at 1 m depth at all sites and were periodically cleaned to ensure good water exchange. The reproduction of snails was assessed in terms of number of eggs laid in each cage after 2 or 4 weeks. During both years we observed significantly less eggs in all marinas compared to the reference sites (eg between 27–99 % less eggs). The nutrients in the water were not limiting at any of the sites and therefore we reckon that the reduction in egg-laying in the marinas was due to other factors. Histopathology of the snails is ongoing for detecting atrophy of the gonads or any other abnormalities. The marina with the lowest snail reproduction in both years also had the highest measured PAH concentrations, i.e. 6 times higher than at a reference site. The chemical analysis of the water revealed the highest copper and zinc concentrations in the respective marina but further evidence is needed to confirm that these metals are the main cause of the observed toxic effects. Analysis of metallothionein concentration in snails is ongoing for measuring a direct biological response to metals such as copper and zinc. This unified investigation of effects at different levels of biological organization will likely help us quantify the contribution of metals vs other organic contaminants to the overall toxicity.

238 Biological effects of an offshore oil and gas installation on local fish populations: The Norwegian Water Column Monitoring programme

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The biological effects of an offshore oil platform on local fish populations were assessed as part of the Water Column Monitoring (WCM) programme for 2014. The Njord A platform was chosen as the study location, which was not in operation and had no current discharge of produced water. Demersal fish species were targeted since they were believed to be less likely to migrate away from the platform than pelagic fish. By targeting organisms deeper in the water column and selecting a platform currently not in operation, the impact of drill cuttings and other sediment sources including leakages from well deposits were the main sources of contaminant. Wild fish including ling (Molva molva), tusk (Brosme brosme), redfish (Sebastes sp.) and saithe (Pollachius virens) were caught with baited rod and line from within the 500 m safety zone of the Njord A platform during the summer of 2014. Reference fish were caught on a separate research cruise in the Icelandic fishing grounds. In the Laboratory of the Norwegian Institute for Water Research, the biological effects of oil and gas activities. Contaminant body burden and a suite of biological effects endpoints were measured in all fish groups and included DNA adducts, DNA strand breaks by comet assay, acetylcholine esterase (AChE) inhibition, ethoxyresorufin 0-deethylase (EROD), vitellogenin (VTG), lysosomal membrane stability (LMS), liver and gill histopathology, PAH metabolites, and PAH body burden. The biomarker data were integrated using the integrated biological response index (IBR/n). Despite low and/or undetected concentrations of PAH and PAH metabolites in fish fillet and bile respectively significant responses in AChE, comet and DNA adducts were found. The biomarker responses indicated exposure to both neurotoxic and genotoxic chemicals in fish inhabiting the lower water column with influence from sediment sources around the Njord A platform. Integration of the biomarker responses (IBR/n) found that all four fish species that were caught from around the platform had markedly higher IBR/n values than their respective reference population. The study shows the advantage of using a suite of biomarkers for assessing the biological effects of low concentrations of complex mixtures with biological effects observed despite low concentrations of PAH measured.

239 The Integrated Biomarker Response: a suitable tool to evaluate toxicity of metal-based nanoparticles

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The current situation goes to show a dramatic change in the field of science and technology with applications in a wide range of areas such as electronics, biomedical applications, bioremediation, energy, cosmetics, water treatment. Among engineered nanoparticles (ENPs), metal-based ENPs comprise the largest number of ENPs such as silver, gold, copper oxide (CuO), zinc oxide (ZnO), cadmium sulfide (CdS) nanoparticles. The toxicity of metal-based ENPs may be either due to their specific physical characteristics or to the release of metal ions from the nanoparticle released from ENPs under environmental conditions. In this study we evaluated toxicity effects of ENPs (Ag, Au, CuO, CdS, ZnO) and their ionic control part on two endobenthic species: the ragworm *Hediste diversicolor* and the bivalve *Scrobicularia plana*. The selected exposure concentrations for Cu, Ag, CdS, and Au (respectively 0, 10, 100, 1000 μg L−1) were selected to cover the range of metal in highly contaminated marine environments. For Au, because this metal is very inert, the concentration (100 μg Au L−1) was chosen in order to induce some biological responses. For ZnO NPs, we selected exposure to 3 mg L−1 ZnO kg−1 sediment since this level is a realistic prediction of the environmental concentration in sediments (Tidek et al., 2009). The implementation of a suite of complementary biomarkers as early warning signals of the presence of contaminants has been successfully applied to provide integrated information on the toxicity of ENPs in aquatic species. A common challenge in multimedia biomarkers studies is to go beyond an independent interpretation of each one, and to really assess a global response of individual species. The aim of the present study was to test the suitability of the Integrated Biomarker Response (IBR) in both species exposed to the different metal-based ENPs studied or to their soluble metal counterpart to provide efficient and easy tools for environmental managers. We evidence that metal-based NP lead to an overall difference in biological responses from the control and their soluble counterpart. The IBR could thus be considered as an efficient tool to transfer some research results to stakeholders.

Environmental risk assessment of chemical mixtures: the steps ahead (I)

240 Applying mixture toxicity modelling to assess the contribution of detected chemicals to in vitro effects: An example from the Danube River

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Surface water can contain a diverse range of organic micropolutants stemming from a number of sources. Targeted chemical analysis alone is unable to detect all micropolutants present and *in vitro* bioassays can be applied complementary to determine the biological effects of complex environmental mixtures. The aim of this study was to test the hypothesis that the *in vitro* effects in large human-impacted rivers are primarily driven by the mixture effects of many chemicals, rather than being dominated by a particular chemical. In this study a suite of bioassays indicative of activation of the aryl hydrocarbon receptor (AHR), activation of the pregnane X receptor (PXR), activation of the estrogen receptor (ER), adaptive stress responses to oxidative stress (Nrf2), genotoxicity (p53) and inflammation (NF-kB) and fish embryo toxicity were applied to large volume solid phase extracted (LVSPE) samples from the Danube River. This was complemented with targeted chemical analysis of 272 chemicals, with available effect concentrations for the detected chemicals collected from the literature or the US EPA ToxCast database. The contribution of detected chemicals to the *in vitro* effects was assessed using the biochemical equivalent concentration (BEQ) concept. Available effect concentrations for between 0 to 13 detected chemicals were found for the different assays. Up to 80% of ER activation was explained by five detected chemicals, with the hormone estrogen and the phytoregulator genistein contributing significantly, while up to 71% of AHR activation could be explained by three chemicals. In contrast, less than 0.2% of the effect was explained by the detected chemicals for PXR activation, oxidative stress response, p53 response and fish embryo toxicity. As a significant fraction of the effect could not be explained by detected chemicals, this study emphasizes the importance of applying complementary test sets of chemical classes in risk assessment and monitoring. The study was limited by the availability of effect concentrations for the detected chemicals and consequently further fingerprinting of water pollutants is recommended.

241 Comparison of approaches to assess the risk of micropolutants in Swiss
243 Mixture risk assessment for surface waters: Comparative assessment of current approaches for assessing the environmental risk in five Swiss catchments

M. Aligari, Swiss Centre for Applied Ecotoxicology EAWARE - EF/Ecoz Centre, T. Doppler, Plattform Wasserqualität VSA; J. Wittmer, Plattform Wasserqualität VSA, c/o Eawag; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology On 01.01.2016 a new water protection ordinance enters into force in Switzerland. In addition to introducing environmental quality standards (EQS) for single compounds, it allows for the consideration of mixture assessment as well as the initiation of measures if a surface water body is at risk of being ecotoxicologically impaired by a mixture of substances. Such measures can be taken even if no single substance EQS is exceeded. In the near future a guidance document will be developed that provides simple but sound methods for assessing the environmental risk of contaminant mixtures in surface waters. For this purpose, the current methods for mixture risk assessment were compared using data from a Swiss surface water monitoring study and chronic EQS proposed for Switzerland. The RQmix predictions always turn out higher than the simple sum of all individual RQ. Most likely, this is due to the extrapolation of the chronic mixture RQ from acute mixture toxicity predictions by using an AF of 1000. The lowest mixture RQ resulted using a method based on similarity of action (RQsim). Since mixture RQ were restricted to substances for which concentration additivity had experimentally been shown, only three mixture RQ groups were assessed: (i) photosystem II inhibitors (triazine and phenylurea herbicides), (ii) inhibitors of very long chain fatty acids (chloroanilines herbicides) and (iii) herbicides with auxin action (phenoxy acids). The RQmix-met method based on identifying the most sensitive trophic level(s) is likely to overestimate the mixture risk if dominant substances have different target taxa within the trophic level. However, the quantitative differences between the methods become marginal, if not all substances present in the sample are analyzed or detected. This was shown by taking passive sampling results for phytoplankton into account, showing that the RQ can be two orders of magnitude higher. All mixture RQ indicate that a risk assessment based on single RQ will underestimate the actual risk. However, the current methods for mixture risk predictions still have some shortcomings. For regulatory purposes a combination between the RQ_sim and the RQ_log method seems to be promising. This may define the range of the actual mixture risk. Furthermore, it is important to include highly toxic compounds which are used in the watershed in any risk analysis.

244 Using prospective assessment to compare municipal wastewater effluents


Municipal Waste Water treatment plants (WWTP) can be a source of “down the drain” chemicals that are targeted by diverse regulatory agencies concerned with potential environmental effects of these chemicals both independently and as mixtures. For the prospective assessment, the chemical mixture is based on domestic activities that are an important source of the chemical mixtures in municipal wastewaters. Starting with activities associated with domestic emissions of chemicals, we identified key products that are used and representative chemicals of those uses. Chemicals were selected based on their reported importance in ecological risk assessments and availability of loading and effects information in Europe. We evaluated a scenario in which a WWTP receives only domestic waste from a fairly small population in order to separate domestic wastewater mixture effects from other influences (e.g., urban stormwater). Chemical loadings to the WWTP were identified based on per capita per day information. Removal rates for each contaminant were based on their physicochemical and biodegradation properties, as well as 3 types of treatment (trickling filter, activated sludge, and advanced oxidation), to determine predicted effluent chemical concentrations (PECs). PECs were compared with predicted no effect concentrations available from the literature. Concentration addition was used to estimate the mixture risk for each scenario. Given activated sludge or better treatment, very few of the representative chemicals individually presented a risk. The aquatic life even when PECs were increased by a factor of 10. Consequently, the chemicals used in this analysis and a concentration addition approach, mixture risk for the activated sludge treatment with or without the addition of advanced oxidation was driven by very few chemicals: galaxolide, EE2, sulfohematoxazole, and carbamazepin. These chemicals have different modes of action and when evaluated using response addition indicated little or no mixture risk given this analysis. Trickling filter treatment resulted in much greater potential for mixture risk in our analyses. Scenarios such as those used here can provide testable hypotheses for studies that include the use of biomonitoring or retrospective-based mixture assessments.

245 Predictability of the Aquatic Toxicity of Realistic Mixtures in Wastewater
Effluents and Their Risk Assessment

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The concept of Concentration Addition (CA) is widely accepted as a default approach for predicting combined toxicity of mixtures of chemicals. However, it can only be applied to mixtures of clearly defined components for their clearly defined mass proportions in the mixture, which limits its applicability to a routine risk assessment of highly complex and variable wastewater effluents. In order to explore the relevance of a mixture assessment compared to a single-substance assessment of residues in wastewater effluents, mixtures of up to ten substances were assessed at two different concentration ratios, including the experimental verification of their predicted toxicity in the absence and presence of wastewater background. The mixtures were tested at an effect-based ratio and at an exposure-based ratio that resembled a realistic mixture scenario. The toxicity of the mixtures could be reasonably well predicted for chronic endpoints both in green algae and in Daphnia magna. There was a rather small tendency to overestimate mixture toxicity by the CA concept than to underestimate it, which demonstrates that the CA approach would be predictive for a mixture risk assessment without being over-protective. The predictability of the mixture toxicity was not influenced by wastewater effluents, i.e. by the presence of other substances at very low concentrations. The presentation will also compare preliminary risk assessments based on measured environmental concentrations (MEC) and predicted environmental concentrations (PEC) and on chronic endpoints of single substances as well as mixtures together with assessment factors for each test organism. In addition, preliminary predicted no effect concentrations (PNEC) will be derived for single substances and the mixtures, and the results will be compared to the calculated MEC and PEC, which will demonstrate the difference for such mixture assessments conducted separately for each trophic level or across trophic levels.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

246 Retroactive monitoring of butyltin in fish and mussels from German marine and freshwaters

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Tributyltin compounds (TBT) and their degradation products dibutyltin (DBT) and monobutyltin (MBT) have been proven to have strong ecotoxicologically effects on aquatic organisms (e.g. imposex of neogastropods, shell malformations of mussels). Therefore, a ban of TBT-containing antifouling coatings for small vessels under 25 m was imposed by the European Commission in 1989 (89/677/EWG) as a first measure. Since 1st January 2003, the usage and distribution of antifouling preparations containing organotin compounds is prohibited by the Commission Directive 2002/62/EG. Furthermore, in 2008 this ban was extended to EU-foreign flagged vessels. A retrospective monitoring of organotin compounds (including TBT, DBT and MBT) in archived freshwater and marine biota samples from the German environmental specimen bank (ESB) has been carried out earlier. The time series for butyltin concentrations in muscle tissue of freshwater fish (bream, Abramis brama) and marine fish (eel, Zoarcus viviparus) as well as in marine mussels (Mytilus edulis) tissue were extended from 2003 to 2011 and samples from 2012/2013 are under investigation. The marine samples were collected from the North and the Baltic Seas, and the freshwater samples from the German rivers Elbe, Danube, Rhine, Murde, Saale, Saar as well as from Lake Belau. After digestion, derivatization and extraction, trace levels of organotin compounds in the pooled and homogenized biota samples were analyzed by a validated sensitive SID-GC/ICP-MS method (species specific isotope dilution – gas chromatography – inductively coupled plasma-mass spectrometry). The aim of this project was to answer the following questions: 1) Does the ban of organotin compounds as antifouling agent show positive effects on the butyltin concentration in aquatic biota? 2)? Are there any differences in concentrations between the various sample sites and the various biota samples? 3) Are there any temporal trends in the concentrations of TBT, DBT and MBT? 

247 Determination of water quality criteria for uranium that account for bioavailability in freshwaters

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Uranium (U) is a chemo- and radio- toxic substance of concern for the aquatic environment, with potential releases to freshwaters due to e.g., mining, processing and waste disposal. In France, a ‘generic’ Predicted No Effect Concentration (PNEC) for freshwater ecosystems has been set to 0.3 µg/L of dissolved U in the water column in addition to the background, based on an exhaustive review of available ecotoxicity data and the use of assessment factors. However, as many other metals, it is well known that U toxicity depends on its bioavailability which is in turn mainly driven by its speciation. For such elements, the last European guideline for deriving Environmental Quality Standards recommends now explicitly to take into account their bioavailability. Therefore, we developed a method based on theoretical simulation of U geochemical speciation in freshwater (VMinuto v3.0, considering U complexion with dissolved organic matter and with alkaline earth and carbonate ions). Based on the assumption that the bioavailable species of U were UO₂⁻, UO₂(OH)²⁻, UO₂(OH)²⁻ and UO₂CO₃⁻, a statistical regression tree analysis was performed to identify physico-chemical domains representative of French freshwaters where different PNEC might apply. Considering these first relevant chemical components, we showed that the physico-chemical domain identified allowed us to derive four PNEC values (ranging from 0.3 to 30 µg/L) conditional to four physico-chemical domains that differ by their alkalinity (cut-off at 75 mg HCO₃⁻/L, pH (cut-off at 7.5) and DOC content (cut-off at 6 mg/L). These findings were formalised in a determination tree. Further analyses were performed to evaluate the robustness of this tree and its domain of applicability. These conditional PNEC values for freshwaters could be used as a refined step in any ecological risk assessment for uranium. However, their applicability in any monitoring programme would need some adaptation in the monitoring practices: not only the dissolved fraction of uranium, but also the influent physico-chemical parameters (pH, alkalinity, DOC) should be measured together, with a sufficient accuracy and sensitivity.

248 A new approach to diagnosing the ecological effects of metal-contaminated sediment in natural systems

J.P. Murphy, Queen Mary University of London / School of Biological and Chemical Sciences; J.J. Jones, QMUL / School of Biological and Chemical Sciences; P. Rainbow, The Natural History Museum / Dept of Zoology; K. Spencer, Queen Mary University of London / School of Geography; A.L. Collins, Rothamsted Research, North Wyke; A. Moorhouse, the Coal Authority; V. Agapito, DEFFRA; P. Edwards, Southern Illinois University - Environmental Specimen Bank and Elemental Analysis; F. Parsonage, DEFFRA; H. Potter, Environment Agency; P. Whitney, Environment Agency / Evidence Pollution from abandoned metal mines is a serious impediment to rivers meeting the water quality targets set out by the EU Water Framework Directive. Recently, there has been recognition that these risks require approaches to setting environmental guidelines that are based on a consistent definition of exposure in systems where bioavailability may vary, thus allowing more robust and useful ecological effect thresholds to be identified. We have used a new approach to relate the levels of toxic metals in sediments affected by mining to ecological damage based on bioavailability. The concept is to use widespread biomonitor species as chemical probes for bioavailable metal, and to relate this measure of metal stress to variation in invertebrate community composition (a WFD-compliant measure of ecological condition). This approach bridges two established methodologies that are traditionally used independently (community-level field observations and laboratory toxicity). We selected 99 river sites in England and Wales, across 20 spatially-independent, replicate river catchments impacted by metal mining. Within each catchment, sites were located upstream and downstream of abandoned mine facilities. At each site we sampled the macroinvertebrate community, collected biomonitor specimens for metal bioaccumulation analysis, and collected a fine sediment sample for metals content, organism (on and part of a new site) using mass spectrometry. We then quantified the response of the macroinvertebrate community to variation in metal bioavailability, having first taken into account the confounding effects of other environmental factors. We identified the position of taxa across the gradient metal bioavailability, providing the basis for a new diagnostic metric to metal stress in rivers. The new metric was tested on an independent dataset drawn from existing data sources. Our aim is to provide a WFD-compliant method for detecting where contaminated sediments are causing ecological impacts.

249 Ten years on: Soil screening values for ecological risk assessment in the UK

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A presentation on the “Use of soil screening values for metals for ecological risk assessment in England and Wales” as presented at SETAC Europe in 2006. Since then, both scientific knowledge and regulatory priorities have changed in the UK in regards to soils and ecological protection. This presentation, 10 years on, provides an outline of those key changes and demonstrates how the continued practical and pragmatic application of complex scientific concepts can deliver solutions to regulatory challenges that face many in Europe and across the globe. The presentation provides a review of the ecological indicators and trace element criteria and in particular the implementation of a bioavailability framework to derive soil screening values (SSVs), to provide a generic screening tool to ‘rule out’ the further assessment of potentially contaminated sites. Just 10 soil screening values (SSVs) for prioritised chemicals were derived at the time. Regulatory priorities have now changed from contaminated land, to assessments for contaminated land. SSVs are needed for many more and different chemicals that 10 years ago. However,

SETAC Europe 26th Annual Meeting Abstract Book

59
through the use of new guidance, the ECHA dissemination portal and industry collaborations SSVs have been developed that utilise new data and research findings. This presentation will show that the lag between scientific developments and regulatory implementation can be relatively short. Changing regulatory needs can keep pace with scientific developments.

250 Assessing the integrated terrestrial ecotoxicity of Cu and Ni species for life cycle assessment (LCA)
A. Vádhal, CIRAIG; L. Deschenes, École Polytechnique de Montréal / Chemical engineering department; C. Riquet, CIRAIG - ESG - UQAM / Strategy corporate social responsibility; L. Deschenes, École Polytechnique de Montréal / Genie Chimique: Life cycle assessment (LCA) validity of metals toxicity can be significantly distribution of obtained BF values. Moreover, BFs ranking was compared and the regression method. The BFs included in the calculation of CFs for Cu and Ni have model is able to calculate bioavailable fraction of Cu and Ni with uncertainties of less than two or three orders of magnitude, respectively, for a wide range of soils distributed globally. It is in better match with the experimental data in term of absolute value than the empirical regression results. To address this variability, 518 archetypes and 13 groups of archetypes are created to show the global distribution of obtained BF values. Moreover, BFs ranking was compared and the soluble Cu and Ni Spearman rank correlation coefficients showed a significant correlation between BFs obtained by WHAM and BFs calculated by the regression method. The BFs included in the calculation of CFs for Cu and Ni have been applied in terrestrial ecotoxicity and the level of regionalization required to adequately represent the spatial variability of CF has been defined. The obtained results from two different versions of the model (WHAM 6.0 and 7) were also compared and the results for WHAM 7.0 show that the model is more sensitive for Cu and Ni that the model for WHAM 6.0. The median factor between the most and the least sensitive species is 1.48. The median factor between the most and the least sensitive toxicity data (1-7 days) from laboratory studies performed on aquatic invertebrates, Daphnia magna.

251 Quantitative risk assessment of mercury contamination to ecosystem in a mercury mining area
Y. Lin, Norwegian Institute for Water Research NIVA; G. Qiou, Institute of Geochemistry Chinese Academy of Sciences; H. Braaten, Norwegian Institute for Water Research NIVA; X. Feng, Institute of Geochemistry Chinese Academy of Sciences; T. Larssen, Norwegian Institute for Water Research NIVA; X. Feng, Institute of Geochemistry Chinese Academy of Sciences; T. Larssen, Nordic Ecological Assessment Programme (NEAP); Wanshan Mercury Mining Area (WMMA) which is located in Southwest China has a long history of Hg exploration and smelting. Hg contamination has been a serious local problem for both human and environment for decades. The risks of Hg to human health and ecological environment have been reported in several studies, and dietary intake rather than inhalation has been identified as the main exposure pathway for the local residents. However there has been less focus on the risk of Hg contamination on the ecosystem. The scope of this study is therefore aimed at quantifying the risk related to Hg pollution from an ecosystem perspective. The endpoints of the assessment will be plants and biota rather human. This study aims at developing a site specific index system to identify the risks of Hg contamination on major biota species (rice, invertebrates and birds). The exposure level in this study is determined by direct sampling and measurement of Hg concentrations in different references species at WMMA (soil, rice, earthworm, grasshopper, spiders, snails). Total Hg concentrations in the samples collected from the studied species was selected as proxy for comparing the concentration levels. No adverse effect concentration (NOAEL) is used as the risk assessment benchmark for the different species. Risk quotients (RQ) are then derived based on comparing the Hg concentrations in each species with benchmark values. Four levels of risk were proposed based on RQ values: Level 1: Background. If all RQ values are smaller than 1, and RQ are smaller than reference site, the species is then considered to be living under a similar condition as in the background site. Level 2: Minimal risk. If all the RQ values are smaller than 1, the species is then considered having minimal risk. Level 3: Moderate risk. It is possible that RQ of tissue concentration is smaller than 1, but RQ of diet is bigger than 1. This indicates that the species is having a risky diet, while tissue concentration has not reached risky level. Level 4: High risk. If RQ based on tissue concentration is greater than 1, it can be confirmed that the species is at risk. The plan for remediation at WMMA is under development with local authority, the outcome from the risk assessment will then supply important basis for the remediation method development, and possible measures to reduce the risk will be then proposed based on the results.

Higher tier tests in the risk assessment of plant protection products (II)
252 An SSD-based investigation of the relative sensitivity of Daphnia magna - Implications for risk assessment
M. Gustavsson, University of Gothenburg; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences The species sensitivity distribution can be used to quantify the spread in sensitivity between different organisms. A large width of a distribution implies that there is a large difference between the most and the least sensitive species tested. Currently approximately 30% of the PNEC’s from the single substances registered under REACH can be traced back to Daphnia magna. In order to determine whether the current risk assessment practice is protective, we compared the sensitivity of Daphnia magna to the sensitivity of other aquatic invertebrates.

The data was thereafter filtered and only those compounds that contained acute toxicity data (1-7 days) from laboratory studies performed on aquatic invertebrates were kept. Finally the species sensitivity distributions were fitted using the R package “drc” for all compounds where 8 or more species had been tested. In total 157 species sensitivity distributions could be generated. The results show that the sensitivity of Daphnia magna, in relation to other tested aquatic invertebrates, varied randomly. The median factor between the most and the least sensitive aquatic invertebrate for each individual compound is estimated to be around 1300. The species sensitivity distribution for three specific compounds show that 2.3% (malathion) and 2.3% (endosulfan) of all aquatic invertebrates are assumed to be more sensitive than Daphnia magna. The difference between the relative sensitivity of Daphnia magna for these three compounds demonstrates the necessity in comparing the available species sensitivity distributions to the currently used assessment factors within chemical risk assessment. The presentation will provide further details on the spread of the species sensitivity distributions and the distribution of sensitivity steepnesses over all the tested chemicals will be analysed.

253 EFSA Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface waters
S. Duquene, UBA, Federal Environmental agency; A. Aagaard, Pesticides Division / Danish EPA; T. Brock, Alterra, Wageningen University and Research Centre / Environmental Risk Assessment Team; B. van Hattum, Institute for Environmental Studies, VU University, de Boelelaan; M. Klein, Fraunhofer IME; I. Sundh, Swedish University of Agricultural Sciences (SLU), Department of Microbiology, Uppsala; R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; M. Egmose, EFSA

Some aspects, proposals and/or recommendations described in the EFSA scientific opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water are presented here. They include elements of exposure as well as effect assessments since the ERA is a combination of both. The issues highlighted here are the following: Triggers for sediment ecotoxicity testing and a decision scheme based on a tiered effect assessment approach for different types of organisms; How to link regulatory acceptable concentrations (RACs) to predicted environmental concentrations (PECs), using the ecotoxicologically relevant concentrations (ERCs) concept that is influenced by the choice of sediment layer depth, exposure metric and test duration; Methodology for introducing an accumulation factor to account in a conservative way for effects of multi-year applications; Assessment of impacts of bioaccumulation, biomagnification and secondary poisoning; Issues related to uncertainties of the current and/or proposed ERA approaches. The specific protection goals (SPGs) for sediment-inhabiting organisms are based, as for the aquatic organisms in edge of field waters, on two options, i.e. (1) the ecological threshold option (ETO), accepting negligible population effects only, and (2) the ecological recovery option (ERO), accepting some population-level effects if ecological recovery is expected within an acceptable time period. For several reasons, for the time being it is suggested that the ETO is the best option to provide adequate protection of benthic organisms.

254 Effects of sediment-spiked lufenuron on benthic macroinvertebrates in single-species tests and outdoor microcosms
T. Brock, Alterra, Wageningen University and Research Centre / Environmental Risk Assessment Team; D. Bas, L. Bibbe, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics IBED; D. Belgers, M. Boerwinkel, Alterra Wageningen UR / ERA team; J. Jilesen; C.J.A.M. Posthuma-Doodeman; C.E. Smit; J.J.M. van der Steen; G.M.A. van Hattum, Wageningen University and Research Centre / Environmental Risk Assessment Team; B. van Hattum, Institute for Environmental Studies, VU University, de Boelelaan; M. Klein, Fraunhofer IME; I. Sundh, Swedish University of Agricultural Sciences (SLU), Department of Microbiology, Uppsala; R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; M. Egmose, EFSA

The publication of the proposed EFSA risk assessment of plant protection products for arthropods and solitary bees highlights the need for suitable test designs and the handling of the test organisms. The aim of this study was to establish suitable test designs and the handling of the test organisms for the purpose of the experiment, i.e. to derive regulatory acceptable concentration (RAC) and fertile. Fenoxycarb can be used to introduce brood termination in eggs and larvae of solitary bees. The arthropod fauna of such ecosystems is generally examined after the end of exposure. The development of the progeny was examined. After the end of exposure the development of the progeny was evaluated as ecologically active. The hatching success was recorded. The first results show, that it is possible to obtain the desired results with the newly proposed EFSA risk assessment of plant protection products for arthropods and solitary bees. The newly proposed EFSA risk assessment of plant protection products for arthropods and solitary bees includes for the first time not only honey bees as test organisms but also other bees species. The aim of this study was to introduce brood termination in eggs and larvae of solitary bees. The arthropod fauna of such ecosystems is generally examined after the end of exposure. The development of the progeny was examined. After the end of exposure the development of the progeny was evaluated as ecologically active. The hatching success was recorded. The first results show, that it is possible to obtain the desired results with the newly proposed EFSA risk assessment of plant protection products for arthropods and solitary bees.

SETAC Europe 26th Annual Meeting Abstract Book
255 Applying the MDD concept to terrestrial NTA studies

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Natural terrestrial ecosystems such as meadows adjacent to cropped fields may be exposed to spray drift. The arthropod fauna of such ecosystems is generally abundant and species richness and diversity are higher in comparison with a terrestrial background fauna. The risk assessment of pesticides in natural (i.e. unbounded) terrestrial off field systems as described in De Jong et al.[2] typically these experimental systems follow a replicated (n=4) plot design, with >30 m plots and multiple sampling methods over a period of 8 weeks following a single application event. A large number of arthropod taxa (800-1000) is assessed and typically at least 80-100 are sufficiently abundant for statistical hypothesis testing. As a consequence of inherent parametric constraints we use non-parametric tests for these analyses. Our primary objective is to test whether the criteria in the aquatic scheme can be applied to derive regulatory acceptable exposure levels for terrestrial systems as well. We use the design scheme in Brock et al. (2015) to data obtained in a large number of off-field studies. MDD’s were calculated using the technique provided by Van der Hoeven (2008)[3]. Potentially sensitive taxa were defined using empirical criteria, in particular whether a specific or related taxon was consistently affected by the reference treatment in different studies. Ecologically vulnerable taxa were defined similarly as taxa consistently affected by the reference item for four or more weeks in different studies. [1] T.C.M. Brock; M. Hammens-Wirtz; U. Hommen; T. G. Preuss; H-T. Ratte; I. Roessink; T. Strauss; P.J. Van den Brink. Environ Sci Pollut Res (2015) 22: 1160-1174. [2] F.M.W. de Jong; F.M. Bakker; K. Brown; C.J.T.J. Jilesen; C.J.A.M. Posthumus-Doodeeman; C.E. Smit; J.J.M. van der Steen; G.M.A. van Eekelen. 2010 ISBN/EAN: 978-90-6960-245-5 [3] Van der Hoeven, N. 2008. Ecotoxicol. Environ.Saf. 70:61-66

256 Development of suitable experimental designs for semi-field trials with solitary bees

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The publication of the proposed EFSAs risk assessment of plant protection products for pollinators led to an increasing demand for experiments with non-Apis pollinators. However, no official guidance for the standardized semi-field trials exists so far. To overcome this lack of guidance, a semi field study was performed to continue our research and increase the knowledge about suitable test designs and the handling of the test organisms. The aim of this study was to implement a test system for trials under semi-field conditions with solitary wild bees. In the study the potential effects of exposure of bees and their brood to test item treated and untreated plants and a based statistical evidence of validity were the focal points. During the exposure and after end of the exposure all relevant parameters were recorded. The test design is in accordance with a proposal from the ICPR non-Apis working group. The semi-field study with the red mason bee Osmia bicornis (Hymenoptera, Megachilidae) was conducted in winter oilseed rape. Dimethoate can be used as a toxic reference to show acute effects on adult wild bees. The endpoints chosen were useful for a study design and the variability was low with regard to the observed effects. The two rates of dimethoate tested showed already the maximum effect so that dimethoate can be used as a toxic reference in semi-field studies at the lower rate. Furthermore the statistical analysis showed that the test design is valid and repeatable.

257 Experimental design for semi-field trials to test brood affecting plant protection products with solitary bees


The newly proposed EFSAs risk assessment of plant protection products for pollinators includes for the first time not only honey bees as test organisms but also non-Apis pollinators. However, there is no official guideline for standardized semi-field trials. To support a tiered risk assessment a semi-field study design was developed and performed in 2015 based on available publications and advice from an ICPR workshop. The results of these studies make it possible to improve future designs and recommendations for the handling of the test organism can be given. The objective of this study was to develop a semi-field test design for plant protection products affecting brood of solitary wild bees. In the study the potential effects of exposure of adult bees and their brood to an insect growth regulator were examined. After the end of exposure the brood survival and development was followed until the following spring and the reproduction success was evaluated as an endpoint. The semi-field brood trial with the red mason bee Osmia bicornis (Hymenoptera, Megachilidae) was conducted in a Phacelia crop. The test design included a water treated control and two treatment groups. Each treatment group was replicated with 4 tunnels. The exposure period started at the beginning of July 2015. The treatment applied was fenoxycarb, an insect growth regulator also used as a reference substance in honey bee brood studies. Two rates were tested with 150 g a.i./ha (T1) and 350 g a.i./ha (T2). The following endpoints were observed in the study: to evaluate sub-lethal effects on adult bees, the nectar consumption of females and the flight activity was documented. In order to evaluate brood effects, the cell production, the cocoon production and the brood termination rate were assessed. To ensure equal starting conditions in all treatment groups the hatching success was recorded. The first results show, that it is possible to perform a semi-field brood study with the red mason bee Osmia bicornis in Phacelia. The bees can be stored until June and hatched specimens are still viable and fertile. Fenoxycarb can be used to introduce brood termination in eggs and larvae of red mason bees. The endpoints chosen are useful to evaluate effects on reproductive success and the variability between replicates was low. The lower rate of fenoxycarb showed already the maximum effect so that fenoxycarb can be used as a toxic reference in semi-field studies.

Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy

258 Comprehensive assessment of fruits and vegetables human health effects in a LCA context

K. Stylianou, University of Michigan - School of Public Health / Environmental Health Sciences; O. Jollivet, University of Michigan; P. Funke, Technical University of Denmark / Quantitative Sustainability Assessment Division Purpose: Nutritional effects of ‘use stage’ of food items (i.e. unbounded) can have a substantial effect on human health; yet, they are often not considered in life cycle assessment (LCA). In our study we explore the case of increased fruit and vegetable consumption, a healthy dietary option – that could result in higher exposures to a wide variety of pesticides – and investigate the trade-offs between associated environmental and nutritional health effects. Methods: We employ the Combined Nutritional and Environmental Life Cycle Assessment (CONE-LCA) framework that evaluates and compares in parallel the environmental and nutritional effects of foods expressed in Disability Adjusted Life Years (DALYs). For the environmental health assessment we consider impact categories such as global warming and particulate matter (PM) as well as chemical exposure due to pesticide residues. Global warming and PM are assessed following a traditional LCA approach. For the pesticide residue exposure, we use publically available health impact scores derived from toxicological studies of numerous pesticide active ingredients. For the nutritional assessment we focus on the various health outcomes considered in the global burden of disease, which are based on epidemiological studies. Results and discussion: Adding one serving of fruits or

outdoor sediment-spiked microcosm experiment and those of 28-d sediment-spiked single-species toxicity tests with Chironomus riparius, Hyallela azteca and Lumbricus variegatus. Twelve weeks after the construction of the microcosms mean measured exposure concentrations in the sediment were on average 87.7% measured initially. Treatment-related effects of sediment-spiked luminescent bacteria were observed on the mollusc species and one observed elevated populations of insects and macrocrustaceans (lowest NOEC for benthic insects 0.8 μg a.s./g OC and for benthic crustaceans 8.5 μg a.s./g OC). Treatment-related increases in abundance (indirect effects) were observed for benthic oligochaote worms (lowest NOEC of 0.8 μg a.s./g OC). The 28-d sediment-spiked laboratory toxicity tests resulted in EC50 values of 0.49 μg a.s./g OC for C. riparius, 1.20 μg a.s./g OC for H. azteca and 211 μg a.s./g OC for L. variegatus. These data also explain why oligochaete worms did not suffer pronounced toxic effects in the microcosm experiment, but could increase in abundance due to the decline of sensitive benthic arthropod populations (release of competition). The chronic laboratory toxicity data measured during the OECD tier-1 assessment approach proposed by EFSA for benthic organisms and sediment-bound insecticides, using the lowest chronic EC10 value for the combination C. riparius and H. azteca and an assessment factor of 10, is protective for the insecticide fenuron and the responses observed in the sediment-spiked microcosm test. a.i./ha dimethoate. The following end points were observed in the study: nest occupation by female individuals, flight activity, reproduction capacity by means of produced cells and cocoons and brood termination rate. Hatching success was also recorded to assess the viability of the used test specimens. The first results showed clearly the potential to perform semi-field studies with the red mason bee Osmia bicornis in winter oilseed rape. Dimethoate can be used as a toxic reference to show acute effects on adult wild bees. The endpoints chosen were useful for a study design and the variability was low with regard to the observed effects. The two rates of dimethoate tested showed already the maximum effect so that dimethoate can be used as a toxic reference in semi-field studies at the lower rate. Furthermore the statistical analysis showed that the test design is valid and repeatable.

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vegetables to the current average diet in Europe may lead to substantial nutritional health benefits. These nutritional benefits are slightly increased when we consider substitution scenarios in which the substituted food items are associated with negative health effects, such as red meat and trans-fat. Overall, environmental health impacts associated with this addition are substantially smaller compared to nutritional benefits of such scenarios. Even when considering an uncertainty factor of 400 for the impacts of pesticide residues. Conclusion: The present study illustrates the importance of considering nutritional effects of food items in LCAs. Our preliminary results suggest that nutritional health effects of food items can be substantial and comparable to environmental impacts, especially for nutritional foods such as fruits and vegetables. This approach could be used for making recommendations about sustainable diets and food choices.

259 Pesticides’ impacts of bananas from different regions
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Most bananas sold in Europe nowadays have some sort of sustainability label. Consumers and supermarkets therefore may have the impression that the labelled bananas are produced in a sustainable way. Sustainability covers many social and environmental themes and several of these are addressed by the labels only to a certain extent. However, large amounts of pesticides are still used in conventional banana production, resulting in potential human health and ecosystem impacts. Using no xenobiotic pesticides at all as is the case for organic bananas is not an option for the large scale, because it needs very specific climatic and logistic conditions. Different climatic, soil conditions and production practices between farm types can lead to large variations in the pesticide use. In this study, the toxicity-related impacts of pesticides applied in banana production were calculated with the consensus model USEtox version 2.0 for three case studies: an organic farm in Peru, smallholder farms in Ecuador, and a conventional plantation in Panama. The resulting toxicity-related pesticide footprints show that the use of large quantities of myclobutanil, chlorpyrifos and mancozeb in Panama case have the highest contribution to the human health impact profile. The use of azoxystrobin and mancozeb in this case shows the largest share on the impact profile for organisms in freshwater ecosystems. The footprint of the pesticides used in banana production from the Panama case is about 20 to 30 times larger than in the Ecuadorian case. In a sensitivity analysis, in each case, regionally specific landscape parameters were used to calculate specific factors for each of the pesticides used. This increased the score of the Panama case by about 300%, while the score of the Ecuador case did not change significantly. Default values for these parameters are provided by USEtox for several regions, among which for a region covering Central America and one covering Peru and southern Ecuador. Calculations with an alternative impact assessment model (USE-LCA) generally confirm the results with USEtox, but shows that the impact on terrestrial and marine ecosystems, which are not included in USEtox, can be significant.

260 Towards a consensual method to assess climate change impacts from bio-based systems
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This study focused on climate change impact assessments of systems involving compartments of the biogenic carbon cycle, addressing the issue of assessing the actual climate impacts of the life-cycle emissions of bio-based systems. A critical review was carried out on seven different characterisation models dealing with these environmental mechanisms, including five methods dealing with Greenhouse Gas (GHG) emissions - conventional Global Warming Potentials (GWP) from IPCC according to a carbon neutrality approach or to a full accounting approach, time-adjusted GWP, biogenic GWP and biogenic accounting factors - and two methods dealing with land occupation and/or transformation - ILCD / IPCC recommendation to account for carbon stock changes from land transformation and Müller-Wenk proposal adopted in the land use framework. These models were rated over eight criteria divided into four categories: completeness in terms of environmental mechanisms covered, scientific soundness, genericity, and easiness of use. Results showed that the currently recommended methods are conventional GWP according to a full accounting approach for the assessment of greenhouse gas emissions, and the ILCD / IPCC recommendation to account for carbon stock changes from land transformation. However, despite the potential of their genericity, these methods fail to take into account the dynamic nature of the biogenic carbon cycle and new methods have then been developed for this purpose. These methods present many gaps but two were identified as promising characterisation models: time-adjusted GWP and Müller-Wenk proposal. Finally, a new method that benefits from the advantages of these two last methods is proposed. This method relies on the land use framework related to carbon sequestration potential with the time-adjusted GWP embedded to better reflect the dynamics and reversibility of the biogenic carbon cycle. It is compatible with full accounting approach for GHG emissions and temporary carbon storage valuation. It also remains sufficiently practicable.

Which functional unit to assess environmental impacts of dairy system intensification?
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262 Environmental Impact of food consumption in EU
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In order to comprehensively assess the impact of food consumption at EU level, a detailed product-based LCA from ‘cradle to grave’ has been conducted aiming at: i) identifying the most representative food and beverage products consumed in the EU-27 via a statistical analysis of food consumption, selecting 2010 as reference year, ii) evaluating, via an LCA, the life cycle environmental impact of the average food consumption of an EU-27 citizen in one year following the ILCD recommendations for impact assessment, iii) developing a strategy for using the BoP food as baseline scenario for testing ecoinnovation options for impact reduction. The methodology developed for assessing the impacts of intensive food consumption in EU, based on a basket of food products, includes the following steps: 1) Quantitative and qualitative analysis of the structure of the EU consumption category of nutrition – during the years 2000-2010 – including international trade and selection of a basket of representative products for the consumption category of nutrition for the year 2010. 2) Collection and development of process-based LCIs for the selected representative products. 3) Calculation of the environmental impact results, based on the results of the previous steps. 4) Quantitative and qualitative analysis of the environmental impacts of the selected nutrition basket, with conclusions and recommendations for future policy. The overall results indicate that in the majority of the impact categories the most burdensome consumed foods are meat products and dairy products. The agricultural phase is the most impacting lifecycle stage of the daily food, due to the contribution of agronomic and zoo-technical activities. Food processing and logistics follow in importance, due to the energy intensity and the related emissions to atmosphere, occurring during the production of heat, steam and electricity and during transport. Regarding the end of life, human excretion and wastewater treatments are posing burdens related to eutrophying substances. The impact assessment results of this study could be used as a means to provide an index for monitoring and analysis, in order to evaluate the effect of possible changes in future consumption patterns and to indicate priority areas; a further step of the analysis should be to develop scenarios of eco-innovation and behavioural changes to test their effect at the EU scale and to prioritize their implementation.

Persistent and mobile contaminants in the aquatic environment: how to identify, analyse and regulate a...
potential threat for drinking water resources (I)

263 Identification, analysis, removal and regulation of persistent and mobile organic chemicals in the drinking water cycle - The approach of the EU project PROMOTE

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Surface water and groundwater are the two major sources for drinking water in Europe. Their chemical quality may be affected by human activities, among them the release of chemicals that we are producing and using. If these chemicals are poorly degradable (persistent) and polar (i.e., mobile in the aquatic environment), or if they are transformed into poorly degradable and polar compounds, then these chemicals may specifically be of concern with respect to contamination of water used for drinking water production and, eventually, for the quality of drinking water itself. We denote such compounds persistent mobile organic chemicals (PMOC). PMOC are generally substances of low molecular weight with a high portion of heteroatoms. They may even be ionic at neutral pH. These properties make PMOC extremely challenging to analyze, because they hamper enrichment from water as well as chromatography. Consequently, knowledge on the occurrence of PMOC in aquatic compartments (including source waters used for drinking water production) is very limited. It is thus questionable whether sufficient protection of drinking water resources with respect to PMOC is in place. PROMOTE (PROtesting water resources from Mobile Trace chemicals) is a recently launched research project under the European Union Joint Programming Initiative “Water Challenges for a Changing World” (Water JPI). PROMOTE focuses on PMOC in environmental water cycles and in drinking water production. The objectives are to close the significant knowledge gaps with respect to (a) identification and prioritization of the PMOC of highest concern, (b) trace analytical methods for screening and quantitative determination of PMOC in water, (c) occurrence and levels of PMOC in groundwater and surface water, (d) environmental emissions and (e) clean-up strategies or transformation of PMOC in the drinking water production. Based on the expected results, PROMOTE strives to develop recommendations with respect to chemical regulation (REACH) and water quality monitoring (WFD watch list).

264 Ranking REACH registered chemicals for Persistence and Mobility: Neutral, Ionizable and Ionic Compounds

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Herein we present a novel set of tools to identify which of the REACH registered compounds are most likely to be persistent, mobile organic compounds (PMOCs), and thereby capable of rapid and sustained distribution in the environment and drinking water supplies. The REACH list was chosen not only because it provides an overview of all substances currently in commerce that can contain environmental data related to mobility and persistence. We therefore conducted a literature search of these REACH dossier as well as the peer-reviewed literature, and employed a swift of quantitative structure-activity relationships (QSARs) to assemble persistence and mobility parameters. As many ionisable compounds could not be handled by standard QSARs, and only few had experimental data available, we developed simple QSARs that gave broad estimations on the likelyhood of persistence and mobility. Further, hydrlosysis products were also assessed for the likelihood of being a PMOC. These data and tools can be used to identify previously undetected drinking water contaminants, or for preventing them from occuring.

265 Screening of polar chemicals in water by liquid chromatography-high resolution mass spectrometry employing mixed-mode columns

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The populairization of liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) has permitted the exploitation of its high mass accuracy and resolution capacities for screening of a large set of organic pollutants without the need of having pure standards and chemical-specific targets. For this, most LC-HRMS screening methods rely on reversed-phase LC (RPLC), which is quite limited for the detection of very polar chemicals. Hence, many very polar (organic) chemicals may have not been recognized as water pollutants of concern, yet, because the analytical methods developed so far are unable to detect them. However, these polar pollutants are highly mobile in the water cycle and spread and even reach drinking water, if they are persistent, i.e. “persistent mobile organic pollutants” (PMOC). In this context, the research project PROMOTE (http://www.promote-water.eu/), funded by the Water JPI, aims to improve the knowledge on these PMOC in the water cycle, including improving analytical tools. Thus, the goal of this study was to improve the analytical detectability of PMOC by using mixed-mode LC (MMLC) combined to HRMS, for screening purposes. MMLC provides the combination of RP and non-exchange functionalities, which allows the unambiguous determination of analytes with extremely different properties (i.e. ionic, basic, acidic and neutral), so it is a promising technology for analyzing very polar chemicals. To reach this objective, a group of over 40 very polar model chemicals with different acid/base functionalities was employed for exploring the retention behavior and then a suspect screening approach was employed for detecting PMOC in different water samples, with several commercial and non-commercial accurate mass libraries. Some of the detected chemicals include well-known PMOC, as asaculuffam, perfluorobutanoic acid, trichlorophosphyl phosphate or metformin, and some newly detected PMOC. Acknowledgements - This work is financed by MINECO (PI2013-117), in the frame of the collaborative international consortium “WATERJPI2013-PROMOTE” of the Water Challenges for a Changing World Joint Programming Initiative (Water JPI) Pilot Call. We also acknowledge the support of Xunta de Galicia (“Consolidação” funds) and FEDER.

266 Identification of persistent and mobile contaminants impacting raw and drinking waters

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Highly polar organic substances may be able to penetrate natural and artificial barriers and are thus mobile in the water cycle. If these mobile organic contaminants (MOC) are not addressed, they can cause chronic and even carcinogenic effects. Their removal during waste water treatment and drinking water purification may prove difficult. When these substances are present in high concentrations, toxic or undergo toxiation, problems for the aquatic environment and human health may arise. As a consequence of the lack of established analytical methods for MOC, only limited information about their occurrence and origin is available, which severely exacerbates the sophisticated monitoring and effective regulation of PMOC and their precursors. To identify potential PMOC, and thus begin to close this gap in knowledge we analysed 20 water samples form five European countries, including WWTP effluents, surface waters, ground waters and drinking waters. Two independent pre-treatment methods were deployed to ensure a broad coverage of potential PMOC for a subsequent HILIC-HRMS non-target screening. As sample pre-treatment methods, evaporation and reconstitution and an SPE method utilizing WAX, WCX and OCB materials in one cartridge were performed. Thus the pre-concentration of the samples and the solvent exchange necessary for the sensitive analysis of water samples with HILIC-HRMS were achieved. Detected substances were prioritized based on their signal intensities and frequency of detection, whereby substances detected in drinking water were given the highest priority. To identify high priority substances, their fragmentation behaviour was investigated and H/D exchange was performed to further facilitate identification. Once a tentative identification was achieved, the fragmentation pattern as well as the retention time were compared with a reference substance, if available. If the comparison of fragmentation patterns was inconclusive or the fragmentation of a substance yielded only few or very common fragments, the retention times of the analyze and the reference substance were compared on a second column with a complementary selectivity to increase the confidence level of identification.

267 Pyrazole, a polar new emerging industrial contaminant

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In the Netherlands around 40% of the drinking water originates from surface water, taken mainly from rivers. Therefore a study was conducted to warn monitoring stations safeguard the quality of water taken in by an array of sensors, daphnia, algae, mussels and instrumental techniques like automated HPLC Diode Array Detection (DAD). The different monitoring stations employ a common best practice protocol HPLC-DAD screening, the so-called UV fingerprint screening which is performed daily. A uniform interchangeable UV-spectrum database helps to identify unknowns without the need for a standard. Known and unknown compounds are followed by using their retention time index, their UV spectrum, and internal standard equivalents. This summer a daphnia sensor and the mussel monitor were triggered in the river Meuse. A compound was measured by crossing over between a broad peak emerging with a relative short retention index (7.62) indicating a contaminant with a highly polar nature not present in the UV database. This resulted in a closedown of the water intake for the production of drinking water. The aim of this study was to identify this new emerging compound by hyphenating the HPLC-DAD to the LTQ-FT-Orbitrap-MS. Non-target screening LCMS is most often employing electron ionization (EI) as detection method due to the high sensitivity of the compounds could not be targeted by ESI and hence the switch was made to atmospheric chemical
ionisation interface (APCI). This resulted in a clear intense corresponding signal (34 fold higher in counts than HESI) in the mass spectrometer corresponding with the exact retention time on the HPLC-DAD, but no fragmentation was observed. The structural formula was found to be C₂H₄N₂, and two possible suspects were selected based on structure and log Kₐₐₚₑₐ₅ namely imidazole and pyrazole. The latter was effective in establishing a calibration curve the concentration in the alarm sample was found to be ~100 µg/L. Pyrazole is widely used as an starting product for the synthesis of pharmaceuticals and pesticides and a known industrial by-product. Now that pyrazole has been identified as an industrial contaminant in surface water, the toxic properties of the substance can be elucidated in order to establish health based guideline values for (sources of) drinking water.

268 Threat of drinking water resources pollution in the vicinity of Novi Sad
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The available information and results about pollution of raw water used for abstraction of drinking water in Novi Sad municipality are significant for good eco-status of surface water in river Danube, risk management as well as for abstraction of drinking water in Novi Sad municipality. The risk management plans and strategies against hazards, which might be caused by those results the list of 300 relevant organic and inorganic compounds was foreseen (some of) the steps, or performing appropriate testing to fulfil the need for both public regulatory authorities and industry to assess the NM properties and the exposure pathway will affect the behaviour and fate of nanomaterials in a read-across case. Since then, progress has been made in both regulatory and scientific communities. To discuss the most recent advancements an EU-sponsored OECD Expert Meeting on ‘Grouping and read-across for nanomaterials’ is held over two days in Brussels on 13-14 April 2016. The main focus of the OECD Expert Meeting is on producing practical, concrete recommendations on grouping and read-across for MNs for the best uptake in a regulatory context. This oral presentation will provide an overview of the state-of-the-art on grouping and read-across of MNs based on current documents and the OECD Expert Meeting as well as best practices and lessons learnt from existing case-studies. In addition, preliminary findings from the discussions in break-out sessions will be reported and discussed with the aim of outlining possible ways forward.

270 A strategy for read-across between nanoforms

Grouping of substances and read-across are valuable approaches in regulatory frameworks to minimise costs and animal testing. Where experimental data are insufficently available for hazard characterisation one may in some cases predict the properties of a substance based on data from structurally similar substances. All of nanomaterials, read-across and grouping approaches may be an important means to reduce costs of information generation and addressing data gaps. Based on existing knowledge and approaches, we developed a systematic strategy to substantiate read-across for nanoforms of the same substance with a focus on compliance with the EU REACH Regulation. The strategy comprises six different steps, including (1) identification and characterisation of the nanof orm(s), (2) when possible formation of initial groups of nanoforms based on physico-chemical parameters (e.g. aspect ratio, or water solubility and dissolution rate), (3) identification of available information and data gaps for each nanof orm per endpoint, (4) hypothesis driven identification of source materials to read across from, (5) where necessary additional testing to substantiate the read-across, and (6) assessing the new data and remaining uncertainties to conclude on the read-across arguments. Where read-across cannot be substantiated, the strategy foresees reiterating (some of) the steps, or performing appropriate testing to fulfil the information requirement(s) in REACH (or other regulatory framework). The presented strategy points towards the availability of data on physico-chemical parameters of each nanof orm as the crucial starting point to obtain a better understanding on its (environmental) behaviour, fate, toxicokinetics and toxicity. This is the cornerstone in developing a scientific, robust justification for grouping and read-across. To improve understanding, further (international) coordination and collaboration in research is advisable. Furthermore, data quality is critical, and monitoring of physico-chemical parameters during testing is therefore a key element. This also requires harmonisation and standardisation of test methods for physico-chemical, toxicokinetics and hazard endpoints.

271 Grouping of nanomaterials regarding their fate and behaviour in the environment - first hypotheses and future work
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A challenge of nanomaterials in products is their optimal use and at the same time the safe handling for humans and the environment along the whole life cycle. Several studies showed the release of NM and their transport to all environmental compartments. The application of the nanomaterial based product, the NM properties and the exposure pathway will affect the behaviour and fate of the NM in the environment and as a result the exposure of the environment, as exposed consumer. The toxicological behaviour in the environment and their concentration are important information for a thorough risk assessment. The potential risk has currently to be assessed case by case since no tested grouping or read across is available for the large varieties of NM. This case by case approach is time and cost consuming. Therefore, a grouping of NM and read across between different nanoforms is insufficently and one may target for a risk assessment. The grouping of nanomaterials and the exposure approaches for release, exposure, hazard potential and risk analysis in view of all relevant protective targets, workers, consumers and environment are explored. The aim is to develop usable and reliable approaches within the framework of pre-regulatory tests. One of the first steps is the set-up of different criterion catalogues based on literature.
data. These catalogues will comprise NM relevant parameters such as physical chemical properties, the respective potentials of exposure, including release and environmental fate, hazard (eco- and human toxicity) and risk. Based on these catalogues grouping hypotheses for NM will be derived and evaluated in a second step. The presentation will focus on the grouping hypotheses for NM in view of environmental fate and behaviour. The first concept based on literature data, about NM transformation and mobility in air, water, sediments and soils. The concept and the planned experiments for verification or falsification of the hypothesis will be presented and discussed with the scientific community. We see the input by the scientific community at this early stage as very valuable to complete this approach and increase the likelihood of the group concept in the end. The results are generated in framework of the project nanoGRAVUR which is founded from the German BMBF, Grant No.: 03XP0002

272 The use of NM libraries for read across estimation - Organism and gene effects in E. crypticus exposed to TiO2-Fe doped NM library

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Nearly all ecotoxicological studies with nanomaterials (NMs) are focused on testing one or a few NMs. More systematic approaches to compare effects across many NMs with different but related characters/descriptors (e.g. different aspect-ratios or surface activities) are rare in the ecotoxicology. Therefore, there is an urgent need to provide tools that allow to develop an (eco)toxicological dataset containing comprehensive coverage of NP toxicity, with the aim of extrapolating acquired information across NM. NM libraries are an excellent tool for alternative testing and modelling. In the present study a custom-designed Fe-doped TiO2 NM library was used, covering a wide spectrum of properties. Additionally, the reference TiO2 materials from JRC were tested (NM 103-5). *Enchytraeus crypticus* (Oligochaeta) was used as test species since it is both environmentally relevant (as an important soil representative oligochaeta and a standard species) and also a genomic model, with a high-throughput (HTP) transcriptomic library. Effects were assessed at the organism (survival, reproduction) and gene level (differential gene expression, microarray). Gene expressions were linked to population effects by using population Effect Concentrations (e.g. ca. EC50) as exposure levels. Material-specific effects were observed. The microarray study across the 11 materials showed that the profiles of DEGs varied among the materials. Analysis of gene ontology terms showed sub-clustering of the different materials, whereas the study of affected pathways provided the basis for mechanism interpretation.

273 Predicting Nanoparticle uptake into cells by easily measurable Nanoparticle properties

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The increasing number of NP products poses increasing human health risks by particle exposure. Therefore concerns about toxicological and environmental effects have been raised because in several in vitro studies adverse effects have been shown in various cell lines. Unfortunately, effects are often measured after a single fixed time point based on exposure dose, neglecting uptake kinetics and time-dependent internal cellular concentration. Two fundamental biological processes can be employed by NPs to enter cells: Endocytosis by all eukaryotic, non-phagocytic cells, and phagocytosis in specialised cells. When looking at NPs, this can be considered a so called Trojan horse effect, because cells take up the particles mistaking them for nutrients. This can result in high levels of NPs inside the cell. Various researcher show the effect of different NP properties, e.g. size and charge, on uptake into the cells. Patterns are albeit uncertain due to the lack of systematic studies and lack of determination of uptake in toxicity studies, but patterns are strongly emerging. To overcome the problem that not every of the numerous NPs can be tested in the laboratory (for uptake and/or toxicity), we aim to combine empirical studies with modelling for a cost- and time-effective risk assessment. For this reason, a key parameter for the uptake rate constant in dependence of NP size and charge from published experimental results. We distinguish between phagocytic and non-phagocytic cells. For non-phagocytic cells there seems to be a size optimum for uptake of 50 nm NPs based on NP numbers, smaller and larger NPs are taken up to a lesser extent. This optimum size effect is often observed as well as the core material of the NPs. An increase in positive or negative surface charge leads to increased NP uptake in comparison to less or uncharged NPs. Positively charged particles are taken up more extensively compared to negatively charged NPs when the absolute zeta potential is similar. Understanding NP properties that determine their transport across cell membranes will improve our understanding of their toxicity and potential health effects. A previous investigation showed a great variation which was attributed to a decreasing trend observed in the uptake rate constant (k).

274 Application of a multiplexed quantitation of protein biomarkers in the invertebrate species Gammarus fossarum: interest to environmental monitoring

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Sub-organismal information provided by the measurement of molecular biomarkers is a relevant approach for environmental biomonitoring (e.g. OSPAR marine strategy). Changes in molecular biomarkers can be related to an exposure to chemical compounds and some of them provide early warning indicators of possible effects on the ecosystem. However, the routine use of these tools in biomonitoring is confronted with several limitations, specifically in invertebrates. The most relevant limitations consist of the lack of specific quantification methods for each species, and the fact that each biomarker measurement involves a specific method, leading to very expensive biomonitoring strategies in time, cost and biological samples. Recently, an approach called “proteogenomics” emerged as a relevant strategy for the discovery of proteins in non-model organisms. With this approach, our consortium created a database consisting of 1873 experimentally validated proteins of the amphipod crusteacean *Gammarus fossarum*, a sentinel species for continental water biomonitoring. Using this protein database, the objective of the present study was to setup an innovative approach to permit fast and specific identification and simultaneous quantification of proteins of interest in this invertebrate species. We applied a mass spectrometry based multiplexed quantitation methodology (Selected Reaction Monitoring – SRM) to study 55 proteins of interest. Identification of specific proteotypic peptides, physiological monitoring of associated proteins and their interest as biomarkers in *G. fossarum* were assessed by studying their change through male and female reproductive cycles, during food privation or after exposure to contamination (model compounds in the laboratory and in situ caging). This novel approach for multi-marker quantification was successfully applied for ecotoxicological analysis in an invertebrate species known for its relevance in environmental monitoring. The concentrations of several biomarkers of interest were simultaneously monitored during the physiological processes and their sensitivity to toxic contamination was demonstrated, showing the relevance of this innovative analytical approach for invertebrate ecotoxicology. In addition, we applied the measurement of molecular biomarkers for environmental biomonitoring. This breakthrough methodology in ecotoxicology constitutes a valid alternative to the time-consuming, biomarker-specific strategies currently used.

275 Bioconcentration and biotransformation of selected pharmaceuticals in the freshwater amphipod, Gammarus pulex

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The aquatic environment is continually exposed to contaminants via wastewater treatment plant effluents. These compounds may have adverse effects on reproduction, development and behaviour in biota. A previous investigation has shown occurrence of several pharmaceuticals in the freshwater amphipod *G. pulex*. However, the potential for these xenobiotics to accumulate is not well understood. Herein, the uptake and elimination kinetics of selected pharmaceuticals in *G. pulex* is presented using both liquid scintillation counting (LSC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Gammarids were exposed to 16 pharmaceuticals covering classes of antibiotics, anticonvulsants, non-steroidal anti-inflammatories (NSAIDs), histamine H2 receptor antagonists, beta-adrenergic agonists and beta-blockers individually for 96 h with a 48 h uptake and depuration phase, respectively. Analysis was performed using either LSC or LC-MS/MS in positive and negative electrospray ionisation modes. Uptake and elimination kinetics were determined and bioconcentration factors (BCFs) subsequently estimated. Both methods of analysis were in good agreement and overall the bioconcentration of these pharmaceuticals remained low with BCFs ranging from 12 - 212. Different methods of modelling used to estimate the kinetic parameters showed large variation which was attributed to a decreasing trend observed in the uptake rate constant (k). Furthermore, the LC-MS/MS method allowed the confirmatory identification and quantification of several biotransformation products resulting from exposures to propranolol, carbamazepine and diazepam. The biotransformation indicates that these organisms are capable of Phase 1 and 2 metabolism, supporting potential further use as a replacement of fish models. Finally, the results suggested that the...

276 Seasonal sensitivity of Gammarus pulex towards cypermethrin K. Dahlhoff, University of Copenhagen / Department of Plant and Environmental Sciences; J.J. Rasmussen, Aarhus University / bioscience; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences

One of the most commonly used classes of pesticides in Danish agriculture is the synthetic pyrethroids which constitute approximately 90 percent of the total insecticide usage. The pyrethroids can enter surface waters by spray drift, colloid facilitated transport or leaching through tile drains and even short pulses of pyrethroids have been shown to be highly toxic to different aquatic invertebrates with EC50-values in the ng L-1 range. The purpose of the current study was to investigate the seasonal influence of short term exposure to the eutrophicGammarus pulex towards a short (90 minutes) pulse of the pyrethroid insecticide cypermethrin. We hypothesise that the gammarids are most sensitive during spring and the least sensitive during fall due to the general increase in fitness during summer and fall such as formation of fatty acids and proteins. To test the hypothesis, gammarids were collected in a local stream, acclimated for three days and subsequently exposed to a 90 minute pulse of cypermethrin before being transferred to clean medium for recovery, where immobilization and death were followed for seven days. To get an indication of the fitness levels of the exposed gammarids the composition of fatty acids, protein content and in vitro cytochrome P450 activity were measured in non-exposed gammarids collected at the same dates as the exposed gammarids. The results of the preliminary test period in February through October seem to support our hypothesis as the estimated EC50-values were lowest during spring and early summer with the exception of March and May. The seven days EC50-estimates varied from 0.29 ± 0.06 to 1.94 ± 0.60 µg L-1 with the lowest estimate observed in April and the highest in October, respectively. This several fold difference in pyrethroid sensitivity may be explained by different fitness levels of the collected gammarids. Fitness levels measured as their lipid and protein content, are expected to increase during fall due to the increase in food sources caused by the leaf-fall. An increased protein level may very well result in a higher level of cytochrome P450 activity, which potentially can explain the decrease in sensitivity during fall, whereas an increase in lipid content might increase the proportion of cypermethrin being contained in an inactive form in the lipid fraction.

277 Silver absorption kinetics in hemolymph of the marine amphipod (Parhyale hawaiensis) exposed via water M. Vannuci-Silva, S. Cadore, UNICAMP; G. Ubbuzeiro, SCHOOL OF TECHNOLOGY -UNICAMP / LEAL

Nowadays, silver’s release into the environment is becoming an environmental concern, due, specially, to its large incorporation in nanomaterials. Absorption kinetics studies can be helpful to elucidate the toxicity mechanism of these contaminants. Therefore, the determination of silver concentration in the hemolymph of exposed organisms can be an interesting exposure measurement but, because of the relatively small size of these organisms, this task is quite challenging. The aim of this study was to evaluate the silver absorption kinetics in organisms exposed to silver nitrate via water. As a next step, organisms exposed to silver nanoparticles will be studied. Experiments were performed using adult organisms of Parhyale hawaiensis (8 months) individually exposed. The Ag concentrations of exposure were 0; 5; 10; 25; 50 and 100 µg L-1 prepared with AgNO3 and reconstituted saline water (salinity 30). The exposure times were: 14, 24, 48, 72 and 96 hours. After exposure, hemolymph (approximately 0.5 µL per organism) was collected and weighted. Three pooled samples of 4 organisms were tested per exposure concentration and per time. The pooled hemolymph was diluted in 1 mL of HNO3; 0.05%. Copper concentration in the hemolymph was also determined and used as a possible internal standard because this metal can be found in hemocyanin. An Agilent 7700x Inductively coupled Plasma Mass Spectroscopy was used for Ag and Cu determinations. The limits of detection for Ag and Cu were 0.043 and 0.027 µg L-1, respectively. Silver concentrations in the hemolymph increased with the increase of Ag in water and with higher exposure times, reaching 14 ng mg-1. Silver seems to be regulated by either Ag uptake inhibition or active exclusion in these organisms, especially when the ion concentration is higher than 50 µg L-1 in the water, regardless the time of exposure. Copper concentrations remained from 60 to 120 ng mg-1, regardless of variation in silver concentration in the water. The observed variation is probably due to the physiological state of the organisms as molting stages, but additional studies are required to verify the adequacy of this measurement as an indicator of the condition of molluscs. The manipulation of molluscs organisms can be an interesting tool and it will be applied in silver nanoparticles toxicity studies.

278 Effects of low dose radiation on reproduction and behaviour of the amphipod, Echinogammarus marinus

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Impacts of environmentally relevant doses of ionising radiation on aquatic biota are poorly understood. Significant data gaps for a range of organisms coupled with highprofile nuclear incidents such as Chernobyl and Fukushima have driven a re-examination of the impacts of radionuclides. Echinogammarus marinus is a widespread intertidal amphipod which has gained prominence as a model species in both ecological and ecotoxicological studies. The aims of the present study were: 1) to develop a method for ecotoxicity testing of contaminants on the reproduction of model amphipods; 2) to investigate the impact of chronic, low-dose ionising radiation exposure on the quantity and quality of spermatozoa in E.markus; 3) To elucidate the impact of radionuclides on crustacean behaviour patterns. E. marinus were exposed to beta radiation (phosphorous 32; P32) at a range of activity concentrations from 0 to 69 Bq/mL. Mean dose rates over the 14 day exposure period of 0.2, 1 and 9 mGy/d were calculated. Subsequently, behavioural assays were performed using video tracking software (Ethovision XT) over an 8 minute alternating light/dark cycle. Sperm quantity and quality were ascertained using dissection and fluorescent (LIVE/DEAD) staining techniques. Following light stimulation, a significant reduction in swimming velocity was observed during dark phases of the assay at all dose rates. Exposure to P32 resulted in a decrease in sperm viability across all dose rates compared to the control although this was only significant in the 1 mGy/d exposure (~11% reduction). No significant differences in total spermatozoa numbers were recorded in any of the treatments. This study has developed sensitive biomarkers highlighting possible impacts of sperm quality but not quantity. A number of studies have suggested that a reduction in sperm quality may be detrimental to crustacean species at higher levels of organisation therefore ongoing research will aim to determine the presence of DNA damage and the knock-on effects on fertilisation success. These preliminary findings indicate that ionising radiation has the potential to alter male fertility and behavioural patterns at dose rates below those encountered in radioactively contaminated environments. Such studies will improve our understanding of the impacts of large scale nuclear incidents such as Chernobyl and Fukushima.

Discussion

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Recent Developments and Current Issues in Bioaccumulation Assessment

280 First insight into bioconcentration of Ionic Liquids - an in vitro approach J. Meszkowska, University of Bremen / Center for Environmental Research and Sustainable Technology; P. Stepnowski, University of Gdansk / Department of Environmental Analysis; J. Thoeming, M. Markiewicz, S. Stolte, University of Bremen / Center for Environmental Research and Sustainable Technology

Thousands of different Ionic Liquids (ILs) have been synthesized and few few hundreds are commercially available. ILs are used nowadays from small-scale through pilot plant to large-scale industrial applications. This comes with the increased probability that they will be continuously released into the biosphere, for example, as process effluents or consumer products, or in larger amounts as accidental spills. Hence they should be considered as potential pollutants (PPs). Bioaccumulation is of the highest concern for environmental risk assessment of chemicals, since it is known to cause far-reaching hazards to wildlife and human health. Generally, the experimental measurement of bioaccumulation is time-consuming, expensive, and due to ethical concerns regarding animal welfare not feasible for large sets of chemicals. Thus prediction models - mainly based on easily determinable physicochemical properties such as the octanol-water partition coefficient - are usually used. However the existing prediction models often give inaccurate predictions and inaccuracy in terms of quantifying compounds and permanently charged organic chemicals. This is due to the fact that classical bioaccumulation models neither sufficiently consider ion–macromolecule interactions nor interactions of cations and anions in solution - both strongly influencing the transport, uptake and bioavailability of ions. Therefore the main aim of this study was to understand the interactions of organic ions, and ion pairs in particular, with in vitro biological systems and their consequences in terms of bioconcentration. On the basis of obtained results strong evidence for the influence of alkyl chain length and thus hydrophobicity was presented. Moreover the influence of hydrophobic counterion on lipid membrane partitioning was determined. The study also showed that lipid membrane partitioning of ILs is concentration – dependent. Hydrophobic ILs had rather high affinity to lipid membranes suggesting significant bioconcentration potential. Since hydrophobic counterion clearly exhibited membrane partitioning of IL cation, influence of co-contaminants on bioconcentration potential should be always considered.

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281 Improving fish bioaccumulation assessment for ionogenic compounds by in vitro measurements of the liver 59 clearance rates

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Under the chemical legislation REACH, adequate estimates of the bioaccumulation potential of thousands of chemicals, of which 50 % is ionogenic, are required. Only recently, a bioconcentration model specifically for ionogenic organic chemicals (BIONIC) in fish has been developed. Bioaccumulation data on ionogenic organic compounds (IOCs) are scarce, while the OSARs used to parameterize the critical uptake rate constant, tissue distribution and biotransformation rates are poorly validated for IOCs. The available in vivo fish biotransformation rates for IOCs do not cover many fully ionized structures, and also do not equally cover all different types of acids (phenolates, carboxylates, sulfonates, sulfates) of bases (primary, secondary, and tertiary amines, and quaternary ammonium salts). We used a straightforward in vitro biotransformation assay with a batch of 9 S9 Rainbow trout liver homogenate (Rt-S9) in order to derive consistent measurements of intrinsic hepatic clearance rates (CLint mennes) on 50 IOCs, covering nearly all monoprotic IOC types. Since the Rt-S9 is well characterized and standardized, this assay appears to be a valid tool for phase I and phase II enzymatic conversions, this data set covers the baseline ability of uninduced fish to biotransform xenobiotic ionomic compounds in an environmental exposure, which would be a valuable conservative approach. The next challenge is to extrapolate these values to whole organism transformation rates, after which they can be applied to the BIONIC model. The 50 IOCs include many simple chemical structures that allow for a differentiation of selectivity on CLint mennes, of (A) the ionizable group, (B) the hydrophobicity (non-polar moieties) of IOC structures, (C) the position of the charged group in the IOC (interior or exterior of the structure), (D) branching (related to C), and (E) to some extent specific polar functional groups. This data set on IOCs pose an important shortcut to current unmeasured in vivo biotransformation processes, followed from in vivo measurements on Rt-S9, and could present a framework to establish a more extensive Rt-S9 QSR that specifically addresses all kinds of ionizable contaminants. The in vitro Rt-S9 data can further be used to evaluate and support the BIONIC bioaccumulation model kint SARs that are based on in vivo data.

282 Identifying obstacles to the application of the one concentration approach for fish bioconcentration testing

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Fish bioaccumulation studies assist in determining the potential for substances to bioaccumulate. The resulting bioconcentration factor (BCF) value is used as part of Persistence, Bioaccumulation and Toxicity (PBT) and secondary poisoning assessments. International data requirements for general chemicals and plant protection products (PPPs) include triggers for determining BCF values, generally where biocentration might be expected. Biotransformation tests are time consuming, expensive, and use a minimum of 108 animals per study. Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations. The 2012 revision of the OECD Test Guideline for BCF testing (TG 305) provides the option to use only one exposure concentration, when justification is provided, although two exposure concentrations may still be required for some regulatory purposes. Recently this justification has been demonstrated for general chemicals and PPPs, where it was shown that one concentration can be tested without compromising the BCF determination. This provides a strong data-driven rationale for using the one test concentration approach for BCF assessment of general chemicals and PPP active substances, and where applied would reduce the number of fish used for this purpose by one third. In 2015 we conducted a survey across academia, regulatory bodies and industry to determine how often the one concentration approach is being applied in practice, and identify the barriers to its widespread uptake. Fifty-eight participants from Asia (n=3), Europe (n=21), North America (n=30), and Latin America (n=4) completed the survey. Of these, 31 respondents were scientists from organisations which conduct fish bioaccumulation testing for regulatory purposes, of which 29 responded to the question ‘How often does your company apply the one concentration approach in practice?’ (Table 1). The majority of these scientists (48%) occasionally apply the approach, with 41% never utilising the approach, and only 10% using it routinely. Twenty-two of these scientists identified barriers to its uptake. These were cited as a lack of global regulatory acceptance (64%), followed by scientific concerns (32%), and inertia (4%). In this presentation we will explore the barriers in more detail and discuss potential strategies to enable greater use of the one concentration approach in practice.

283 Study of biomagnification of poly- and perfluoralkyl substances on fresh water food webs

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Poly- and perfluoralkyl substances (PFAS) are surfactants that have been synthesized and used in a wide variety of applications since the 1950s. As a consequence, PFAS are introduced via multiple pathways in the environment and some of these compounds have been detected in all compartments including aquatic systems. Even if PFAS concentrations are rarely superior to µg/L in surface waters, elevated concentrations have been reported in aquatic organisms (up to mg/kg) demonstrating their bioaccumulative properties. Recent works highlight the importance of the use of metrics such as the Toxic Magnification Factor (describing the behavior of a compound along a food web) for bioaccumulative assessment of persistent organic pollutants. In this study, the biomagnification potential of (TMF) of 23 PFAS was investigated in freshwater riverine food webs, for the first time. Thus, various biota species including fish (barrels Barbus barbus and chubs Squalius cephalus) and invertebrates representing different trophic levels (primary consumers, omnivores, herbivores, carnivores or filter feeders) were collected across 4 sites in the Rhône basin and 1 site in the Loire Basin (Eastern France). Analysis of stable isotopic ratios (δ13C and δ15N) allowed for the determination of trophic levels (TL). Several regression approaches were compared for TMF calculation, including a classical regression, and regressions taking into account sample heterogeneity and/or left-censored values. At each site, particular care was devoted to the choice of the organism at the baseline of the food web. Fish were consistently at the top of the food chain (TL = 3–4.5) and were significantly more contaminated than invertebrates (2PFAS = 6–3231 Vs 0.8–205 ng.g−1 ww). The most frequently detected PFAS were of types methanesulfonates, sulfates, sulfonates, and perchloroethanes (PC2). Shorter chain PFAS were never detected. TMFs ranged between 1.5 and 6 for long-chain PFAS, strongly suggesting their biomagnification potential in the riverine food webs investigated. TMFs could vary substantially among sites, and according to the regression model used.

284 Measuring Trophic Magnification Factors: Role of Spatial Concentration Gradients, Disequilibrium and Field Sampling Design

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Trophic magnification factors (TMFs) are field based measurements of the bioaccumulation behavior of chemicals in food-webs. TMFs can provide valuable insights into the bioaccumulation behavior of chemicals. However, like other bioaccumulation metrics, TMFs are subject to considerable uncertainty and the influence of numerous confounding variables. This study seeks to investigate the role of spatially-variable concentrations in water and sediments on the determination of the TMF. For this purpose, a multi-box food-web bioaccumulation model was developed to account for horizontal and vertical spatial concentration gradients and species movement on chemical concentrations in aquatic biota of food-webs and TMFs. Model testing through a comparison of measured and calculated and observed biotransformable phthalate esters in a marine aquatic food-web (subject to spatial variations in chemical concentrations in water and sediments) showed good agreement between model calculated and observed TMFs. Model testing showed no systematic bias and good precision in the estimation of the TMF for PCB congeners but an apparent underestimation of the TMFs for phthalate esters. A model sensitivity analysis showed that species sampling designs that ignore the presence of concentration gradients can misidentify the TMF. The determination of the TMF is most sensitive to concentration gradients and species migration patterns for substances that are subject to a low degree of biomagnification or trophic dilution (i.e., TMFs around 1). TMFs were found to follow a strong relationship with log KOW and to be sensitive to biotransformation rates. Model calculations indicate that systems with relatively homogeneous exposure are best suited to determine TMFs that are representative of chemical bioaccumulative properties. The model is useful in anticipating the effect of spatial concentration gradients on the determination of TMF; guiding species collection strategies in TMF studies; and interpreting the results of field bioaccumulation studies in study locations that are subject to spatial differences in chemical concentration.

285 Interactions between the bioaccumulation assessment using a weight of evidence approach and Toxicfox as an example

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The assessment of the bioaccumulation potential of a substance according to current regulation requirements such as REACH is based on the logKow (octanol-water) for the first instance, followed by a fish bioconcentration factor (BCF) study following OECD 305 test guidelines for the final assessment. The
standardized fish BCF study results are usually considered definitive for the “B” assessment and the potential for accumulation along the food chain (secondary poisoning). Normalized to a 5% lipid content and corrected for growth, this value is considered as representative for aquatic species (i.e. fish). However, in some cases where BCF values from several tests are available, these values can be highly variable indicating an uncertainty among those data. For example, in case of Triclosan, an antimicrobial active substance, at least three guideline fish BCF studies are available revealing BCF values from about 100 to about 8700 L/kg. Other “B” metrics such as biomagnification factors (BMF’s) and trophic magnification factors (TMF’s) and toxicokinetic information such as the total elimination half-life (t1/2) can also be considered as lines of evidence in a weight of evidence approach for “B” assessment to address uncertainty in measured BCFS and to more fully characterize the bioaccumulative properties of a chemical. Taking all of these lines of evidence into account, it can be concluded that Triclosan does not exceed the commonly used BCF biomagnification criteria (thresholds). Furthermore, models parameterized with the available data calculate biomagnification factors that are less than 1; Triclosan does not biomagnify in food chains.

Environmental risk assessment of chemical mixtures: the steps ahead (II)

286 Mixture toxicity assessment using tissue concentrations - experiences from analysing real-world monitoring data
J. Baars, Waeterschoot, Eurometaux / Department of Biological and Environmental Sciences; D. Yngells, University of Gothenburg / Department of Biological and Environmental Science; M. Gustavsson, University of Gothenburg Norway has been monitoring populations of cod and trout since 1998 for the presence of organic contaminants including classic POPs from the "dirty dozen" such as PCBs, polychlorinated dibeno-p-dioxins and -dibenzo-ringfurans, but also more "modern" pollutants such as flame retardants, alkyphenols, triclosan and perfluorinated compounds. Aim of the presented study was to assess whether the joint body burden of Norwegian cod and trout populations indicate an impairment of fish population health. Two sets of monitoring data were made available by the Norwegian Environment Agency for this purpose: (i) data on the pollution burden of trout populations monitored between 2010 and 2014, and (ii) data collected between 1998-2013 for cod populations. In total the body burden of 151 organic compounds was monitored. For each compound estimates were collected on bioaccumulation (BAF) and chronic toxicity to fish (QSAR estimates, experimental data, PNEC and EQS values). These were then combined with the biomonitoring data in order to estimate the overall risk of the exposed fish populations using Concentration Addition. For this purpose all aquatic concentrations were first re-calculated to internal body burdens by multiplying each aequous concentration with the corresponding bioaccumulation factor. Results indicate that final risk quotients are well below one for the majority of cases, although the analysis pipeline is fraught with substantial uncertainties. In particular (i) there is a paucity of reliable toxicity data for a majority of the compounds, (ii) the application domain of existing QSAR and bioaccumulation models is too restricted to encompass highly lipophilic compounds such as e.g. several of the brominated flame retardants that were commonly found pollutants in

287 Hierarchical Bayesian approach to reduce uncertainty in the aquatic effect assessment of realistic chemical mixtures
R. Oldenkamp, Radboud University Nijmegen / Department of Environmental Science; H.W. Hendriks, Radboud University / Department of Applied Stochastics; D. van de Meent, RIVM / DMG; A.M. Ragas, Radboud University / Department of Environmental Science
Species in the aquatic environment differ in their toxicological sensitivity to the various chemicals they encounter. In aquatic risk assessment, this interspecies variability is often quantified via species sensitivity distributions. Because the information available for the characterization of these distributions is typically limited, optimal use of information is essential to reduce uncertainty involved in the assessment. Here, we show that the credibility intervals on the estimated potentially affected fraction of species after exposure to a mixture of chemicals at environmentally relevant surface water concentrations can be extremely wide if a classical approach is followed, in which each chemical in the mixture is considered in isolation. As an alternative, we propose a hierarchical Bayesian approach, in which knowledge on the toxicity of chemicals other than those assessed is incorporated. A case study with a mixture of 13 pharmaceuticals demonstrates that this hierarchical approach results in more realistic estimations of the potentially affected fraction, as a result of reduced uncertainty in species sensitivity distributions for data-poor chemicals.

288 Evaluation of pesticides monitoring programmes using a process based mixture model
J. Baas, Centre for Ecology & Hydrology; M.G. Vijver, CML Leiden University; J. Rambouhl, M. Dunbar, Environment Agency England and Wales; M. van’t Zelfde, CML Leiden University; D. Spurgeon, Centre for Ecology & Hydrology; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology
A number of European countries run large scale pesticide monitoring schemes aimed at identifying and evaluating the presence of pesticides in the environment. We compared data from a regional monitoring programme in the UK with the country wide monitoring programme in the Netherlands. In total over 15,000 samples containing over 1 million individual measurements were evaluated for effects, using a process-based mixture model that relates observed concentrations to chronic survival of Daphnia magna. Survival thresholds for the monitoring data were analysed for effects, including and excluding below detection limit results. It showed that there are only a small number of places where we can expect to have effects on daphnids, based on measured concentrations. However, the most polluted samples would cause extinction of a daphnid population within only 30 hr. The detection limits are not included in the analysis (like the Water Framework Directive prescribes), in up to 35% of the Dutch samples the effect of the simultaneous exposure to all pesticides is such that a direct threat to the survival of daphnids exists. Our analysis also showed that detection limits in the monitoring programmes are basically too high to exclude additive mixture effects and so to make a statement on whether or not the environment is actually protected. To improve on this predictive mixture modelling can be used in combination with a more focussed monitoring programme.

289 Thresholds for synergy: How to define at which concentration a synergist starts acting as a synergist
N. Cedezruen, University of Copenhagen / Plant and Environmental Sciences; E. Becerra, D.S. Paludan, K. Dalhoff, M.A. Bjergager, University of Copenhagen / Department of Plant and Environmental Sciences
Synergy has long been a topic of concern in cumulative risk assessment. If toxicants act as synergists, they might enhance the effect of other chemicals beyond what is predicted with mixture models, which assume no interactions between chemicals. Tests for synergistic interactions are, however, most often performed with concentrations of the synergists being much higher than the concentrations occurring under natural conditions. It is likely that there is a lower threshold concentration below which the action of the synergist is negligible. The aim of this study was therefore to explore different ways of determining lower thresholds for synergistic interactions, using the known synergistic fungicides prochlor granopropiconazole together with the pyrethroid insecticide alpha-cypermethrin. Different setups and statistical methodologies were tested and lower thresholds for synergy on survival were determined for the two azole fungicides in combination with alpha-cypermethrin for the aquatic macroinvertebrates Daphnia magna, Chironomus riparius, Gammarus Pulex and Hyallella azteca. Synergy was defined as happening in mixtures where either EC50 values decreased more than two-fold below what was predicted by concentration addition (horizontal assessment) or as mixtures where the frequency of immobile animals increased more than two-fold above what was predicted by independent action (vertical assessment). Finally, survival over time was evaluated using a GUTS TKTD model letting the elimination rate constant, k, depend on the azole concentration. Horizontally assessed thresholds were 1.2-4.7-fold lower than vertically assessed thresholds for D. magna. By evaluating the effect of time, the vertical threshold was found to decrease with increasing test duration, with 0.026±0.013 μM and 0.425±0.089 μM for prochlor granopropiconazole in the 48h standard tests, to 0.015±0.004 μM and 0.145±0.025 μM, in the 14-days tests on D. magna. The threshold concentrations for the three other species were within an order of magnitude of the D. magna thresholds. The GUTS modelling showed a strong decrease of k, with increasing azole concentration, indicating that effects on k might also be used for setting thresholds for synergy.

290 Combined toxicity considerations in a constituents-based environmental risk assessment of multi-metallic UVCBs
K. Oorts, F. Iaccino, ARCHIE; V. VEROU/GSTRAETE, Eurometaux / EHS; H. Waetenschoot, Eurometaux
There is a clear need to include a generic approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs, for which the assessment was made the last 40 years ago. The list driven approach has driven the high toxicity of UVCBs as environmental constituents, resulting in parallel risk assessments of the constituents. Upon ECHA’s request REACH registrants of inorganic UVCBs intermediates committed to improve the combined toxicity assessment in their registration dossiers. However, an appropriate standard approach to address combined toxicity of metals in a regulatory framework is still missing: most standard approaches yield indeed over-conservative results, such as risk scenarios at background concentrations when several metals are combined. Numerous research efforts have been performed during the last years in the field of combined toxicity of metals in the environment. This research is usually focussing on high effect concentrations, specific combinations of up to maximally 4 metals and on few standard test organisms, and restrictions are studied for limited metals, such as Co, Zn, Cr and Ni, with well characterised ecotoxicological effects to organisms in the water, sediment.
and soil compartments. However, multi-metallic substances like the inorganic UVCBs can contain more than 10 constituents, including ‘data poor’ metals such as As, Pd and Te. No clear conclusions have yet been formulated on a generic approach, i.e. extrapolated to any combination of metals and to all organisms within an environmental compartment. A one day workshop was therefore organised in October 2015 with participants from academia, regulatory bodies and industry. The aim of this workshop was to bridge the gap between the scientific developments and current regulatory requirements on combined toxicity of metals. This workshop resulted in a proposal for a generic tiered approach, starting from the standard concentration addition evaluation based on summation of the PEC/PNEC ratios of the individual constituents. Several options for refinements of this standard approach, e.g. taking into account bioavailability, using msPAF calculations or assessment per trophic levels, are proposed in higher tiers, as well as screening approaches for evaluating the relative importance of the data poor constituents. This tiered approach is being tested for some case studies. In addition, several open research questions have identified for future improvement of the standard concentration toxicity assessment of metals.

Expanding LCA: looking at organizations and at new policies

291
The EU Organisation Environmental Footprint Sector Rules (OEFSR) for the retailer sector
S. Humbert, Quantis
The European Commission started a pilot to create a “Single Market for Green Products”, that aims at facilitating better information on the environmental performance of products and organisations. 26 pilots made of companies, industrial and stakeholder organisations are drafting respectively 24 Product Environmental Footprint Category Rules (PEFCR) and 2 Organisation Environmental Footprint Sector Rules (OEFSR). One of the pilot is drafting the OEFSR for the retail sector and is composed by retailers (Carrefour, Colruy, Decathlon, Picard, Kering, Office Depot), public agencies (EAA from Austria, ADEME from France and ENEA from Italy), one NGO (Global 2000), one association (PERIFEM) and one LCA consultant (Quantis). As of November 2015 an assessment of the impacts of an average retailer has been performed using the 15 impact categories required. A first OEFSR has been drafted, submitted for public consultation and accepted by the Steering Committee. The results of the assessment, the draft and its main methodological points (e.g., for direct, as well as upstream and downstream indirect contributions), as well as the benefits of this OEFSR for companies will be presented highlighting latest developments and feedback, including from the supporting studies. These points also include the issue pertaining to consistency with the product approach for a sector as interdisciplinarily as the retail sector. As an example of results, an average general retailer supplying products for 3’000’000 people can have a carbon footprint in the order of magnitude of 10’000’000 t CO2/yr per year, most of it being associated with the life cycle of its products sold. Interaction between OEFSRs and PEFCRs such as cross cutting issues and consistency will also be addressed. As an example of methodological agreement that has been reached among sectors is how allocation among meat, milk, pet food and leather should be performed among cattle co-products. Such type of agreement is key for a sector like the retail to be able to consistently perform its Environmental Footprint. One of the significant differences with traditional corporate footprint is that assessment and reporting for OE goes beyond the traditional carbon footprint and includes impact categories such as biodiversity, water and land impact, or (more and more frequent) impact on human health through environmental pollution. Pressure on biodiversity or deforestation throughout the supply chain is also included.

292
Arla Foods Environmental Profit and Loss Account (E-P&L) - Organisational LCA with Monetisation
J. Schmidt, 2.0 LCA consultants; A. Flysjö, Arla Foods
Arla Foods is among the world’s largest dairy companies. To document the total life cycle environmental impact of their product portfolio, Arla Foods has conducted an Environmental Profit and Loss Account (E-P&L). The E-P&L expresses Arla Foods’ environmental impacts in monetary units, in addition to the underlying physical units. The functional unit of the study is Arla Foods’ product portfolio in 2014. Arla Foods intends to use the results to evaluate their environmental strategy and in various communications. This paper discusses the results and learnings from the E-P&L. Especially, the similarities and differences with traditional LCAs as well as the added value of monetising the impacts are discussed. An E P&L can be described as a means of placing a monetary value on the environmental impacts along the entire supply chain of a given business. The only difference between an organisational LCA (OLCA) and an E-P&L is that the E-P&L uses monetisation as weighting in the life cycle impact assessment, while commonly LCAs are not. One study included LCA’s environmental impacts were compared with Arla Foods’ sites’ (99 sites in 12 countries). Production and use of raw materials, energy carriers, packaging and transport are included, as well as treatment and utilization of by-products and wastes. In addition, products and services not directly used in production, such as computers, furniture and travelling are covered. The downstream parts of the life cycles (retail and consumers) are also included. The study presents results calculated using both consequentiell and attributional modelling in the life cycle inventory. Indirect land use changes are included. The identified significant impacts, i.e. Arla Foods revenue at 10,600 million EUR2014, which indicate the created value. When monetarising the impacts, the consequentiell and attributional approaches show a contribution at 1840-5850 and 2240-4980 million EUR respectively. The intervals represent different valuation methods. The consequential results are generally higher than the attributional. This is because they include indirect land use changes and thereby include an impact on nature, occupation (biodiversity). The E-P&L gives a broad and deep insight in the impacts from the full life cycle of Arla Foods product portfolio, and it provides a good basis for comprehensive sustainability reporting and for identifying options for improving the performance and reducing the impact.

293
Does ex ante application undermine the usefulness of LCA?
M. Villares; J. Guinee, University of Leiden / Institute of Environmental Sciences; A. Mendoza Beltran, CML Leiden University
Introduction/Background for exploring options for safe recovery of metals from WEEE include: Increasing development driven demand for metals/ Wastefulness of metal loss into the environment/ Deleterious environmental effects of unsafe disposal/ Bioleaching, a natural process mediated by microorganisms, may become a promising emerging technology contributing to safer secondary metal recovery from WEEE/ Initial experimental bioleaching results on EEF printed circuit boards show efficient metal recovery yields/\textit{Methodology} LCA methodology was applied at an early stage to the novel bioleaching process to embed it in a life cycle context, linking it to upstream and downstream flows. A short term future scaling up scenario was defined using a proxy technology and estimated data/ Environmental hotspots of this scenario were identified and its evaluation showed promise/ A current industrial pyrometallurgical technique/\textit{Results} Potential hotspots were related to energy and material inputs for the bioleaching process and solvents for copper recovery/\textit{Conclusion} An existing technology returned an inferior environmental performance, even after further optimisation. These results could not be considered robust given the precariousness of application, yet they served as valuable preliminary information/\textit{Discussion} The uncertainties also prompted further enquiry about the chosen product system boundary and the comparability of the technologies/\textit{Conclusions} The ex ante application of life cycle assessment on an emerging technology created new knowledge on its potential development/\textit{Ex Ante} LCA and an exploratory scenario brings a systematic rigour and discipline to an ambiguous situation/\textit{Conclusion} With much conjecture involved, it is a valid mock-up of a plausible future providing useful provisional insights to be built upon. Despite uncertainties, LCA displays potential environmental hotspots/ The developmental milestones for the emergent technology gain definition at an early stage. The LCA approach broadens the research scope, bringing a systems approach, long term view, environmental aspects, and alternative perspectives on the novel technology to the research domain/\textit{Conclusion} An exploratory scenario is of great service as a developmental design tool.
identified to assess the socio-economic aspect of carbon fiber recycling from resource perspective. These are: supply risk due to geological availability (CML-Resource Depletion), supply risk due to geopolitical factor (GeoPolRisk) and importance (waste flows obtained from the material flow analysis (MFA)). For the economic aspects the net present value (NPV) that allows to consider stakeholders and potential investor's view was selected. The paper highlights the possibility to bring aspects of criticality assessment as a new resource dimension that can be integrated with the traditional environmental assessment tools to address the sustainability performance of a newly created carbon fiber recycling sector. The future perspective is to apply the proposed indicators to a specific case study.


Enhancing resource efficiency is a major goal of sustainable development. Manufacturing industries are responsible for around 25 per cent of the primary resource use and a third of the global electricity use [1]. As to metalworking industries in Germany, major potentials to increase energy and resource efficiency at company have been identified in previous studies. However, the implementation of these potentials is slow. In the research project ‘TU Darmstadt learning factory—resource efficiency in production—pilot project machining processes’ [1] we investigated technological parameters and indicators as basis for guidance documents for metal working companies. The focus of our research project was 1) to define relevant parameters of machining processes (drilling and milling) on company level, which have a high impact on resource and energy consumption and 2) to create concepts of indicators for assessing resource efficiency of machining processes on life cycle level. European resource policy defines the term of resources as materials extracted from nature but also the carrying capacity of the natural environment [2]. Given this definition, Life Cycle Assessment is a suitable methodology to assess resource consumption as well as flows to the environment connected to impact categories. However, enhancing resource efficiency in the manufacturing industry requires robust and transparent indicators of resource consumption which can be easily communicated within companies. Using the LCA as assessment methodology we found out that during the examined machining processes the consumption of electricity, compressed air, cooling lubricants and the MQ oil are relevant parameters for resource efficiency. On this basis, we derived indicators which can be used for metal working processes on the company as well as on policy level. References [1] UNEP. 2011. Manufacturing. Investing in energy and resource efficiency. http://www.unep.org/greeneconomy/Portals/88/documents/ger/GER_7_Manufacturing.pdf Accessed 23.11.2015. [2] European Comission. 2010. A strategy for smart, sustainable and inclusive growth. COM (2010) 2020.

296 How to improve the valorisation process of End-Of-Life vehicles? LCA as a tool to help decision

S. Belboom, University of Liege - Chemical Engineering / Chemical Engineering PEG; G. Lewis, P. BAREEL, Comet Treatments SA; A. LEONARD, University of Liege / Dpt of Chemical Engineering PEG
This paper undertakes an environmental evaluation of hybrid vehicles recycling, using industrial data from Comet Traitment SA in Belgium. Three business lines have been modelled and analysed. The first one is relative to the business as usual with a dismantling to recover batteries and engines followed by shredding and post shredding treatments. The second one considers, in addition, the removal of electronic control units (ECU) before shredding followed by same steps than in the first line and the last one is relative to the additional removal of big plastic parts before shredding and business as usual post shredding treatments. Environmental impacts are assessed using both attributional and consequential LCA methodology both ILCD 2011 midpoint and ReCiPe 2008 methods. This study tends to answer this question: “Are the recovery of plastics and ECU prior shredding environmentally relevant in the end-of-life vehicle recycling process?”. Using these environmental impacts results, important steps of recycling in both alternative routes will be highlighted. This study will also allow a comparison between these routes using several environmental criteria. This information could be coupled with the results of this paper and will give an idea relative to this implementation of processes in Belgium and then information for policy decisions. Removal of ECU and plastics before shredding show an environmental gain in all categories compared to the business as usual scenario but differences are non-significant, lower than the uncertainty of the results, excepted for ECU where it depends on the yield valorisation. Concerning plastics, additional environmental gain is always negligible. This LCA shows the limit of dismantling in terms of environmental benefits and the highlights given by this tool for policy making.

Ecotoxicological assessment and water quality monitoring in support of marine and freshwater legislation in Europe

297 Marine contaminants and biological effects assessments for policy purposes; OSPAR’s work at the regional scale

J. Foden, OSPAR; T. Burgeot, IFREMER / Department of Biogeochemistry and Ecotoxicology
This talk will provide a policy perspective, presenting OSPAR’s work in assessing contaminants and biological effects in the marine environment, at the regional-scale. It will highlight how OSPAR’s Contracting Parties of the North-east Atlantic maritime area cooperate to share the burden of monitoring and assessment. Data are freely available from an ICES data portal, and are used by OSPAR, HELCOM, AMAP and EEA groups. In the management of chemical and biological data for regional marine assessments, OSPAR’s expert groups and publishes annual assessments of chemical, http://www.ospar.org/work-areas/cross-cutting-issues/cerp). The talk will describe OSPAR’s Intermediate Assessment 2017 (IA2017) and its constituents of ‘common indicator’ and ‘thematic’ assessments. The dual purpose of the IA2017 as a ‘roof report’ to assist OSPAR Contracting Parties that are EU Member States in their reporting commitments for the EU’s Marine Strategy Framework Directive will be explained.

298 SIMONI: Smart integrated monitoring of the water quality

R. van der Oost, Waternet / Onderzoek en Advies; G. Sileno, Waternet / Research and Development; L. Moria, Waternet / Water Systems; M. Thao Nguyen, Waterproof; M. Suarez Munoz, University of Amsterdam EBED Institute / Institute for Biodiversity and Ecosystem Dynamics
SIMONI: Smart integrated monitoring of the water quality is an alternative effect-based chemical monitoring strategy has been developed over the last five years. The objective of this SIMONI strategy (Smart Integrative Monitoring) is to get more information on the water cycle quality for less money than traditional methods (e.g. Water Framework Directive). This project, that aims to bridge the gap between laboratory scientific research and real field monitoring promoting massive sampling with standardized in situ, in vivo and in vitro bioassays. The first version of the SIMONI model for classifying microchemical risks of surface waters will be demonstrated. A simple ‘Toxicity traffic light’ is developed for policymakers and regulators, indicating low, potential and high risks of microchemical pollution for the ecosystem. Phase 1 of the model is a hazard identification that makes the distinction between low and potential ecological risks. Hazards are mainly determined with a suite of reliable, fast, user-friendly and inexpensive bioassays. The selection of endpoints for toxicity profiling was directed towards the indication of potential risks from a broad spectrum of chemical micropollutants to aquatic organisms (e.g. nonspecific toxicity, endocrine disruption, antibiotics activity, genotoxicity, oxidative stress, dioxin-like toxicity). In order to indicate ecological risks, effect-based trigger values (ETV) were developed for all relevant bioassay responses. A three-step approach was used for ETV development, aquatic toxicity data search, species sensitivity distribution of toxic equivalents (TEQ), and benchmark field studies. Measured bioassay data were incorporated into a simple model that compares the responses with their ETVs and adds a weight factor to different types of bioassays, thus generating a quantitative hazard classification of the entire mixture of micropollutants. Results of field monitoring studies will be presented that demonstrate the feasibility of this strategy for identifying hotspots of chemical pollution. It appeared that highest ecological risk occurred at the agricultural greenhouse areas, most likely due to pesticide emissions, while lower risks were observed in waters receiving wastewater effluents. Due to low costs and high relevance, this model has the potential to become the first bioanalytical strategy to be applied in routine water quality monitoring. Key words: toxicity profiling, passive sampling, ecological risk assessment

299 Environmental monitoring: industry requirements and needs

L. Niisnen, Statoil ASA and Norwegian University of Science and Technology / Environmental Technology
Environmental monitoring related to the offshore petroleum industry has traditionally been carried out through ship campaigns with varying temporal and spatial resolution. Typical parameters measured are physical parameters such as grain size and total organic material (TOM), chemical parameters such as heavy metals and hydrocarbons as well as biological parameters such as taxonomy, species diversity and condition. National requirements are highly variable but the main purpose is to define the polluted area and trends within and between various areas. Linking environmental monitoring to the industry’s relevant risk picture, however, traditional monitoring may not be the most cost-efficient. The reasons are several and they all originate from the need of flexibility. The activities on the different assets vary, the nature of the discharges varies and they are located in areas with various environmental conditions. In a risk-based system, monitoring, risk modelling and mitigation measures are the cornerstone. Modelling can help optimising the environmental monitoring programme by focusing on the most critical aspects and sampling strategy. New technology has led to new opportunities for a holistic environmental monitoring approach adjusted to purpose, object or specific area of interest. A variety of fixed and mobile sensor
platforms now enables gathering of relevant amounts of data with the temporal and spatial resolution needed. Combining monitoring and modelling with appropriate mitigation measures, targeting the most critical component(s) of the discharges, will lead to improved environmental performance.

300

Use of effect-based tools in the context of the Water Framework Directive (WFD) – presentation of past and on-going projects

S. Schaan, European Commission - DG Environment / Water unit

Effect-based tools are drawing increasing interest in the context of the implementation of the Water Framework Directive (WFD), in particular because of their ability to detect effects caused by mixtures of pollutants and because of their potential use to bridge the gap between a substance-by-substance risk assessment (used to assess the chemical status in the WFD) and the integrative biological indicators used to determine WFD ecological status. This presentation will describe past and on-going activities on the use of such tools led under the Common Implementation Strategy for the Water Framework Directive, and outline some future perspectives.

301

Developing a framework for the assessment of contaminant impacts in marine ecosystems

K. Hylland, University of Oslo / Department of Biosciences; I. Davies; M. Gubbins, Marine Scotland; C. Robinson, Marine Scotland Science; T. Lang, Thünen Institute; D. Vethaak, DELTARES / Marine and Coastal systems; C. Martínez-Gómez, Instituto Español de Oceanografía / Marine Contamination and Biological Effects; T. Burgeot, IFREMER / Department of Biogeochemistry and Ecotoxicology; J. Thain, Cefas

European marine ecosystems are subject to a range of pressures, including fisheries, habitat change and contaminant inputs. Available data suggest that contaminants affect marine organisms, particularly linked to inputs from large rivers and areas with high offshore activity. Despite such knowledge, there has been no widely accepted guideline by which to assess contaminant effects. An integrated framework has been developed by ICES/OSPAR working groups (WKMON/SGMC) to enable an assessment of contaminant impacts in coastal and offshore ecosystems. The framework is based on assessment criteria for contaminant concentrations and effect methods, i.e. identifying background assessment concentrations (BACs), and environmental/ecotoxicological assessment criteria (EACs) for selected contaminants and biological effects methods. The framework comprises abiotic and biotic components, i.e. sediment, fish, mussels and gastropods. In addition, additional measurements to support the assessment, e.g. hydrographical data, are included. A methodology has been developed to combine results from different matrices/species and analytical methods, producing indices that may be integrated across methods/mechanisms of toxicity, locations or regions. The index for any single location or region using the framework can be integrated with assessments performed within the Marine Strategy Framework Directive.

302

Panel discussion

Science Integrity and Publication Bias

303

Introduction to Research Integrity – Academic Perspective

M. Zeegers, Maastricht University

There is a broad consensus among academic institutions, government and industry that more systematic and explicit attention should be paid to scientific integrity. Responsible Conduct of Research (RCR) is not only an ethical responsibility. It is also a relevant operational priority in times when there is increased scrutiny from government, regulatory authorities stakeholders and the public, and the reproducibility of research findings appears to be low. Previous studies, however, have shown however that on average 2% of scientists conduct fraud and 34% are involved in Questionable Research Practices (QRP), which due its high frequency may have a larger organizational impact. This whistleblowing presentation discusses the current play-field and proposes some policy changes to reduce ‘research waste’.

304

Research Integrity Codes – An Overview in a Governance Perspective

L. Sydnes, University of Bergen

Science is an enterprise that is based on trust and transparency. Knowledge is supposed to be generated, communicated, and distributed a verifiable and provable way that is assumed to prevent fraud and other dubious practices and ensure that the science community is self-correcting [1]. Of course, we know that the system has never been completely flawless, but overall it has functioned so well that most knowledge made publicly available has been reliable and trustworthy. However, the last couple of decades we have witnessed a sharp increase in the number of violations of this vulnerable and delicate system. This is alarming because the value and benefits of research as well as the sciences’ ability to serve society and humankind are vitally dependent on the trustworthy researchers performing investigations with integrity [2]. To meet this crucial challenge and try to curb scientific misconduct, discussions and developments of Code of Conducts have increased considerably in recent years and made the science community aware of the need to be proactive and teach and reflect on what constitute research integrity as the framework for science changes. In the lecture we will present a reflexive approach about the key issues and conclusions from a single country’s experience with publication bias in the scientific literature. There is no widely accepted guideline and awareness of the importance of this issue is increasing. In particular, the on-going policy process on science integrity by the Norwegian Research Council. This paper outlines some of the main principles and experiences from that process. Finally, we will present some preliminary results from a survey of researchers in Norway on their experiences and views on research integrity.

305

Sound Science – Industry Perspective

D. van Wijk, Euro Chlor

Chemical industries and their products are essential for societal prosperity and innovation. Innovation depends on effective scientific progress, which is also a key determinant in how the assessment of chemical safety for human health and the environment is performed in order to enable evidence-based decision-making. Therefore the scientific investigation and publication process and the utilisation of scientific results are essential for industry and the entire society as a whole. In a society with different interests, science has to provide the level playing-field and common ground and therefore it is essential that science integrity is maintained at all levels during science’s route from concept to law. In practice though, there are vulnerabilities in the process that may occur at different application levels, in a multitude of different ways. To identify and illustrate good and bad practices at all levels from investigation to policy, a matrix approach has been developed which will be discussed. As a worst-case example, the incorporation of poor studies into poorly reviewed journals that are ‘cherry-picked’ into review articles, may lead to selection by a prejudiced scientific committee for inclusion by legislative bodies who are motivated by popular opinion (based on tabloid/ social media ’scare-mongering’). This may result in regulations that incorrectly restrict societal use of essential substances. The presented matrix can help to identify those best practices that are required at each stage in directing impartial, apolitical and scientifically correct, high quality decision-making.

306

Perspectives of Academic Journals on Scientific Integrity and Publication Bias

G. Burton, University of Michigan / School of Natural Resources

There is an increasing awareness of a lack of scientific integrity and publication bias in peer-reviewed journals. This has been documented in the highest ranked journals. Retraction rates have dramatically increased due to plagiarism or falsification of data. This increasing trend is likely due to both improved detection methods and pressures to publish. The rates of bias are less clear and more difficult to measure and are linked to sector affiliation, gender, age, institutional reputation and geography. The majority of those surveyed support the double-blind review process to reduce bias, but there is no consensus across scientific journals. Promoting publication from the disenfranchised is a noble call – but requires concerted efforts from scientists and professional scientific societies. ET&C is addressing these challenging issues via a number of avenues, including their “Perspectives” columns, routine dialogue with their Editors to identify and respond to bias, and actively recruiting submissions from under-represented regions. ET&C will continue to be vigilant in maintaining its high level of scientific integrity, and will continue to look for methods to reduce the inherent human tendency of bias. Organizing a mentoring approach for scientists in under-represented regions has been of an ad hoc nature and should be expanded in the future.

307

SETAC Ethical Code of Conduct - followed by panel discussion

P. M. Gimney, Retired / S C Johnson & Son, Inc / Molecular and Environmental Toxicology Center

SETAC has maintained and upheld a Code of Ethics for many years. In 2015, the SWC revisited this Code of Ethics as part of their Long Range Planning process and decided to focus on this important topic as a key initiative. A Scientific Integrity Subcommittee was formed under the auspices of the Global Communication Committee. This Steering Committee for this time group met and discussed at least three issues we thought SETAC could significantly influence in the near future.
a positive way. First, we felt that there was an increasing lack of personal integrity. Certainly there are institutional biases, mostly in the organizations that are supposedly “protectors of the environment.” But underlying that is the question of personal integrity. What are the data really telling us? We need to know; good, bad, and ugly. Secondly, we felt that there was a general lack of understanding industry. Most people have “things” but don’t realize all that is involved in the manufacture, distribution, sale, and end-of-life management of “things.” There has been an increasing fervor from the business sector of SETAC that there is industry bias. This is an issue that SETAC with its long history of tripartite representation is in a unique position to address. Finally, is the issue of publication bias including what might be called the rise of the on-line journal. Many SETAC scientists get numerous emails requesting that they publish their work in speedy on-line journals at low costs. The on-line publishing world discovered a great “pay to play” model, where almost anyone can get almost anything published in a “peer reviewed” journal. Of course, their Impact Factors are at or below zero, but many times that’s irrelevant. Judges don’t know about Impact Factors, so a publication in any journal holds a lot of weight in a court of law. When you are in a “publish or perish” institution, any journal is better than none. In addition, because of the plethora of journals, it is becoming nearly impossible to find good reviewers for papers. The current Code of Ethics also mixes two distinct issues: namely personal ethics and scientific ethics. In this context, personal ethics means how someone responds to someone else and scientific ethics means the quality of our research. SETAC has a duty to address both types of ethics. Our medium to longer term, is to separate the two types of (un)ethical behaviour. Thus we are proposing changes and enhancements to both the SETAC Code of Conduct and the Code of Ethics.

308 Panel discussion Ctd.

Tendency towards higher complexity in environmental risk assessment of Plant Protection Products: to accept or to avoid?

309 Introduction

T. Frisch, Federal Environment Agency (UBA) / Section Plant Protection Products

310 Issues raised during the brainstorming-workshop “Reflecting on the increasing complexity in environmental risk assessment of Plant Protection Products” organized by the UBA, ANSES, KEMI (12th – 13th November 2015 in Berlin, Germany)

V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health and Safety

311 How to handle decreasing time lines and complex risk assessments of plant protection products - a proposal from KEMI

H. Sundberg, Swedish Chemicals Agency

312 Reasons and possible solutions for complexity in risk assessment

F. Streissl, EFSA / Pesticides Unit

313 Risk management decisions regarding plant protection products: information needs and regulatory context

K. M. Niemstedt, European Commission - DG SANCO / PPR

314 Models are neutral, funding is biased, complexity is real

R. Ashauer, University of York / Environment

315 Simplicity, Sense, and Scrutiny in Environmental Risk Assessments of PPPs

P. Kubow, BASF SE

Key messages from an NGO perspective

M. Wang, Greenpeace Research Laboratories

317 General discussion

T. Frisch, Federal Environment Agency (UBA) / Section Plant Protection Products

Persistent and mobile contaminants in the aquatic environment: how to identify, analyse and regulate a potential threat for drinking water resources (II)

318 How do chemical and environmental properties affect the retention of organic contaminants in urban watersheds?

A. Parajulee, Y.D. Lei, A. Kananthalingham, University of Toronto Scarborough / Department of Physical and Environmental Sciences; L.W. Yeung, University of Toronto / Chemistry; A.O. De Silva, Environment and Climate Change Canada; C.P. Mitchell, University of Toronto Scarborough / Department of Physical and Environmental Sciences; F. Wania, University of Toronto Scarborough / Physical and Environmental Sciences

Prominent characteristics of the urban landscape include impervious surface coverage and sewer networks that both serve to increase runoff delivery to streams and decrease infiltration. This can lead to rapid, often untreated inputs of contaminants to water bodies during snowmelt and rainfall. Of particular interest are how chemical properties and environmental properties interact during such events to influence contaminant delivery to streams. This study investigates the relative importance of chemical properties, urban infrastructure, and physiography on the transport of a broad range of organic contaminants in urban Canadian watersheds. In Toronto, Canada, we have three sampling sites in each of two watersheds that differ widely in their degree of urbanization, and hence the types and configurations of their land cover and city infrastructure. We measured dissolved and suspended sediment-bound streamwater concentrations of polycyclic aromatic hydrocarbons (PAHs), neutral and acidic pesticides, and benzotriazoles during two rainfall events and one snowmelt event. We also concurrently measured suspended sediment, dissolved and particulate organic carbon, and electrical conductivity. Preliminary results from one rainfall event show peak concentrations of particle-bound PAHs that are 20-94 times higher, and area-normalized fluxes 6 to 24 times higher, at the sites in the more urbanized watershed relative to the largely agricultural watershed. Furthermore, while these peak concentrations increase downstream in the urbanized watershed, there is little to no change in peak concentrations in the other watershed. The total watershed particle-associated PAH loading to Lake Ontario was also 25 times higher. We will additionally present the results of ongoing sample analyses that answer the following questions arising from these initial findings: (1) What drives the higher concentrations and loadings in the highly urbanized watershed? The explanation could be one or both of the following: first, that the urbanized watershed has greater within-watershed sources of PAHs and second, that the mostly agricultural watershed has a greater retention capacity for PAHs. (2) Are the observed patterns consistent across snowmelt and rainfall events, for all analytes, and why or why not?

319 Screening of organic contaminants in raw and drinking water from source to tap

L. Ahrens, Swedish University of Agricultural Sciences (SLU) / Dept of Aquatic Sciences and Assessment; R. Troger, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; P. Klockner, Swedish University of Agricultural Sciences SLU; J. Lundqvist, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; A. Rosennai, T. Le Godec, Swedish University of Agricultural Sciences; A. Oskarsson, SLU Dept Biomed Sci Vet Publ Health / Dept of Biomed Sci and Vet Publ Health; K. Wiiberg, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment

The occurrence of synthetic organic compounds (OCs) in drinking water can pose a threat to human health. In this study, water samples were collected from drinking water source areas, inside a drinking water treatment plant (DWTP) and in finished drinking water from the surroundings of Uppsala, Sweden. The water samples were examined for 178 residues of pharmaceuticals and personal care products (PPCPs), perfluoroalkyl substances (PFASs) and pesticides. The contaminants were extracted using large volume solid phase extraction (SPE) and analysed using liquid chromatography coupled to a time-of-flight spectrometer (LC/TOF-MS). The main source of the targeted OCs was found to be a sewage treatment plant (STP) effluent. Concentrations decreased in two lake samples (lake Ekoln and Gvrln) connected to the DWTP due to dilution effects and potential degradation. Within the DWTP, concentrations remained stable and contaminants were not removed efficiently with the conventional treatment
techniques of flocculation, sand filtration, granular activated carbon (GAC) and disinfection with UV light and chloramine. A pilot plant with membrane technology is ineffective as well, except when coupled to new GAC filter. Overall, the occurrence of contaminants in drinking water emphasizes the need for research and to improve our knowledge about their risks for human health and ecosystem.

320 Advanced water treatment assessment and novel monitoring techniques S. O’Reilly, K.W. WaterTec Research Institute; K.A. Baken, K.W. WaterTec Research Institute / CWG; I. Breitung, IAWR; G. Stroomberg, RIA River Water Works
To control the emission of chemicals in the environment and to meet the requirements of the WFD, emissions of chemical compounds are more and more regulated, for example by the REACH and other substance specific regulations. Next to that, emission point sources of WWTPs are being upgraded towards the elimination of chemicals of emerging concern (CECs). More specifically, in the Rhine area, several communal waste water treatment plants aim to increase the removal of these CECs. Here, additional treatment steps such as advanced oxidation processes (AOPs) and/or in combination with Granular Activated Carbon (GAC) are being considered or have been implemented. Numerous studies describe the successful removal of substances in waste water, hence higher removal rates by these treatment steps. While the intention is to lower emissions of CECs, resulting transformation products may be overlooked. Concise information on the formation and toxicological relevance of transformation products is lacking and it is unclear to which extent transformation products contribute to overall toxicities of treated waters. This review will collate existing information on the risks of AOPs in the light of large scale applications of these new waste water treatment techniques. Also, the review will identify research questions that arise, specifically the impact of the formation of polar persistent transformation products. The review will highlight existing and novel monitoring techniques, both to identify toxicity as to recognize unknown and known chemicals. As drinking water utilities apply similar techniques for the production of safe and healthy tap water, information may be usable for different water managers and researchers.

About 50% of the halogenated material formed during water disinfection is still unknown[1]. Several chemical classes of low molecular weight iodosinated disinfection by-products (iodo-DBPs) have been reported to be within the aforementioned unidentified fraction. The presence of iodo-DBPs in drinking water is of concern, because these compounds have been found to be in some cases more genotoxic and cytoxic than their corresponding brominated and chlorinated analogues[2-12]. Recent developments in gas chromatography (GC) - mass spectrometry (MS) allow the use of high resolution-accurate mass (HRAM) Orbitrap mass analyzer to further explore and characterize the volatile and semi-volatile compounds present in the disinfection by-product (DBP) mixtures, providing the resolving power, mass accuracy, and sensitivity required for these tasks. In this context, the main goal of the present work was to apply the GC Orbitrap MS technology to characterize iodo-DBPs in chlorinated and chloraminated DBP mixtures. To this end, chlorinated and chloraminated DBP mixtures were generated from a reference aquatic natural organic matter (NOM) solution, i.e., Nordic Reservoir NOM, fortified with bromide (50 ppb) and iodide (50 ppb), and Llobregat river water, and analyzed with a GC-Orbitrap MS. Iodo-DBP characterization was based on HRAM data and fragmentation rationalization. The workflow applied allowed identification of up to 11 different iodo-DBPs in the DBP mixtures analyzed, including one new iodo-DBP. Overall, it could be also observed that iodo-DBPs were formed to a larger extent after chloramination of waters; however, the type of NOM, in terms of aromaticity, and the bromide and iodide content the water were found to be relevant factors affecting the formation of these compounds during disinfection treatments.


322 Do cyostatic drugs reach drinking water? The case of mycophenolic acid H. Franquet. Environmental Chemistry; F. Ventura, IDAEA-CSIC; M. Boleda, Aigues de Barcelona / Organic Chemistry Department; S. Lacorte, IDAEA-CSIC / Environmental Chemistry
Mycophenolic acid (MPA) has been identified as a new river contaminant according to its wide use and high predicted environmental concentration in the river (PEC_{concer1}). In Catalonia (NE Spain), its consumption ranged from 4.7 to 4.9 tons during 2010-2012, which represents 40% of the total drugs administered, and the calculated PEC_{concer1} was 77.4 ng L⁻¹ [1]. Therefore, the aim of this study was to monitor the impact of MPA in a drinking water treatment plant (DWTP) that collects water downstream Llobregat River (NE Spain) in a highly densified urban area. An UPLC-MS/MS method was developed and validated to quantify this compound in water samples. During a one week survey MPA was recurrently detected in the DWTP intake (17 - 56.2 ng L⁻¹). The presence of this compound in river water was associated to its widespread consumption, high excretion rates and low environmental persistence. As MPA was always detected after 7 consecutive days, its fate in waters at each treatment step of the DWTP was studied and complete removal was observed after pretreatment with chlorine dioxide. In addition, degradation experiments were performed using CI₂O₃ at different concentrations and degradation products were identified. MPA does not reach drinking water considering the current treatments carried out in this particular DWTP. However, its retentive presence associated with its consumption in anticancer treatments is of relevance to highlight the importance of monitoring this compound. [1] Franquet-Griell, H., Gomez-Canelo, C., Ventura, F., Lacorte, S., 2015. Predicting concentrations of cyostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain). Environ. Res. 138, 161-172.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (J)

323 Detection and quantification of Carbon Nanotubes along the life cycle, using a thermo-optical method A. John, Institute of Energy and Environmental Technology IUTA eV / Air Quality Sustainable Nanotechnology; M. Renker, Institute of Energy and Environmental Technology IUTA eV / Air Quality Sustainable Nanotechnology; T.A. Kuhlbusch, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality Sustainable Nanotechnology; C. Nickel, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality Sustainable Nanotechnology
Carbon nanotubes (CNT) are already used as additives to various structural materials, due to their extraordinary thermal conductivity and mechanical and electrical properties. A release during production, handling, usage, transport or end of life seems possible. Therefore, the detection of CNT along the whole life cycle in different compartments is very important to assess a potential risk for human health and the environment. Despite the increasing number of application and release mechanisms CNT may enter different environmental compartments in varying concentrations. Thus, different requirements for the sample preparation and detection have to be considered. One main challenge dealing with the detection of CNT in the different environmental compartments is the high background concentration of natural carbon based materials and the differentiation between CNT and these materials. By using a thermo-optical method a differentiation between different types of CNT and natural carbon based materials and CNT was possible. For this, we adapted a thermo-optical carbon analysis which is routinely used to determine elemental carbon from filters probed with e.g. ambient or workplace air. This method determines total carbon (TC) and uses a specific temperature protocol to fractionate TC into organic carbon (OC) during first temperature steps in pure helium and elemental carbon (EC) during the second temperature steps in a helium/oxygen atmosphere. For the detection of CNT an established NIOSH5040-like protocol „quartz.par“ was modified. Two additional temperature steps were integrated in the low temperature EC section to achieve a good differentiation between CNT and natural carbon based materials, like humic acids. During this presentation results for different life cycle stages are presented, starting with the release of CNT in air from products during mechanical stress, the detection of CNT in water and soils, the uptake into organisms and the release of CNT during incineration at the end of life. The results were generated within the CarboLifeCycle project which was founded from the German BMBF, grant 03X0114D.

324 The devil is in the details: Tomography to characterize nanoparticle-plant interactions at low population concentrations. F. Schwab, CEREGE / Interfast Transferts; A. Avellan, CEREGE; A. Turner, Duke University; C. Santaela, CNRS/CEA/Aix-Marseille Université / Bioscience and biotechnology Institute of Aix Marseille; J. Bottero, Labex Serenade/CEREGE/Aix-Marseille Université; J. Rose, CEREGE / Interfaces Transferts; C. Levard, CEREGE; M. Auffan, CEREGE / International Consortium
for the Environmental Implications of Nanotechnology
The current understanding of the uptake processes of engineered (nano)materials (ENMs) in plants strongly relies on two-dimensional visualizations of (nano)particles in or on plant tissue, and quantitative analyses of plant digestates. Most of these techniques require the plants to be exposed to very high ENM concentrations (mg kg⁻¹) and are therefore not suitable for in situ fieldwork.

Most of these techniques require the plants to be exposed to very high ENM concentrations (mg kg⁻¹) and are therefore not suitable for in situ fieldwork. Such high concentrations may exceed the physiological and environmental limits of the plants and may result in non-specific responses.

However, it is challenging to use 2D-imaging and digestate analyses to answer questions on the extent and mechanism of internalization of ENMs, or on ENM-plant interactions at environmentally more relevant, subacute exposure conditions. The system is also capable of taking simultaneous size distributions of nanoparticles (PSD). The current work presents an overview of previously developed data processing methods and discusses how such data can be processed and the limits of several approaches.

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real-world environmental samples, while highlighting the limitations and challenges for two case studies. TiO2 ENMs were successfully measured in the presence of high Calcium concentrations; however, this method is not able to measure TiO2 particles smaller than 80 nm. CuO ENMs were also detected in spiked colloid extracts; we choose a rather conservative approach for particle detection with the goal of avoiding falsely identifying background signal as engineered CuO NP particles. In this context, the need for comparing and correcting detected particles spikes against the presence of false positive spikes is also discussed.

**Ecological traps for wildlife driven by pollutants**

329 Eutrophic wetlands receiving effluents from wastewater treatment plants may act as ecological traps for endangered waterfowl

R. Matao, U.C.L.M.-C.S.I.C. / Instituto de Investigacion en Recursos Cinegeticos; C. Gil, R. Guitart, Universitat Autonoma de Barcelona; A.J. Green, EBD-CSIC / Wetland Ecology

Animals can select a habitat according to characteristics of the environment that correlate with better survival and reproductive success. In rapidly changing environments, these natural preferences can become ecological traps if the selected aspects of the habitat are accompanied with adverse environmental factors that affect individual fitness. Pollutants can be one of the important drivers of ecological traps nowadays. One of the potential scenarios that matches the concept of ecological trap are the eutrophic wetlands receiving effluents from wastewater treatment plants. These wetlands are highly productive and therefore attractive for waterbirds, especially those feeding on aquatic invertebrates like the white-headed duck (Oxyura leucocephala). These wetlands may also receive a myriad of pollutants from urban and industrial sewage that can accumulate and affect the ecological dynamics and the health status of the waterbirds living there. Here we discuss the potential effect of these ecological traps for the white-headed duck and the marbled teal (Marmaronetta angustirostris), which are globally endangered species under threat in their remaining breeding areas such as Spain. The studied birds were marbled teals (n=28) and white-headed ducks (n=12), but also the invasive ruddy duck (Oxyura jamaicensis) (n=13) and their hybrides (Oxyura leucocephala x jamaicensis) (n=5). Organochlorine pesticides and PCBs were measured in liver and adipose tissue samples after extraction with hexane, clean-up with sulfuric acid and analysis by GC-ECD. Birds of the genus Oxyura showed higher organochlorine levels than marbled teal, especially in adipose tissue. No significant differences were found between both Oxyura species and their hybrids, between geographical areas (Andalusia, Valencia or Central Spain), by sex or by age. Several potential biotic i.e. pathogens) and abiotic agents (i.e. chemical pollutants) may drive negative influences on the populations of endangered waterfowl through a mechanism in eutrophic wetlands that can be defined as an ecological trap. In addition to the mortality caused by botulism outbreaks and lead poisoning, white-headed duck shows a low reproductive outcome that could be caused by pollutants accumulated in the eutrophic lakes frequently selected by this species.

330 Can POPs contaminated sites be ecological traps for top predators?

J.E. Elliott, Environment Canada / Science Technology Branch; C. Nelson, University of British Columbia / Animal Science; D. Guertin; A.C. Huang, Forest Conservation Sciences

Numerous contaminated sites are documented in North America and many other parts of the globe. Among the main contaminants at such sites are legacy persistent organic pollutants (POPs) such as PCBs and organochlorine insecticides. Wildlife, including top-predatory birds and mammals may be attracted to such sites as the habitat often appears suitable. In the process, species such as river otters, can bioaccumulate POPs with potential consequences on health and fitness. Previously we used fecal samples collected from river otter communal marking sites (latrines) at the federal contaminated site of Victoria Harbour, BC, Canada, reported levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Following up on that study, we again used scats to show variation in PCB exposure in time and space, and the population implications. In the present study we again used fecal sampling but combined with radio-telemetry to further investigate the exposure and to infer population level effects in river otters in that contaminated system. We also examine the question of whether the harbour constitutes an ecological trap situation. The implications of population structuring relate to the extent and impacts of contaminant exposure based on where the otters spend their time foraging and whether or not they are able to sustain a population in a contaminated site environment.

331 Are ecological-traps for birds because of pollutants?

R. Schellker, University of Franche-Comte / ChronoEnvironment; J. Bailly, University of Burgundy / Biogeoeciences; N. BERNARD, University of Franche-Comte / Laboratory of ChronoEnvironment; B. Faivre, University of Burgundy / Biogeoeciences

Cities have higher temperatures, louder noise, lower parasite prevalence and higher levels of pollutants than rural habitats. They are considered as ecological traps because studies dealing with the impact of urbanization on bird fitness have shown that breeding parameters are affected. Among the mechanisms that may explain the responses of birds to urbanization, the impact of pollutants although cities are characterized by the circulation of many different pollutants. We hypothesized that NO2 concentrations in the air and/or concentrations of non-essential trace elements (TEs) in blood were positively correlated to reduced reproductive outputs and/or nestling body condition in urban populations of Great tits (Parus major) compared to forest ones, while concentrations of essential elements in blood were negatively related to breeding outputs and/or individual performances if their concentrations exceeded threshold values or, on the contrary, if they did not meet physiological (homeostatic) requirements. Two cities and two forests were studied in eastern France. Each site received 150 artificial nestboxes that were inspected from March 2010 to record breeding success from 2010 to 2014. In 2013, passive NO2 samplers were put on nestboxes and the concentrations of 25 essential and non-essential elements were determined in 13-day old nestlings. In urban sites, breeding success, nestling growth rate and body condition were reduced. Atmospheric concentrations of NO2 significantly differed between the four sites but no significant correlation was detected between NO2 and TEs concentrations. Based on the results of a PCA on metal concentrations, we used the coordinates on the first axis as an index of richness in essential elements (REE), with negative values indicating high concentrations in essential TEs, and positive value indicating low concentrations. Brood body mass was positively correlated with REE. No other significant correlation was detected between REE and biological parameters. Our study brings new data about pollution exposure of TEs and NO2 concentrations in birds from urban and forest habitats and their positive relation to nestling body mass and condition. To our opinion, this is a clue to suggest that food availability and/or quality may be a causal mechanism for a reduced growth of nestlings in urban areas, an issue that would need further investigations in urban ecology and in ecotoxicology in general.

332 Short-term and residual effects of pesticides on mosquito oviposition site selection and larval performance, and on aquatic community structure

C. Dachet, Institute of Evolution / Department of Evolutionary Environmental Biology; G.M. Moraru, University of Haifa / Department of Evolutionary Environmental Biology; K. Saurav, University of Haifa / Department of Marine Biology; A. Gershberg Hayoon, University of Haifa / Department of Evolutionary and Environmental Biology; R. Shapir, University of Haifa / Department of Evolutionary Environmental Biology; L. Steinler, University of Haifa / Department of Marine Biology; L. Blaustein, University of Haifa / Department of Evolutionary and Environmental Biology

Pesticides can have immediate toxic effects on targeted pest species as well as on non-target species for broad-spectrum pesticides. Broad-spectrum pesticides may also have positive effects on mosquitoes after the pesticide no longer has direct toxicological effects by altering the community structure – i.e. by reducing abundances of mosquito competitors and predators, and via a trophic cascade, increasing food resources for mosquito larvae. Moreover, in some cases, pesticides can act as an ecological trap for some taxa, attracting colonizer insects to habitats that are ultimately lethal for them. The present study was undertaken to assess mosquito oviposition habitat selection, mosquito larval performance and community structure alterations after pesticide applications. The experiment was conducted in outdoor mesocosms assigned to one of four treatments: (1) control – no pesticides; (2) Bacillus thuringiensis israelensis, a mosquito larvicide well-known for its selectivity for Nematoceradipterans; (3) temephos, a mosquito larvicide of the organophosphate class; (4) pyriproxifen, a pyridine-based pesticide of the Insect Growth Regulator (IGR) class. All three of these pesticides have short activity periods. Invertebrates were sampled 2 days before treatments, 4 and 7 days after treatment and then, every two weeks for a period of 2 months. Environmental parameters (water temperature, pH, conductivity, chlorophyll a) were measured in every 2 weeks to control the sampling date. Mosquito oviposition was monitored every 3 days. After applications, the number of egg rafts decreased in all the treated pools, but remained stable in the controls. Species richness and invertebrate abundance were also initially strongly reduced in the broad-spectrum pesticides (temephos and pyriproxifen) when compared to both the control and to the individual R. thuringiensis israelensis treatments in terms of chlorophyll a concentrations. One month after the treatment, mosquito oviposition was higher in the pyriproxifen-treated pools, in comparison to other treatments. However, mosquito larval survival remained lower in pyriproxifen-treated pools, even though pyriproxifen was not detected in the water samples 7 days after the application. Our results suggest that pyriproxifen appears to provide an ecological-trap, attracting mosquito oviposition due to altered community structure (fewer antagonists and more algal resources) but causing high mosquito larval mortality.
Due to reductions in winter food resources, newly sown cereal seeds have become a key component of many bird species’ diet, but these seeds are often treated with pesticides that may cause toxic effects. In order to complete an appropriate risk assessment, data on treated seed toxicity need to be combined with information about the risk of exposure of birds in the field. The aims of the present work are to characterize the exposure of farmland birds to pesticide-coated seeds in the wild, and to estimate the risk of poisoning of animals as a consequence of such exposure. To do this we analysed crop and grain contents of hunted partridges (n=189) to detect residues of pesticides used for seed treatment. Moreover, we measured the contribution of cereal seeds in the winter diet of partridges in order to assess the potential risk of exposure to pesticide-treated seeds. We also studied the abundance of pesticide-treated seeds available for birds in the field (n=48), the pesticides and their concentrations in these treated seeds, and the bird species that feed on them. Seed availability was influenced by the location of the crop within the field, regardless of the sowing technique, there were more seeds on the surface in the headland (43.4 ± 5.5 seeds/m²) than in the field centre (11.3 ± 1.2 seeds/m²) (t = -4.67, p < 0.001). Six pesticides, mostly fungicides, were detected in the seeds collected from recently sown fields. 55.2% of the seed samples contained detectable levels of pesticides, with tebuconazole being the most frequently found active ingredient. During the sowing period, 31 species were observed feeding on sown cereal seeds. We detected pesticide residues in the digestive content of 32.3% of the analysed red-legged partridges, including insecticides in a 3.7% of the analysed samples and fungicides in a 29.6% of them. As observed in seeds, tebuconazole was the most frequently detected pesticide (10.5% of the sampled seeds were contaminated). Residues of the other pesticides were found in the digestive content of red-legged partridges. The present study demonstrates that the use of pesticide-treated seeds constitutes an important way of exposure of farmland birds to pesticides, and that there exists a potential risk for these birds to suffer toxic effects from ingestion of treated seeds in the wild.

**334 Mange, anticoagulants, and felids: Investigating immune response to low dose brodifacoum exposure**

K. Honke, J. Kopanke, E. Musselman, K. Beutner, S. Vandewoude, Colorado State University, Fort Collins, CO, USA. Anticoagulant rodenticides are used worldwide to control pest rodents in urban, suburban, and agricultural settings. This widespread use poses potential risk of exposure to non-target species. An analysis of bobcats and mountain lions from Los Angeles, CA detected a correlation between anticoagulant exposure and mange. Exposure to brodifacoum has thus been proposed as a potential mechanism for this effect in felids, though domestic cats have been shown to have high tolerance for anticoagulants. More research is needed to determine potential effects of brodifacoum that a cat would be exposed to by consuming non-target rodents. In this study, cats were offered 0.05 mg/kg brodifacoum once per week mixed into canned food and vaccinated with irrelevant antigen (ovalbumin, OVA) and adjuvant starting at week 1. With booster injections on weeks 4 and 8 to test effects of brodifacoum on recall antigen immune responses. Blood was collected weekly beginning on day 0 to additionally assess PBMC proliferation following exposure to ConA, KLH, and OVA. Whole blood was frozen for brodifacoum concentrations in blood were measured using a high-performance liquid chromatography (HPLC) method. Brodifacoum concentrations in blood were observed feeding on sown cereal seeds. We detected pesticide residues in the seeds collected from recently sown fields. 55.2% of the seed samples contained detectable levels of pesticides, with tebuconazole being the most frequently found active ingredient. During the sowing period, 31 species were observed feeding on sown cereal seeds. We detected pesticide residues in the digestive content of 32.3% of the analysed red-legged partridges, including insecticides in a 3.7% of the analysed samples and fungicides in a 29.6% of them. As observed in seeds, tebuconazole was the most frequently detected pesticide (10.5% of the sampled seeds were contaminated). Residues of the other pesticides were found in the digestive content of red-legged partridges. The present study demonstrates that the use of pesticide-treated seeds constitutes an important way of exposure of farmland birds to pesticides, and that there exists a potential risk for these birds to suffer toxic effects from ingestion of treated seeds in the wild.

**Evaluation of currently available methods for determining log Kow values of surfactants**

F. Bauer, P. Thomas, KREATiS; S. Neunlist, S. Fouchard, Université de Haute-Alsace / Laboratoire de Chimie Organique et Bioorganique EA A and understanding of the log Kow values of surfactants is crucial for the efficient prediction of ecotoxicity using in silico techniques. Different MechaAs will need different parameters or different coefficients to calculate toxicity values. To date, several classification methods, based on molecular structure analysis, exist to determine the Mode of Action (Mea) of surfactants within REACH and OSPAR. Results from this study show that logKow values were a selected set of 12 surfactants. Side-by-side comparisons of values derived using different experimental methods compared against predicted values identified whether certain existing methods give similar results for the same surfactants and recommendations made.

**336 New Mechanism of Action definitions based on Molecular Initiating Events and a classification algorithm based on calculated structure properties**

F. Bauer, P. Thomas, KREATiS; S. Neunlist, S. Fouchard, Université de Haute-Alsace / Laboratoire de Chimie Organique et Bioorganique EA A and understanding of the log Kow values of surfactants is crucial for the efficient prediction of ecotoxicity using in silico techniques. Different MechaAs will need different parameters or different coefficients to calculate toxicity values. To date, several classification methods, based on molecular structure analysis, exist to determine the Mode of Action (Mea) of surfactants within REACH and OSPAR. Results from this study show that logKow values were a selected set of 12 surfactants. Side-by-side comparisons of values derived using different experimental methods compared against predicted values identified whether certain existing methods give similar results for the same surfactants and recommendations made.

**In silico modelling perspective to advance hazard assessment of aquatic ecotoxicology**

An in silico modelling perspective to advance hazard assessment of aquatic ecotoxicology

**335 Evaluation of currently available methods for determining log Kow values of surfactants**

G. Hodges, Unilever Research / Safety and Environmental Assurance Centre; J. Arndt, Dow Chemical Company; B. Bossuyt, HUNTSMAN Europe bvba / PEHS; A. Bouvy, Cefic; C.V. Eastford, Shell International / Shell Health; M. Enrici, SOLVAY / HSE. PRA PS; M. Geurts, Akzo Nobel N.V. / EET; D. Miller, Clariant GmbH; G. Oetter, BASF SE / Materials Physics and Analytics; P. Sun, The Procter & Gamble Co.; J. Venzmer, Evonik

The n-octanol/water partition coefficient (log Kow) is a key parameter in chemical environmental studies as it determines the environmental fate and bioavailability and thus toxicity of a compound. Due to their high water solubility, measurement of log Kow values of surfactants is a technical challenge. The traditional ‘shake-flask’ method (OECD 107) is no longer considered appropriate. Here we compare log Kow values of surfactants were compared using a range of existing experimental and predictive methods which have been used by lead registrants in REACH phases 1 and 2. However, there are concerns that these methods have not been fully validated for surfactants and may not be applicable due to the specific phase behaviour of surfactants, leading to unreliable or unrealistic values. This is complicated by the fact that the solubility is not properly defined for surfactants since they will form a micellar phase above the Critical Micelle Concentration (CMC). As a result, log Kow will be dependent on CMC. In this study, logKow values were a selected set of 12 surfactants. Side-by-side comparisons of values derived using different experimental methods compared against predicted values identified whether certain existing methods give similar results for the same surfactants and recommendations made.

**337 Fragment Model to Predict the Rate of Hydrolysis for Organic Compounds**

R. Köhne, R. Ebert, G. Schüürmann, Helmholtz centre for environmental research - UFZ / Department of Ecotoxicology. Hydrolysis as one of the key degradation processes in the environment is considered to be important for a number of classes of organic compounds as carboxylic acid esters and amides, anhydrides, epoxides, isocyanates and isothiocyanates, carbamates, phosphorus and thiophosphorus esters, alkoxyalanes and alkoxyethers, and halogenated hydrocarbons. However, the number of available experimental data is rather limited. Computer models are a promising alternative to overcome respective data gaps. The overall hydrolysis is a combination of the reaction with neutral water molecules and acid and base catalysed degradation reactions. These processes will take place to a different degree. Since the acid and base catalysed degradation depends on the pH, the role of the individual processes and the overall rate of hydrolysis. The main objective of this work is to test the relevance of the simplified model approach and to determine the influence of fundamental reaction parameters (pH, concentration) on the hydrolysis rate. A novel approach is to express this rather complex system of reactions in terms of simple pseudo-first-order kinetics. A related approach is to express this rather complex system of reactions in terms of simple pseudo-first-order kinetics. The reaction with neutral water is directly addressed by a pseudo-first-order rate that is a constant for a given chemical and temperature and independent of the pH. Acid and base rates are primarily derived as a constant and not dependent second-order rate constant. The simplified model approach is to express this rather complex system of reactions in terms of simple pseudo-first-order kinetics. The reaction with neutral water is directly addressed by a pseudo-first-order rate that is a constant for a given chemical and temperature and independent of the pH. Acid and base rates are primarily derived as a constant and not dependent second-order rate constant.
concentrations. For the fixed pH the overall rate then can be calculated from the combination of the individual rates. In the present study, three independent fragment models are being developed for the individual pH-independent rate constants, and the overall rate constants are obtained by putting them together for a particular pH value. The model is implemented and will be publicly available after its finalisation in the OSIRIS edition of the software system ChemProp (UFZ Dpt. Ecological Chemistry 2015. ChemProp 6.3 http://www.ufz.de/index.php?en=6738). The training set consists of about 2200 compounds of all considerably hydrolysing compound classes. For each 670 substances, rates of the neutral reaction are available, and for the acid and base catalysed reaction there are data for 750 and 1280 chemicals. The models comprise ca. 75, 85, and 125 structural fragments for the estimation of the neutral, acidic, and basic rate. The performance with respect to the overall rate is ca. 0.9 (squared correlation coefficient of regression). The reliability of the model and the comparison to existing approaches will be discussed.

**338 Development of parameters to predict hydrophobicity and sorption of non-ionic and anionic surfactants**

J. Halika, Utrecht University / Institute for Risk Assessment Sciences; J. Hammer, University of Utrecht / IRAS; P. de Voort, University of Amsterdam / IBED; J. Hermens, Utrecht University / Institute for Risk Assessment Sciences

Octanol-water partition coefficients ($K_w$) are often used in environmental risk assessment as a measure for hydrophobicity of organic contaminants to predict bioaccumulation, toxicity, and sorption to soil. The $K_w$ value is therefore an important parameter in predictive modelling of environmental fate and exposure of chemicals. The cut-off value of the octanol-water partition coefficient in predictive models for surfactants becomes problematic because of the amphiphilic nature of surfactants. More knowledge about the environmental behaviour of ionized and surface-active substances is therefore required to expand the chemical applicability domain of the currently used predictive models. Sorption of surfactants to polymer-coated fibres can be used as a measure of hydrophobicity. In addition, retention on stationary phases used in liquid chromatography (e.g., C$_8$, HIIC or ion-exchange phases) can be used as a probe to simulate sorptive interactions of surfactants with relevant environmental sorption phases (e.g., membrane lipids, organic matter, etcetera). The applicability of polymer phases and selected stationary phases to determine the hydrophobicity of non-ionic and anionic surfactants and to predict their bioaccumulation in aquatic organisms will be explored in this study based on literature and experimental data from different surfactant groups. The possibility of using such methods as a measure of hydrophobicity will have important consequences for the regulatory evaluation of surfactants within REACH and OSPAR. Results from this study show that fibre-water sorption coefficients and capacity factors on a C$_8$ phase show great promise for the prediction of hydrophobicity of non-ionic and anionic surfactants. These values that reflect differences in hydrophobicity can be correlated with available data of bioaccumulation, toxicity or sorption. However, a single parameter or a single polymer or hydrophobic phase will not be sufficient in predictive modelling of sorption phenomena of surfactants to environmental phases. Likely, a series of phases are needed that correspond with specific types of interaction.

**339 Replacing Fish Acute Toxicity Tests by QSAR Predictions: How much is realistic?**

M. Nendza, Analytisches Laboratorium AL-Luhnstedt; M. Müller, A. Wenzel, Fraunhofer-Institute for Molecular Biology and Applied Ecology

Alternative methods like QSARs (quantitative structure-activity relationships) can replace some (eco)toxicity testing in regulatory contexts (e.g. REACH). Complete substitution of in vivo animal tests by in silico methods is, however, not realistic. Reliable QSAR predictions from the octanol/water partition coefficient (log $K_w$) are possible only for certain classes of chemicals, the so-called baseline toxicants. Other chemicals that may reveal excess toxicity and/or act by specific modes of action are not well-represented. Other approaches to develop a scheme to group chemicals as either baseline toxicants (= predictable by QSARs) or as potential excess toxicants (= not predictable by baseline QSARs). This scheme was tested with a new data set obtained with diverse fish species. Only for baseline toxicants, it is possible to predict the fish acute toxicity with sufficient accuracy (even for low values). Valid QSARs can replace in vivo testing. In contrast, excess toxicants and chemicals not reliably classified as baseline toxicants, require further in silico, in vitro or in vivo assessments. The stepwise approach identifies baseline toxicants (true negatives) in a precautionary way, not ignoring possible excess toxicants (true positives). At the same time, we tolerate a certain fraction of false positives, i.e. baseline toxicants without specific effects that may be tested instead of predicted. The way to replace many fish acute toxicity tests by QSAR predictions takes 3 steps: First, we use a classification scheme to discriminate between baseline and excess toxicants. Second, we apply high quality QSARs that meet the requirements for regulatory acceptance to replace in vivo fish acute toxicity tests for baseline toxicants with QSAR predictions. Third, the excess toxicants and chemicals not reliably classified as baseline toxicants, have to be assessed by further in silico, in vitro or in vivo methods. Application of the classification scheme to identify potential baseline toxicants to a new heterogeneous data set obtained with diverse fish species results in about 50% baseline toxicants, almost 30% excess toxicants and nearly 20% compounds not classified. Thus, we can conclude that replacing at least 50% of the fish acute toxicity tests by QSAR predictions is realistic.

**340 Can we undertake beyond experimental limits using HA-QSAR methodology?**

P. Bicheno, F. Thomas, KREATIS

In aquatic ecotoxicology water solubility is considered a key parameter since it provides an indication of how much of a substance can be available for uptake by organisms. In general ecotoxicity of very hydrophobic substances are difficult to measure. They are likely to not have reached equilibrium within the experiment’s duration and it is often technically difficult to achieve accurate concentration measurements. On the contrary, various quantitative structure-activity methods is available to assess the miscibility of very water soluble substances. QSAR/QSPR modelling is a semi-empiric approach based on measured data. Their applicability domain tends to be limited by the extreme values in the available dataset. In this presentation we consider whether extrapolations beyond the data boundaries can be justified for both miscible and very hydrophobic substances using High-Accuracy QSAR models. On the hand, for “miscible substances” without any experimental quantification of the solubility, the water solubility values predicted by the HA-QSAR model are completely in agreement with the ecotoxicity values even if they cannot be compared to experimental water solubility. At the other end of the scale predicted QSAR values cannot be extrapolated beyond a recognised solubility/log $K_{ow}$ cut-off value since acute tests are too short to reach equilibrium for very hydrophobic substances. Thus, for certain QSAR models, it is not the availability of the extreme datapoints in the dataset that determine the applicability domain, but other factors such as equilibrium with the test duration that will determine whether a substance endpoint value can be accurately predicted or not.

**Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (I)**

**341 Selecting mechanistic effect models for environmental risk assessment; the link with protection goals and test protocols**

T. Jager, DEBtox Research / Dept of Theoretical Biology

Virtually all frameworks for environmental risk assessment assess risks by combining the results from an exposure and an effects assessment. Estimation of exposure concentrations relies heavily on mechanistic fate models, but for the effects side, in contrast, focus lies with toxicity testing and descriptive statistic treatment of data. Mechanistic effect models do exist, however, and are gaining increasing interest in a regulatory context. A wide range of effect models exists at different levels of biological organisation (individual, population, community). These models differ in their degree of complexity, in their underlying assumptions about the biological and toxicological reality, in their ‘quality’ (as assessed from a good-modelling perspective), and in their data requirements, making it impossible to see the wood for the trees. The selection of the most appropriate models cannot be performed in isolation. Model selection is best viewed in conjunction with two other issues: the protection goals and the test protocols. The protection goals, when explicitly formulated, will make it easy to select (or develop) useful models. And subsequently, the useful models should guide the most efficient design for toxicity testing. At this moment, the linkage between these three components (protection goal, model selection and test protocols) is rather chaotic. Protection goals defined in risk assessment regulations are too vague to be of much guidance, models are often developed by modellers out of scientific interest, and existing test protocols are generally quite useless to support parameterisation of mechanistic models. For these reasons, environmental risk assessments needs a more ambitious agenda to redesign the effects assessment, and that requires an integrated evaluation of the three main issues. Model selection cannot be performed by only considering the ‘quality’ of the models. Instead of focussing too hard on model ‘quality’, we need to consider that the task of model selection (or development of novel models) is intricately linked to the regulatory protection goals and to the process of designing test protocols. In this contribution, I will provide several example cases to demonstrate how these linkages might work out. I hope that this contribution can help to streamline the fruitful integration of mechanistic modelling into a regulatory setting.

**342 Optimizing experimental design for calibration of GUTS models**

S. Charles, University Lyon; Laboratory of Biostudies and Evolutionary Biology; C. Albert, Eawag Swiss Federal Institute of Aquatic Science and Technology; B. Goussen, University of York / Environment Department; T. Jager, DEBtox Research / Dept of Theoretical Biology; S. Vogel, Eawag Swiss Federal Institute of Aquatic Science and Technology; R. Ashauer, University of York / Environment
The General Uniformed Threshold model of Survival (GUTS) provides a theoretical framework for analysing stressor effects on survival over time through consistent model equations based on different assumptions about the stressor quantification, the compensatory processes and the nature of the death process. In ecotoxicology, stressors are toxicants characterised by a dose metric, e.g. the concentration in the medium surrounding cells for some sets of species and compounds including quantity they cause. The key GUTS feature is that mortality is estimated when the dose metric exceeds a certain threshold. Several GUTS flavours can be derived according to the assumption underlying the death process: (i) the threshold is distributed within a population, and when exceeded, the individual dies (individual tolerance, IT); (ii) there is one common threshold for all individuals, and when exceeded, the probability to die increases (stochastic death, SD); (iii) a unification of both previous assumptions (GUTS proper). While more realistic, GUTS proper requires the estimation of one additional parameter. Because environmental risk assessment of chemicals depends on robust estimates of GUTS parameters, we investigated parameter identifiability, in relation to the experimental design of ‘short-term’ laboratory bioassays. In practice, standard survival datasets generally do not contain enough information to estimate all parameters of GUTS proper with sufficient precision. This is because a large number of individuals is required to provide strong information on probabilistic events. Hence, based on simulated datasets we identify appropriate experimental designs suitable to estimate all parameters of GUTS proper with the best possible precision. We show that datasets with a high number of animals per treatment allow for parameter estimation of GUTS proper with reasonable accuracy and precision. Moreover, increasing the number of animals or the duration of the experiment substantially reduce the uncertainty around the mean value of the threshold. It is recommended that statements about optimisation for any chemical, any species, any test duration and/or any exposure concentration profile remain difficult. As take-home message, to the extent possible, we recommend not to use fixed experimental set-up for GUTS analyses, but rather tailor dedicated designs according to the chemical, the species and/or the research/regulatory question at hand.

343 Validation status of TKTD models: predictions of survival after time-variable exposure to pesticides match observations, but not all details
A. Fucks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team
TK/TD models are supposed to deliver relevant information for environmental risk assessment of pesticides, especially for the extrapolation from toxicity observed under constant laboratory conditions to survival under time-variable exposure, as expected for realistic application scenarios. TK/TD models from the GUTS framework describe the expected survival for a number of individuals being exposed to chemical concentrations over time. These models are structurally simple and consist of three or four equations for all species, and species- and compound-specific information is captured by the model parameters. Standard acute toxicity test results are often the only data being available for model parameterisation and can be used to parameterize the individual tolerance and the stochastic death survival submodels, using scaled internal concentrations as dose metric. For an application in risk assessment, it is required to check the quality of model predictions, or, with other words, to validate parameterised models for specific combinations of compounds and species. This study presents validation results of TK/TD models given consistent statements about optimisation for any chemical, any species, any test duration and/or any exposure concentration profile remain difficult. As take-home message, to the extent possible, we recommend not to use fixed experimental set-up for GUTS analyses, but rather tailor dedicated designs according to the chemical, the species and/or the research/regulatory question at hand.

344 Exposure specific species sensitivity - a toxicokinetic-toxicodynamic approach
A. Ceres, gatiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental Social and Spatial Change; R. Ashauer, University of York / Environment; S. Augustine, Akvaplan-niva; A. Nyman, European Chemicals Agency; V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health and Safety; T. Preuss, Bayer CropScience / Environmental Modelling; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; K. Veltman, University of Michigan / Department of Environmental Health Sciences; E. Zimmer, IBACON GmbH
Currently in higher tier environmental risk assessment of chemicals, species sensitivity distributions (SSD) are used to describe and to quantify differences in sensitivity among species. When available for multiple species, normally observed-effect concentrations (NOECs) or ECx (representing x% of an affected population) usually derived from acute and chronic toxicity tests under constant exposure, are being used to calculate SSDs. In turn, these SSDs are used to extract ‘safe’ community effect thresholds in the form of an HC5, i.e. the concentration at which 5%) of the species are affected. In current ERA practice, the ratio of effect measures and exposure, such as the maximum predicted exposure concentration (PECmax) or the time weighted average (TWA), are being employed to characterize risk ignoring that, depending on environmental conditions, the exposure concentrations are highly variable in space and time. Mechanistic models, such as the General Unified Treshold model of Survival (GUTS), take the processes leading to an effect into account and can explicitly cope with time variable exposure. We present the outcome of a GUTS workshop held in March 2015 where species sensitivity distributions under time variable exposure have been studied. Our results point towards an unrecognized problem with the widely used SSDs and HC5 values: they could depend on the exposure pattern and differ a lot for exposures with the same time weighted average concentration but different temporal profiles. By using GUTS to analyze and compare species sensitivities, we can overcome the dependence of time and exposure patterns, and extrapolate toxicity to other exposure scenarios. Furthermore, we can develop a more thorough understanding of the underlying toxicity mechanisms by quantifying organism internal toxicity thresholds in conjunction with compensating processes.

345 How to map ecological risk assessments of chemicals
B. Olesen, University of York / Environment Department; C. Rendal, A. Franco, O. Price, Unilever / Safety and Environmental Assurance Centre SEAC; R. Ashauer, University of York / Environment
The lack of ecological realism is widely recognised as a limitation in current prospecitive chemical risk assessment, as is the failure of making variability explicit and transparent. The integration of ecological scenarios with chemical effect models to achieve quantitative ERA promises to increase ecological relevance. Probabilistic environmental risk assessment (PERA) has been suggested as a way to account for spatial, temporal, and environmental variability. Probabilistic plots area new way of presenting ecotoxicological data whilst accounting also for ecologically relevant parameters. They provide an indication of the maximum population-relevant impact of an effect of interest (e.g. biomass reduction) and the prevalence of this impact. Essentially they answer two related questions: How strong is the effect? In how many locations will we see the effect? We discuss some of the challenges and opportunities involved in bringing these new concepts into everyday risk assessment for down-the-drain chemicals. One of the key questions revolves around understanding the protection goal for anthropogenic stressors in specific ecological scenarios, and indeed whether certain scenarios require specific modified protection goals. Once the specific protection goal has been established, a metric to suit both the specific ecological scenario and protection goal needs to be defined and agreed. The selection of this endpoint must be carefully considered as different options will lead to different interpretation. We present a framework to integrate probabilistic approaches with mechanistic effect models to assess variable chemical and environmental scenarios. We present a hypothetical case study risk assessment for an ingredient used universally in all laundry products across Europe and illustrate the potential benefits of the framework. To do so, we use an individual based model integrating a dynamic energy budget model to assess the potential impact of chemicals associated with local environmental characteristics. We then map the outcomes based on probabilistic plots and on potential policy makers’ decisions of the maximal ecologically acceptable impact and the maximal prevalence of this impact. This new framework has the potential to better present ecologically relevant risk by using integrated biological endpoints and to aid more transparent risk communication.

Application of a coordinated OMICS research program and data into regulatory frameworks: case-studies and perspectives

346 SETAC Global Advisory Group OMICS: Cutting-edge science to solve real world problems
A. Biales, U.S. EPA / National Exposure Research Laboratory; B. Campos, IDAEA-CSIC / Environmental Chemistry; J. Colbourne, University of Birmingham / Bioscience for Life and Environmental Sciences; D. Vidal-Dorsch, VDA LCS / Toxicology
Governments around the world are passing new legislation to regulate chemical substances considering their potential impacts on both human and environmental health. These are critical and complex rules, designed to better ensure public...
Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToXLab unit

Over the last decade, molecular technologies have evolved into robust high throughput platforms yet implementation of these technologies in ecotoxicology and risk assessments remains difficult and is limited to small datasets. As a result, knowledge of stress responses and mechanisms of toxicity across stressors, particularly mixtures remains limited. Here, we report the use of high throughput microarrays to study 48 binary mixture combinations of cyanobacterial stress and insecticide contamination to *Daphnia*. We identified gene networks that correlated significantly with changes in reproduction in *D. pulex*. These gene networks were diverse in size and function, and were represented by a small subset of 10 core or hub genes. This suggests that (1) only a limited number of genes is driving expression patterns that correlate with reproduction at the organismal level and (2) these limited subset of genes can be divided into several independent gene networks. The results highlight the potential of network analysis in combination with high throughput molecular data for environmental risk assessment.

349 Developmental neurotoxicity assessment of chemical exposure in children: weight of evidence approach using in vivo behaviour, metabolomic and proteomic studies

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While a serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants (e.g. pesticides) during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC ’Developmental Neurotoxicity Assessment of Mixtures in Children’ investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. We focus on (subclinical) effects on learning (cognitive skills) and developmental disorders in children (e.g. ADHD, autism spectrum disorders and anxiety disorders). The aims are to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity, and to improve our understanding of chemical exposures and the observed effects.

350 Potential and Limitations of Comparative Genomics for Predicting Pharmacological Effects of Pharmaceuticals in Fish

L. Gnammas, University of Exeter / Biosciences; E. Kristiansson, Chalmers University of Technology / Department of Mathematical Statistics; T. Österlund, Chalmers University of Technology / Mathematical Sciences; S. Owen, AstraZeneca / Safety Health Environment; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; C. Tyler, Biosciences College of Life and Environmental Sciences

Identification of orthologous genes is an important step in the assessment of evolutionary conserved functions. Within the field of (eco)toxicology, information regarding orthologous relationship holds promise to predict conserved, chemical induced, mode of action (MoA) across species and thus help to guide the selection of appropriate test species for further testing. We have started to explore the potential and limitations of a systems approach by analysing orthologous relationships between human drug targets (466) and corresponding proteins in more than 40 vertebrate genomes, including 13 species of fish (11 teleosts). We have previously shown that ~ 85% of the human drug targets are conserved in fish. A growing number of publications comparing pharmacological effects and orthologous gene expression results across species in vitro models to internal outcome measures in vivo suggest that pharmacological effects in fish provide support for orthology detection as a strategy to inform extrapolations of effect data across species. It is more challenging however to identify species and taxa that are lacking orthologs and/or effects. To address this question we focused our predictions on the identification of drugs that are lacking pharmacological effects in fish. We have analysed predictions of orthologous relationships from two databases (Ensembl Compara and Inparanoid), which uses different strategies for the predictions. Only three of 976 drugs lack...
orthologs to all pharmacological targets in all fish studied. In the case of Amlolide, despite the absence of the sequence targets, published studies show it can induce specific, pharmacologically related effects in fish (inhibition of sodium transport), albeit at concentrations several orders of magnitude higher than in humans. This effect (in fish) is mediated via conserved off-targets. The example reflects the major need for development of effective TSC effects in API assessments. The results of the ortholog detection also predict that 84 drugs (46 targets) are lacking all pharmacological targets in at least one but not all species of fish. Additional data, supporting these potential gene losses were however available only for two targets. In conclusion, orthology detection holds good promise for identification of conserved chemokine Moa across species but the lack of predicted orthologous targets doesn't rule out biological effects for drugs, and thus findings from such an approach need to be interpreted with caution.

351 Toxicity testing and transcriptomics with Daphnia magna and application to Danube risk assessment in SOLUTIONS

T. Williams, University of Birmingham / School of Biosciences; M. Hockedy, The University of Birmingham / Biosciences; P. Antczak, University of Liverpool; T. Schulze, Helmholtz centre for environmental research - UFZ / EffectDirected Analysis; M.R. Viant, University of Birmingham / School of Biosciences; J.K. Colbourne, The University of Birmingham / Biosciences

The SOLUTIONS project aims to find new and improved tools, models, and methods to support decisions in European environmental and water policy. In addition to a wide selection of bioassays, transcriptomics assays are being performed using Daphnia. Crucially, the extensive chemical and ecological characterisation dataset from the 2013 sampling campaign can be used to demonstrate the possibility of linking chemical data to adverse ecological outcome via biological responses following the AOP paradigm Large volume solid phase extraction (LV-SPE) was used to concentrate organic extracts from 1000 litres of surface water at 25 sampling sites on the Danube during the Joint Danube Survey 3 (JDS3) project and SOLUTIONS. Acute toxicity of the Danube LV-SPE samples to Daphnia was tested experimentally by a modified version of OECD Test No. 202[3]. Neonates from sublethal exposures were collected and frozen at -80C prior to RNA preparation and transcriptomics. Transcriptomics employed a novel 8x60k *Daphnia magna* Agilent oligonucleotide microarray design. From chemical data, PAHs (benzo[a]pyrene, indeno(1.2.3-c.d)pyrene and fluoranthene) and the AChE inhibiting insecticide diazinon were predicted to pose the most risk of Daphnia acute toxicity, considering all Danube sites. Daphnia toxicity determined experimentally corresponded positively with predicted toxicity (r=0.49, P-value < 0.05), although several outliers were noted. At the Novi Sad site toxicity at the effluent was significantly higher (P<0.05) than immediately upstream. Daphnia transcriptomic responses to the Danube environmental extracts provide data to characterise the similarities and differences between sites of different pollution profiles. Although there was a positive correlation between Daphnia toxicity predicted from chemical concentrations and those determined experimentally, several outliers showed that additional factors influence toxicity. These are likely to include chemicals not detected or considered in the analysis, or for which there is insufficient Daphnia toxicity data, as well as interaction between components of the mixtures. Detailed transcriptomic investigation of Daphnia responses to the different environmental extracts, in combination with chemical, ecological and other bioassay data, have the potential to reveal candidate compounds associated with deviation of toxicity from that predicted by risk assessment.

352 Regulation of cosmetic ingredients under REACH: Is it necessary? - A case study with 3-BC and 4-MBC

P. Kalnes, Umweltbundesamt / Federal Agency of Environment / IV Chemicals Management. Cosmetic ingredients and cosmetic products can enter the environment during use and disposal. Special concern is arising from the group of commonly used UV filter substances in sunscreens and other cosmetic products since they enter surface water bodies directly via wash-off during use and without being processed within waste water treatment plants. The problem here is that the EU cosmetic regulation does not allow for higher tier testing using in vivo assays. Thus, PBT, vPvB and e.g. endocrine disruption, being the most critical hazards arising from chemicals from an environmental point of view, cannot be evaluated based on the data requirements under the cosmetic regulation. The presented case study should demonstrate, using the example of two camphor-based UV filter substances, how the identification of significant hazards and risk management can be addressed under REACH. In this context a research project dealing with the relevance of potentially endocrine disrupting substances for regulation the uses and findings of 3-BC and 4-MBC in the environment were evaluated. Furthermore, a sound evaluation of the SVHC properties of both camphor substances is being performed under REACH aiming at identifying high relevance UV filter chemicals as substances of very high concern for the environment. Further risk management option analysis (ROMO) is performed to identify the most adequate and effective risk reduction approach. Part of this evaluation is also a discussion of the substances within the ECHA expert groups (e.g. the Endocrine Disruptor Expert Group) and a public consultation of the outcome of the ROMO analysis and the SVHC identification. This means a transparent process for all stakeholder groups being involved. The REACH framework provides useful tools to evaluate and to manage cosmetic ingredients of environmental relevance. Especially the option to request higher tier in vivo data under the substance evaluation process within REACH, which is not possible under the EU cosmetic regulation, is of high relevance to clarify hazardous properties of very high concern (CMR, PBT, vPvB and e.g. endocrine disruption) of cosmetic ingredients. Thus, assessing cosmetic active ingredients under REACH can be an efficient approach to minimize their environmental and/or human health risks and be a potent driver for substituting critical chemicals in cosmetic consumer products.

353 UV-filters and musk from Personal care products in coastal regions : Seasonal and diurnal trend in mussels and seawater.

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UV-filters and musks fragrances have come into focus because these compounds are increasingly used in sunscreen products and in many products of daily use, such as cosmetics, skin care products or varnishes. The high possible dermato- and ecotoxic potential and the increasing interest due to their occurrence in the environment and to their potential to cause endocrine disruption. UV-filters have been reported in coastal regions mainly due to recreational bathing activities, including both sea water (Sankoda et al. 2015) and marine mussels (Bachelet et al. 2012). The highest concentrations of UV filters in mussels were reported for two substances, ethylhexylmethoxycinnamate (EHMC) and octocrylene (OC) and occurred in sites with a strong touristic frequention and a geomorphological conformation of the beach closed to. In this context, the objectives of this work were three folds: firstly, a monitoring of the seasonal variations and diurnal trends of these emerging contaminants was conducted during the touristic period. Secondly, the relation between the concentration in water and in mussels was studied within one day for a better understanding of the temporal trends. Finally, the contribution of WWTP releases on coastal contamination was investigated. The extraction of mussel tissues was based on a QuEChERS procedure (Picot-Groz et al. 2014). Sea water was extracted with semi-preparative extraction (SPE) method. Analysis was performed with liquid chromatography coupled to high resolution mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS). The developed analytical method allowed to detect target compounds at low levels under 10 ng/g in marine mussels and under 1 ng/l in sea water samples. A seasonal variation of OC and EHMC was observed with the highest concentrations in mussels reported in July while the strongest touristic pressure. The diurnal trend of UV filters was similar in sea water and in mussels. These results suggest a high bioconcentration potential in mussels, and probably a metabolism ability for these substances. This is in accordance with previous findings where mussels feed with contaminated products containing low UV-filer concentration (Gomez et al. 2012). One UV stabilizer (UVP) and one musk (galaxolide) occurred in mussels from a non protected area, indicating potential threat for protected marine areas. In addition, mussel tissues across protected areas are increasingly contaminated by UV-filters and musks from personal care products. Mussel tissues was based on a QuEChERS procedure (Picot-Groz et al. 2014). Sea water was extracted with semi-preparative extraction (SPE) method. Analysis was performed with liquid chromatography coupled to high resolution mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS). The developed analytical method allowed to detect target compounds at low levels under 10 ng/g in marine mussels and under 1 ng/l in sea water samples. A seasonal variation of OC and EHMC was observed with the highest concentrations in mussels reported in July while the strongest touristic pressure. The diurnal trend of UV filters was similar in sea water and in mussels. These results suggest a high bioconcentration potential in mussels, and probably a metabolism ability for these substances. This is in accordance with previous findings where mussels feed with contaminated products containing low UV-filer concentration (Gomez et al. 2012). One UV stabilizer (UVP) and one musk (galaxolide) occurred in mussels from a non protected area, indicating potential threat for protected marine areas. In addition, mussel tissues across protected areas are increasingly contaminated by UV-filters and musks from personal care products.
(549 ng/g dw in Aulacomya ater, “choro”). In the samples from Brazil, BP1 in the three fish tissues and OC in liver and gills were the predominant and most accumulated compounds. The occurrence of the metabolites, BP1, 4HB and 4DHB in the samples suggest that metabolites also accumulate in fish. Results revealed that UV filters were detected in all samples. Some of them are endocrine disrupting compounds that may cause negative effects on the reproductive function and development of these species which may lead to their extinction and/or to affect humans through ingestion. Metabolites may be both excreted and bioaccumulated in the organisms. Thus, for a reliable ERA, metabolites should be considered. In References [1] Nieto A., Borrell F., Marcé RM., Pocurull E., J. Chromatogr. A 1216 (2009) 5619 [2] Bester K. J., Chromatogr. A 1216 (2009) 4703 [3] Gago-Ferrero P., Díaz-Cruz, M.S., Barceló, D. J. Chromatogr. A 84 (2013) 93 ‘in Acknowledgement - The authors thank Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”).’ We also thank Sylvia Nogueira and Mariana Alonso for the provision of the Brazilian samples.

355

Eco-SUN for Eco-design of Sunscreen Using Titanium dioxide Nanoparticles
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Among cosmetics and personal care products, sunscreen products are of emerging concern due to their environmental and health. The fate and impact of mineral nanoparticulate UV-blockers, such as TiO₂, nanomaterials, is under consideration from a regulatory perspective due to their potential impact. Once leaving the skin either through bathing or everyday usage and cleaning, the nanomaterials contained in the sunscreen can be released into rivers, lakes, sea shores, and/or sewage treatment plants. The nanomaterial behaviour, fate and impact in these different systems is largely determined by its surface properties, (e.g. the nanomaterial coating type) and lifetime. Here we present the first result of the Eco-SUN research program aimed at developing the eco-design of sunscreens through the minimization of risks associated with nanomaterials incorporated into the formulation. Different stages of the cream lifecycle are considered from its manufacture to its end of life, through its use by the consumer and its impact on the exposed environments. Reducing the potential release and/or toxicity of the nanomaterial from the cream is a decisive criterion for its eco-design. Different relevant TiO₂ UV-blockers have been selected to integrate a typical o/w formulation as case studies. The resulting sunscreen were characterised in terms of nanomaterial localisation, sun protection factor and photo-passivation. The risk for the consumer by dermal exposure was assessed using skin biopsies. Inflammation and skin penetration were evaluated. The risk for the aquatic environment directly exposed was assessed both in terms of exposure and hazard. The release of nanomaterials from the sunscreen upon normal use was studied in laboratory through simulated aging procedure. Two biological models, sea urchin and coral colonies, were selected as relevant endpoints to assess the marine ecotoxicity of the byproducts formed. Finally, the risk related to the end of life of the sunscreen through the removal with cleaning water followed by drainage to sewage treatment plants was evaluated. The following parameters were considered: (i) concentration in sewage sludge later spread as fertilizer in agriculture, and (ii) nanomaterial suspension maintained in the treated water and released in river water. Thus, fate and impact in soil and river ecosystems were also studied.

356

Benthic Invertebrate Exposure and Body Burden Analysis for eVMS Materials - A Probabilistic Risk Assessment Approach
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Suspected endocrine disrupting chemicals (EDCs) are now being evaluated by several regulatory authorities. A debate is in progress about whether or not EDCs can be adequately assessed by following the standard approach involving identification of intrinsic hazards, prediction of exposure and consequent calculation of risk, or if hazard alone should be used to decide if chemical registration can be permitted. Now that a number of mammalian, non-mammalian and in vitro test guidelines have been adopted by the Organisation for Economic Cooperation and Development (OECD) and the US Endocrine Disruptor Screening Program (EDSP) has generated significant data, the key challenge remains the integration of scientific evidence to support the hazard and risk assessment of chemicals with known or suspected endocrine activity. For this reason the SETAC-Pestell Workshop29Environmental Hazard and Risk Assessment Approaches for Endocrine-active Chemicals (EHRA) will be held from the 31st of January to the 5th of February 2016. Through the development of NOEC distribution datasets for the eVMS materials. The use of Monte-Carlo analysis yielded descriptions of the probability of overlap of exposure and chronic NOEC, i.e., a quantitative description of the risk posed by these materials to benthic invertebrate organisms/populations. Using both sediment OC-based and lipid tissue-based analyses, the cumulative probability of exceedance of chronic NOEC levels for tested benthic invertebrate species is

357

Towards A New Method for Performing Environmental Risk of Complex Substances (CRANCS).
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Complex mixtures pose a significant problem for environmental risk assessors in Europe as guidance is unclear how to deal with input data. Aquatic ecotoxicity data can be generated using traditional acute tests, but derivation of PNECs in other compartments is not simple. Complex mixtures deposited into receiving water will differ in partitioning between the aquatic and sediment compartments, leading to a different constituent profile from the original mixture. The objective of the “Compartmentalised Risk Assessment for Natural Complex Substances” (CRANCS) project is to develop a predictive method for environmental risk assessment in aquatic medium for Complex Substances, notably essential oils. Concentrations of the constituents of known mixtures will be spiked to characterised sediment-water systems and measured concentrations of each constituent will be compared to those calculated using standard partitioning methods. Alternative partitioning calculations may be developed to quantify the partitioning of constituents between aqueous phase and sediment phase. This information will be used to define the PCaAquatic and PCsediment. It is expected that the pelagic and sediment compartments will contain different mixtures (sub-mixtures) from each other and from the original complex substance. The spiked compartments will be tested for the influence these mixtures have on ecotoxicity, and a risk assessment will be carried out in both compartments: pelagic (water) versus benthic (sediments) at equilibrium using the PECs and acute and chronic aquatic toxicity data. The results will be compared to approaches currently used for estimating risk based on the original complex substance. The experimental phase will be performed on two complex mixtures (one prepared specifically for this study, containing not readily biodegradable stable substances and a second one on a known NCS). The modellers will simulate the results in silico using the WAF mixture calculation on each sub-mixture (pelagic and sediment). An attempt will be made to calculate the sediment WAF validated by the experimental results. Ultimately it is hoped that the model can be used in future risk assessments to predict the appropriate kind of ECR for both the water and sediment phases. This would significantly reduce the amount of experimentation necessary and should also be considerably more precise than the current risk assessment methods available for complex substances.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (I)

358

SETAC Pestell Workshop29Environmental hazard and risk assessment approaches for endocrine-active chemicals (EHRA): Developing technical guidance based on case studies to support decision making.
I. Sutherland, Department for Environmental Food & Rural Affairs / Chemicals Emerging Technologies; A. Leopold, Independent Consultant/ Researcher / Wildlife International Ltd.; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; M. Galay-Burgos, ECETOC; P.D. Guiney, Retired- S C Johnson & Son, Inc / Molecular and Environmental Toxicology Center; H. Holbech, University of Southern Denmark / Department of Biology; T. Iguchi, National Institutes of Basic Biology; K.A. Kidd, University of New Brunswick; A. Kumar, CSIRO / Center for Environmental Contaminants Research; P. Mathiesen, Independent Consultant; E.M. Mihaić, ERIC; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; B.W. Vigon, SETAC; L. Welte, BASF SE / Crop Protection Ecology; J. Wheeler, Dow AgroSciences

In many cases the chemical composition of an endocrine disrupting chemical (EDC) is not well enough characterised to be able to estimate the dose available to each organ. This is especially true when the chemical is a mixture of compounds. The aim of the workshop is to develop an approach to estimating exposure and deriving the most adequate and effective risk reduction measures for a mixture of EDCs, taking into account uncertainties in the data. The integrative approach is based on the use of the SAFER methodology once the names of the EDC or EDC mixture have been determined. In a first session (9:30 – 11:30) a discussion of the methodology and an update of the COST Action 789, “choro” will be presented. In a second session (11:30 – 13:00) case studies based on EDC mixtures will be presented. In a third session (13:00 – 15:00) an overview of the scientific basis of the SAFER methodology will be given. In a fourth session (15:00 – 17:00) a discussion of the use of model-based hazard assessment methods will be presented.
case studies it aims to develop tools that can be used by regulators and other decision makers involved in the assessment of chemicals with endocrine activity. 50 carefully selected scientists from a range of disciplines are working in case study groups each evaluating one of six chemicals known or suspected to have endocrine activity: 17 alpha ethylhexanol, dibromobenzene, perchlorate, propylene glycol, nickel oxide and tributyltin. For chemical approximately 150 publications and publicly available regulatory data have been selected for detailed evaluation. Following a Weight of Evidence assessment focused on endocrine activity, the main environmental hazards will be quantified looking at relevant taxa. Risk assessments for both endocrine-active and non-endocrine-active endpoints will be conducted. While going through this process the case study groups are asked to focus on a number of issues that cut across the different case studies and reflect the challenges that are faced when determining hazard and/or risk of endocrine active substances (EAS). The primary output of the workshop is the Synthesis Document which will be a technical, scientific guidance document produced by the case study groups to support decision making in environmental hazard and risk assessment approaches for EAS. The presentation will discuss objectives, approach, output and regulatory impact of the workshop.

359 Endocrine disruptors - Regulatory decision making process at ECHA’s Member State Committee C. Ajao, ECHA-European Chemicals Agency; L. Wollenberger, European Chemicals Agency; K. Tyle, DK EPA / Chemicals; W. de Wolf, European Chemical Agency REACH is an EU Regulation that requires ‘standard information requirements’ to be provided by manufacturers and importers of industrial chemicals in the EU. Under dossier evaluation, carried out by ECHA, the information in registration dossier is compared with the standard information requirements found in Annexes VI to X of REACH. Further information may be requested if data gaps relevant for the safe use of the substance were identified. On the other hand, under substance evaluation, were Member State competent Authorities (MSCA) carry out an evaluation of a substance prioritised based on risk concerns and listed on the Community Rolling Action Plan (CoRAP), information that goes beyond the standard information requirements of REACH can be requested. Depending on the concern, more sophisticated experimental data or information on exposure or hazard (e.g. information related to Mode of Action like in relation to endocrine disruption or genotoxicity) may be requested for the priority substances. Some tests targeted for endocrine disruption are considered non-standard information requirements (e.g. the fish Sexual Development Test or the Uterotrophic Assay in rats). ECHA’s Member State Committee (MSC) is responsible under REACH for reviewing divergent views among Member States and ECHA as regards the information Registrants and Downstream Users are required to generate to show that their substance can be used safely without harm to man or the environment. Furthermore, MSC can identify substances of very high concern (SVHC) for instance on the basis of CMR or BPT/PvP properties. Endocrine disruptors may be identified on a case-by-case basis as SVHCs, where there is scientific evidence of probable serious effects to human health or the environment, which gives rise to equivalent level of concern to CMR or BPT/PvP substances under Article 57 (f) of REACH. Two case studies to exemplify MSC’s considerations and decision making process will be selected: one from a substance evaluation (benzophenone (BP)) and another from a SVHC identification (Bis(2-ethylhexyl)phthalate (DEHP)). The discussions for each case study at the MSC based on the lines of evidence will be presented explaining the reasoning for the change in the proposed testing strategy for the BP substance evaluation case and for the identification of DEHP as an SVHC with endocrine disruptive properties towards wildlife.

360 Identification of environmental endocrine disrupting chemicals under REACH - Experience and challenges S. Franke, Federal Environmental Agency, Germany; J. Arning, German Environment Agency, Germany Horizontally harmonized criteria for the identification of endocrine disrupting chemicals (ED) are still missing at the EU level. While such criteria are urgently needed to regulate ED substances under the plant protection product and biocidal product regulation (PPPR and BPR), under REACH the identification and regulation of ED is already possible without having harmonized criteria. This is due to the fact that under REACH the identification of an ED can be treated separately from the following risk management, which is not the case for plant protection products and biocides. The work presented here will reflect our experience with the identification of ED in the environment under REACH and tries to work out why from an environmental point of view the identification of ED differs from the approach used for human health risk assessment. The resulting problems concerning the design of harmonized identification criteria are also addressed. The differences and the commonalities of the PPPR and BPR regarding the identification and its consequences are summarised. The process under REACH is discussed in detail and using examples from already identified EDs, representative requirements of this process should be fulfilled without having horizontal criteria for ED identification. It is generally agreed that there are many uncertainties in carrying out a risk assessment of ED. Many are not specific to EDs but some factors may be particularly relevant to some ED. Endocrine Disruptors may cause adverse effects in a variety of species including very different taxa. With respect to this we have the following position: It is at the moment impossible to define which species and endpoints represents the best wildlife with regard to endocrine disruption effects. The available test methods are very limited and especially with regard to invertebrates do not cover sensitive taxa and life stages. It may be possible to overcome these shortcomings in future but based on the already available indications of harmful effects in the environment, it seems not to be adequate to wait this progress. The experience think that the definitions should be based on WHO/IPCS definition and should consider that uncertainties are high for ED with regard to environment. This should result in a hazard based regulation but regulatory consequences for different legislation need to be taken into account (i.e. exemptions needed for plant protection products and biocides).

361 What, oh, what, is the mode of action? Use of the acute to chronic ratio in a weight of evidence for endocrine activity F.M. Mihalcé, ER2; K.K. Craddy, The Dow Chemical Company / Toxicology Environmental Research and Consulting; J. Wolz, Currenta; R. Werner, Covestro Germany AG; P. Stoldt, EnviGo CRS Switzerland Ltd Extensive discussion is occurring in the European Union and internationally on how to identify and potentially categorize endocrine disruptors (ED). There is general agreement that the evaluation of ED should be based on the WHO/IPCS (2002) definition. According to WHO/IPCS: “An endocrine disruptor is an exogenous substance or mixture of substances which through any of their effects (including genotoxicity) may be requested for the priority substances. Some

362 Opportunities and challenges in using rodent data to evaluate ecological hazard of potential endocrine active substance C. Prosseg, M. Lampi, ExxonMobil Biomedical Sciences, Inc.; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division Inter-species extrapolation of toxicity data is a substantial challenge for environmental risk assessment. Due to practical limitation in the number of in vivo assays that can be conducted, it is therefore necessary to rely on regulatory guidelines. The regulatory guidelines commonly rely on few whole-animal studies. Additionally, ecotoxicological hazard assessments most often rely solely on aquatic species (fish and amphibians). This has led some to consider the use of rodent data to evaluate endocrine active compounds for terrestrial assessment. While this has advantages, including a better extrapolation to endemic mammalian species, evaluating such data in the context of endocrine disruption encompasses many challenges and unknowns. For example, macroscopic (e.g. routes of exposure and life histories) and microscopic (cellular receptors and pharmacokinetics) differences between rodents and other terrestrial vertebrates (including avian and reptilian species) potentially exist. Additionally, pharmacokinetics and dermal dermal uptake rates have shown to vary widely between vertebrate taxonomic groups (up to five orders of magnitude). Energetics will influence behavior, biochemical pathways and food intake, all of which may alter hazard potential. Furthermore, rodents in a laboratory will not be subject to the same seasonal influences, which greatly affect the endocrine system, and therefore may not exhibit similar exposure effects. Sensitivities find no read across to the mammalian species, while a read across focused on hazards to individuals, investigating endocrine disruption in the environment.
requires evaluating adverse effects at the population level. Here we further discuss some challenges and opportunities in extrapolating rodent data to adverse environmental effects at the population level. This discussion will inform future research and regulatory action when assessing ecological hazard assessment. While rodent data may be reasonable to evaluate endocrine disruption for terrestrial species, it is not universal, and factors such as exposure route, pharmacokinetics, and taxonomic differences in metabolic pathways must be considered when determining adverse population level effects in the environment.

363 Quality Standards for Nonylphenol and Octylphenol in sediment based on ecotoxicological studies

S. Valacchi, Water Research Institute - Italian National Research Council IRSA-CNRA; H. Clausen, Danish Environmental Protection Agency; M. Mazzoni, M. Rusconi, Water Research Institute - Italian National Research Council IRSA-CNRA; A. Roncan, Water Research Institute - Italian National Research Council IRSA-CNRA / Water Research Institute; R. Bettinetti, University of Insubria; S. Polessolo, Water Research Institute - Italian National Research Council IRSA-CNRA / Water Research Institute Nonylphenol (NP) and octylphenol (OP) are degradation products of alkylphenol ethers (APE), non-ionic surfactants employed for more than 40 years, which elicit endocrine disrupting effects on aquatic organisms. For this reason, since 2001, they have been included in the list of priority hazardous substances of the Water Framework Directive. Quality standards (QS) for different environmental compartments were set out, and NP, OP and APE have been subject to EU restrictive regulation on marketing and use since 2003. The significant decrease in NP and OP concentrations in water in the last years demonstrates the effectiveness of the control measures that the European Union has put in place to control these priority hazardous substances. However NP and OP concentrations in monitored sediments are often above QS for sediments raising doubts about the reliability of these QSs. In the framework of a European initiative for the harmonization and derivation of Environmental Quality Standards, Italy and Denmark collaborated to derive a robust and reliable QSsed for these two priority substances. Toxicological tests with Chromomus riparius, Lambriculus variegatus and Hyallela Azteca were carried out according to OECD and USEPA guidelines for both NP and OP. The test results and literature data were combined and a QSsed for NP and OP based on ecotoxicological studies were derived according to the Technical Guidance Document on deriving EQS (CIS-WFD Guidance n. 27). The estimated QSs for sediment, normalised to organic carbon content, were 37 and 25 mg/kg OC respectively for OP and for NP. These QSs for sediment, based on ecotoxicological studies, are about 100 higher the previously derived QSs which were calculated by Equilibrium Partition (EIQ) approach.

Advancements in life cycle impact assessment method development (I)

364 A first step towards the consideration of habitat fragmentation in LCA

P. Larrey-Lassalle, IRSTEA Montpellier / UMR ITAP ELSA; E. Loiseau, National Research Institute of Science and Technology for Environment and Agriculture - Istrea; E. Blanco-Perez, National Research Institute of Science and Technology for Environment and Agriculture - Istrea / UMR ITAP ELSA; S. Alleaume, National Research Institute of Science and Technology for Environment and Agriculture - Istrea / UMR TETIS; P. Roux, National Research Institute of Science and Technology for Environment and Agriculture / UMR ITAP ELSA; M. Lopez-Ferber, Ecole des Mines d’Alès; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - Istrea / UMR ITAP ELSA Fragmentation is commonly defined as a process in which “a large expanse of habitat is transformed into a number of smaller patches of smaller total area, isolated from each other by a matrix of habitats unlike the original”. Alongside climate change or pollution, habitat change and fragmentation was identified by the Millennium Ecosystem Assessment as one of the five direct drivers of biodiversity loss. Given this fact, it seems to be of paramount importance to include this driver in LCA methods. To this end, the last theoretical frameworks of land-use impact chains proposed to include fragmentation as a human intervention leading to biodiversity loss. Nonetheless, the UNEP-SETAC land-use group recently pointed out that habitat composition and configuration, including fragmentation, seemed to be under-represented in impact assessment models and should be considered, particularly when dealing with Species Area Relationships (SAR). A literature review on the ecology field was performed in order to define fragmentation and its relating environmental mechanism and to identify quantitative approaches that attempt to consider the impacts of fragmentation on biodiversity loss. Among these approaches, recent work on adapting the SAR in order to include fragmentation effects along with area loss seems to be promising. The Species Fragmented-Area Relationship (SFAR) uses the metapopulation capacity c to describe the degree of fragmentation of a landscape. This work proposes to adopt this method to consider fragmentation in LCA. First, we developed a routine procedure to derive quantitative values of c for different landscapes. Second, we applied the methodology to two contrasted Amazonian landscapes to test its feasibility and interest. The results show that fragmentation has important impacts on spatial configuration and should be included in LCA methods. However, one limitation of the SFAR is its relatively recent methodological development, and the fact that it has so far only been tested in an Amazonian ecosystem for birds, while a larger spatial and taxonomic coverage for a practical use in LCA is required. Therefore, it is important to assess the validity of the method in other contexts. In conclusion, this exploratory work aimed at assessing the feasibility of including one of the most comprehensive and operational ecological models for fragmentation into LCA. The next step will be to derive CFs for land-use from the SFAR in order to conclude on its relevance.

365 Advancing marine impact assessment within life cycle assessment (LCA)

F. Verones, NTNU / Department of Energy and Process Engineering; J.S. Woods, NTNU / Industrial Ecolgy R. Bettinetti, University of Insubria; K. Vethamathan, Department of Environmental Health Sciences; M. Huijbers, Radboud University Nijmegen / Department of Environmental Science; E.G. Hertwich, Yale University Assessment of ecosystem damage in life cycle assessment (LCA) has greatly progressed over recent years. However, marine ecosystems remain heavily underrepresented, despite their importance for anthropogenic activities, both as a source of resources and receptor of waste, meaning that impacts on marine ecosystems are often assessed negligible. We identified seven major drivers for marine biodiversity loss: climate change, seabed damage, ocean acidification, marine eutrophication, overexploitation, invasive species and plastic waste. Characterization factors associated to some of these impact categories have already been developed. However, (spatial) detailed and (geographical) taxonomic coverage remain limited. For other impact categories, such as marine plastic debris, no LCA approaches exist yet. Furthermore, others have only case–study-based LCA approaches, for example invasive species. For all of the seven drivers, models predict the distribution of impacts: these drivers are generally agreed that there are many uncertainties in carrying out a risk assessment of ED. Many are not specific to EDs but some factors may be underrepresented, despite their importance for anthropogenic interventions, both temporally and spatially, are available. We therefore review possible quantitative approaches for including, or refining the representation of, these seven impact categories in LCA. We find that it is possible to derive impact indicators for all considered drivers. However, the levels of detail, coverage and uncertainty of the factors may still vary. In addition, fate and exposure factors are more readily developable than effect factors. For plastic debris, for example, models are available that describe the transport of plastic waste from the land to the ocean and the subsequent accumulation procedure within the ocean itself. These approaches can be translated into a fate factor by assigning to each country the fraction of plastic that is ending up in one of the different accumulation zones in the ocean. Models to estimate the effects on biodiversity are less developed and variation in model sophistication is large. Whilst well-established species sensitivity distributions can, for example, be used for marine eutrophication, ocean acidification and particle sedimentation, models for indicating the effect of plastic debris on marine biodiversity are completely lacking. We recommend adapting available quantitative approaches to generate new and refine existing fate models, and to further development of effect factors based on species richness loss, analogous to potentially disappeared fractions of species.

366 Accounting for biodiversity impacts of seabed damage in life cycle assessment

J.S. Woods; F. Verones, NTNU / Department of Energy and Process Engineering; traditionally life cycle assessment (LCA) has mostly focused on estimating impacts on terrestrial and freshwater ecosystems. Due to limited coverage of marine-focused operational life cycle assessment (LCA) methodologies, impacts on marine ecosystems are often assessed negligible. Given the importance of the marine environment for human society, for example through provision of mineral and fish resources, and a desire for LCA to be a comprehensive environmental assessment tool, development of novel marine-focused LCA methodologies is essential. Marine biodiversity is a priority of the Global Biodiversity Framework for the next 20 years. Marine biodiversity loss in marine ecosystems, one of the neglected categories is seabed damage. Some first steps in methodological development for this impact category have been undertaken in the LCA community. However, limitations remained, particularly because of limited consideration for biodiversity-specific impacts. We established a generalised approach for seabed damage assessment. This impact pathway applies for three main modes of impact: 1) abrasion (e.g. benthic trawl fisheries), 2) smothering (e.g. deposition of dredged material, cutting pile formation due to offshore oil and gas production) and 3) extraction (e.g. aggregate dredging). We developed characterisation factors (CFs) using a modelling approach developed by the University of Michigan and life cycle impact assessment (LCA) methods. As such, the overall impact of seabed damage is a function of disturbance characteristics (scale and intensity), initial response of the benthic community, duration of the anthropogenic activity and subsequent physical and ecological recovery of the seabed. Our CFs allow for quantification of temporary impacts in terms of potentially disappeared fraction of species (PDF) due to occupation (PDF(year)-1) and transformation (PDF(m-2.yr-1)) of the seabed as well as for quantification of permanent impacts (PDF.m-2). If a complete recovery is not
expected. The CFs are mode of impact specific and spatially differentiated according to the large marine ecosystem (LME) biogeo graphical system and seabed substrate. Given that most anthropogenic seabed damage occurs within continental shelf areas, our developed methodology is applicable for the global extent of the 66 LME units, which covers all continental shelf areas globally. For the first time we are able to account for biodiversity impacts of seabed damaging activities within LCA on a global scale (LME) and at endpoint level.

367 Habitat suitability: water use impact assessment for ecosystems beyond counting species

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Assessment of water-use impacts on ecosystem quality in LCA is a relatively recent research domain and still undergoing continuous development. The most recent approaches develop characterization factors by differentiating fate, exposure, and effect factors for distinct water sources and ecosystems. Even though, there is no consensus about what are the midpoint and endpoint indicators to be chosen to better represent impacts on the Ecosystem Quality area of protection. Current methods are based on describing ecosystem quality by means of biodiversity loss indicators. If choosing such type of endpoint allows a straightforward representation of results, they still carry a lot of uncertainty due to difficulties in isolating direct and indirect effects from the analysis, for example in support of environmental improvements. Within the work done in support to PEF/OEF, the JRC has performed some studies and some tests in order to identify the most relevant issues to be considered in the interpretation phase. The final aim of this work is to gain insights on cross-cutting issues affecting all the phases of a life cycle study and to provide guidance and support to practitioners and researchers in the interpretation phase. The presentation will show and discuss the results of some preliminary tests done within this framework including i. the influence to final results of the selection and the modeling assumptions in secondary inventory dataset; 2. sensitivity analysis of impact assessment modeling; 3. sensitivity analysis of normalisation, including uncertainty analysis; and 4. sensitivity analysis of weighting.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (I)

370 Detecting POPs profiles across the Atlantic Ocean using polyethylene samplers

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Little is known of the distribution of persistent organic pollutants (POPs) in the deep ocean. Polyethylene passive samplers were used to detect the vertical distribution of truly dissolved POPs at two sites in the Atlantic Ocean. Samplers were deployed at five depths covering 26-2535 m in the northern Atlantic and Tropical Atlantic, in approximately one year deployments. Samplers of different thickness were used to determine the state of equilibrium POPs reached in the passive samplers. Comparable sampling rates were obtained from model derived results (5 & 4.5 L/day) and performance reference compounds derived results (8.7 & 4.9 L/day). Concentrations of POPs detected in the North Atlantic near the surface (e.g. ΣPCB, 0.84 pg L⁻¹) were similar to previous measurements. Currents seemed more important in moving POPs to deeper waters than the biological pump. The ratio of PCB concentrations in near surface waters (excluding PCB-28) between the two sites was inversely correlated with concentration in the water column. This is the first measurement of POPs in the Atlantic Ocean (4.8-26 % of the global HCB environmental burdens), contrasting traditional beliefs that POPs do not reach the deep ocean.

371 Vertical distribution of organic contaminants in harbour waters assessed by passive sampling

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Coastal and estuarine areas receive contaminants from wastewater discharges and littoral activities such as boating, sea trade and fisheries as well as from upstream inputs through riverine discharges and soil washout. Assessing the distribution of these contaminants in water bodies provides insights for the understanding of their sources and fate, hence allowing a better management of our waters. In particular, a vertical profile of pollutants can indicate sources originating from the water
surface, freshwater-derived material if correlated to salinity or surface processes such as photodegradation or evaporation at the air-sea interface. For this purpose, passive sampling is a method of choice for the monitoring of moderately hydrophobic organic contaminants at marine trace levels as they are able to concentrate the analytes in-situ, thus providing limits of detection that can be as low as the pg L⁻¹. In this study, vertical distribution of pristine contaminants were assessed in an English marine from the surface to more than 3 m depth. Polyethylene strips were held vertically and immersed for 18 days from floating pontoons. Following deployment, the strips were sliced to provide 46 depth related sub-samples. A section of approximately 1 cm at the surface allowed profiling of the sea-surface microlayer. Time-weighted average concentrations were calculated for Polycyclic Aromatic Hydrocarbons (PAH) by using passive sampling and the performance and reference compound approach. As an example, results revealed a surface enrichment for phenanthrene and chrysene with a 5-fold increase in the first 30 cm, whilst fluoranthene and pyrene exhibited decreasing concentrations closer to the surface. Time-weighted average concentrations for the PAHs indicated an oil-derived contamination at the surface with enhanced combustion derived PAH concentrations at lower depths. Measurements were further extended to selected antifouling compounds, organophosphorous flame retardants and personal care products. The vertical profiles of detected compounds exhibited comparable distribution to the model PAH’s which aided in the interpretation of their relative sources and fates. This study demonstrates that passive sampling can provide a cost-effective and novel monitoring tool to investigate the sources and fate of contaminants whilst providing important information on compound dependent vertical profiling in contaminated waters.

372 Two activities on passive sampling in water and sediment in the North Atlantic within the OSPAR/ICES community
F. Smedes, Masaryk University; RECETOX Research centre for toxic compounds in the environment; T. van der Zande, Wetsus; Laboratory; H. Bohlań, TNO; Environmental chemistry and sustainable development. Masaryk University; RECETOX Research Centre for Toxic Compounds in the Environment; D. Vethaak, DELTARES; Marine and Coastal systems; I. Davies, Marine Science, Marine Laboratory.

OSPAR Convention is the current framework for international cooperation on environmental protection in the North-East Atlantic and has installed a programme for baseline monitoring of environmental status, covering both the spatial distribution of contaminants and temporal trends. Generally, OSPAR countries monitor chemical contaminants in sediment and/or biota. Both matrices suffer from compositional heterogeneity throughout the OSPAR. To reduce sample variability OSPAR is considering the inclusion of passive sampling in its monitoring programmes, and a number of preparatory actions on passive sampling have been conducted within the OSPAR/ICES scientific community, including the sampling of water as well as sediment. In 2006, the OSPAR/ICES Passive Sampling Trial Survey (PSTS) was organised with 13 laboratories participating and samples from 50 stations distributed across the ICES area applying polymer sheet samplers and bottles with 10 µm silicon polymer coatings for sediment equilibrations. Participants exposed sheet samplers were exposed to water at each of the selected stations equilibrated sediments in the laboratory. Analysis of duplicate samples (sheets and bottles jby the participants and the central laboratory gave information on the between laboratory variability. The data provided by the central laboratory for all participants allowed to evaluate the data in a spatial perspective as well as over matrices. In another project (ICON/2008) sediment was collected at 13 stations between Iceland and the Mediterranean and equilibrated sheet samplers for 6 months. Sheets were analysed for PRCs, PCBs, PAHs, PBDEs, OCPs, alklyphosphates, and dioxins and dibenzofurans. Passive sampling proved to be extremely sensitive and was able to detect many compounds that were not detected by whole sediment analysis. The presentation will demonstrate the power of passive sampling to reveal spatial distributions over large areas.

373 Advancing in situ passive sampling techniques to quantify chemical activity gradients across the sediment-water and benthic boundary interfaces in large water bodies
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Passive sampling is increasingly recognized as a superior monitoring tool for nonpolar organic chemicals in water and sediment. Beyond monitoring, passive sampling offers opportunities to explore contaminant transport mechanisms in large water bodies; and the potential of these opportunities have yet to be fully explored. Recently, research has been focused on the development of simplified in-situ benthic flow-through flux chambers. In this study, organic membranes were coupled with both µm-thin low-density polyethylene (LDPE) films and silicone (PDMS) membranes as passive samplers for polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), offering a 2.5 cm vertical resolution of contaminant concentrations within and across the sediment-water interface and the benthic boundary layer. Passive samplers were designed in order to assess whether chemical equilibrium was reached during the 2 and 5 months exposure time. Passive samplers were also exposed in the water column above the sediment porewater profilers. Parallel deployment of different passive sampling materials proved to be a simple and effective tool to validate obtained passive sampling data. Passive sampling across the sediment-water interface using the newly developed sediment porewater profiler in combination with passive sampler deployment in the water column facilitated a vertical mapping of HOC contamination and allowed a thermodynamically based assessment of contaminant fluxes.

374 Determining the release of hydrophobic organic contaminants from sediment by in-situ benthic flow-through flux chambers

Contaminated sediment may act as a source of hydrophobic organic contaminants (HOCs) to water, such as in the Baltic Sea where high chemical activity ratios between pore water and bottom water for dioxins were observed, demonstrating a potential release of dioxins from sediment to the water column. Quantification of the sediment-water flux of HOCs is important to enable assessment of the risk associated with contaminated sediment. Currently used methods to estimate the flux of HOCs from sediment to water are either indirect or direct by the use of in-situ flux chambers. Existing in-situ flux chambers are closed with no water flow through the chamber. Oxygen is therefore consumed during the deployment and contaminants are released into the chamber and disturbed. This is a drawback of closed chambers since bioturbation may have a significant impact on the sediment-water flux. This study presents a novel in-situ benthic flow-through flux chamber for determination of the release of HOCs from sediment to water. The water in the chamber is kept at ambient oxygen concentration by pumping of bottom water through the chamber. The measured flux thereby includes effects due to bioturbation. Water is pumped through the chamber to oxygenate the water inside the chamber, and passes first a sorbent at the inlet to strip it of any contaminants. At the chamber outlet, a second sorbent captures contaminants that were released from the sediment during deployment. Three benthic flow-through flux chambers were deployed for 3 days in a contaminated Baltic Sea bay (south of Stockholm) for in-situ testing. The flux of PAHs and PCBs [ng m⁻² d⁻¹] was determined from the mass of compound quantified in the outlet sorbent, the area of the benthic chamber and the deployment period. 15 PAHs and 7 PCBs were analysed. Closed flux chambers were deployed in parallel and passive samplers were used to collect the freely dissolved fraction of PCBs and PAHs in bottom water and porewater (work in progress). The flux individual PAHs and PCB congeners from sediment to bottom water ranged between 60-2300 [ng m⁻² d⁻¹] and 6-170 [ng m⁻² d⁻¹], respectively. The low variance among the 3 replicate flux measurements supports the robustness of the in-situ flux measurements using the novel chamber design. The results will be further compared to flux estimates calculated from chemical activity ratios (pore water and bottom water) and with flux measurements from closed chambers deployed in parallel.

375 Application of POCIS and new mixed polymer passive sampler for monitoring organic contaminants in the river Saar and the outflow of a wastewater treatment plant
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A broad spectrum of organic contaminants is released into aquatic environments and affects the water quality. For monitoring of these aquatic contaminants, passive sampling is a promising tool with a number of advantages over grab sampling. The objectives of this study were (1) to determine the dissolved concentrations of organic contaminant in the river Saar and effluent of a wastewater treatment plant (WWTP) which discharges into the river and (2) to compare the performance of passive samplers. Two passive sampler types were deployed over two years (May-June 2015) at three locations along the river Saar, at the confluence of the rivers Saar and Mosel and in a WWTP effluent discharging into the river. One sampler was the POLAR ORGANIC CHEMICAL Integrative Sampler (POCIS), where Oasis HLB was sandwiched between two polypropylene (PES) membranes. The second sampler was the Mixed Polymer Sampler (MPS) made of Oasis HLB embedded in polydimethylsiloxane (PDMS) disks and enclosed in copper mesh to limit biofouling. PDMS is widely used for sampling neutral hydrophobic organic compounds in water due to its reversible sorption and high internal permeability. Compared to POCIS alone, the MPS has an increased affinity towards a wide range of polar contaminants with the added advantage that it makes handling of the powdery Oasis HLB easier. After five weeks deployment, targeted 46 organic contaminants of diverse log Kow=0.3 for atenolol to 6.25 for miconazole were analyzed directly via LC-MS/MS. The measured sampler concentrations were calculated back to time-weighted average water concentration using sampling rates for each compound which were determined in the separate concurrent analysis. Both samplers types showed good well and could detect a range of organic contaminants. Low concentrations (ng to sub µg
per litre) of 27 compounds were detected in both the POCIS and MPS extracts. This includes 6 compounds from the WFD priority list, which were detected at all sampling sites. For the POCIS, some of the compounds accumulated significantly in the PES membranes, which complicates interpretation of the results. The polymer block nature of the MPS made this particularly robust to handle during deployment and extraction. Future efforts are focused on applying the mixed polymer as a passive dosing phase, with a view to directly integrating passive sampling and dosing for measuring environmental mixture toxicity for polar and non-polar organic compounds.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (II)

376

Release of TiO2-NP from construction landfills
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After their use, a large fraction of nanomaterials (NMs) will be deposited in landfills, which are considered as terminal sinks for the NMs. However, experimental data and field measurements supporting this assumption are mostly lacking. Production volumes of NMs are still limited, but TiO2 particles are used in large quantities mainly as white pigments since many years. We, thus, used TiO2 particles as a proxy for other NMs, which may become relevant in the future, and investigated the release of TiO2 particles from a landfill dominated by construction materials. The pH and the temperature of the oxygen saturated runoff were ~7 mg/L and 12°C. Average discharge rates were a few m³/d but increased to more than 100 m³/d after heavy rain events. The total suspended solids (TSS) were mostly between 2–20 mg/L. One liter of runoff was filtered through 0.2 μm polycarbonate filters and acid digested using a microwave assisted acid digestion system. The digest was diluted 1:50 with DDW and Ti was measured on an ICP-OES. The Ti concentrations were ~10 μg/L, expect after one heavy rain event, when more than 150 μg/L Ti was measured in the runoff. This exceptionally high Ti concentration was paralleled with TSS content of more than 70 mg/L. For electron microscopy analysis, runoff was either filtered (0.4 μm) or the supernatant of centrifuged samples (5 min, 700 g) was directly centrifuged on TiO2 grids. About 1% of all particles (> 10'000) detected using automated electron microscopy was identified as TiO2 particles. The particle number of all particles and also of the TiO2 particles showed an exponential increase with decreasing particle diameter down to 0.5 μm, corresponding to the detection limit resulting from the applied microscope settings. More detailed analysis of individual particles from the supernatant in the electron microscope revealed individual TiO2 particles in the nano-range. Single particle ICP-MS analysis revealed a total number concentration of TiO2 particles (< 500 nm) in the order of 10⁸+ / m³. These results indicate that individual TiO2 particles are released from landfills. Although the amount of TiO2 particles released from landfills is currently orders of magnitudes smaller than the natural background of TiO2 in surface waters, the predicted increase in the use of NMs in building materials, consumer products, electronic devices etc. calls for a more detailed analysis of the release mechanisms of NMs from landfill sites.

377

Varieties of nano-textile exposure: comprehensive sequential aging of fabrics to sunlight, washing and landfills
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The potential for nanomaterials to be released from consumer goods is not in itself a new topic, but the basis of scientific understanding of released particles and the transformations they may undergo during the products life cycle (e.g. during storage, use and disposal) is often hampered by the narrow scope of many research endeavours. In this study, we expanded the completeness of characterization using multiple analytical methods. Additionally, studies of sequential aging of products representing multiple stages of the life cycle are scarce. In order to bridge these gaps for the release of nanomaterials from textiles, we conducted a comprehensive suite of studies which allowed us to support some overarching hypotheses for finding important mechanisms and parameters for particle transformations when still adhered to the fabric or when released. Laboratory prepared nano-enhanced fabrics were subjected to sequential combinations of sunlight irradiation, washing (in seven different detergent formulas). Characterization is divided into two groups: analysis of solutions (washing and landfill leachate) and analysis of the NP fraction that remained on the fabric. Analytical techniques included single particle spICP-MS and TEM of the released particles, fabric digestion and total metal analysis after each exposure scenario, and both SEM and XANES analysis of the textiles for the visual integrity and speciation of Ag, respectively. Some broad generalizations suggest that sunlight irradiation hinders further speciation upon washing (as suggested by XANES). More Ag is released from fabrics than Au suggesting additional chemical influences and while a size effect may also be in play, the capping agent plays the largest role. However, when release does occur, a large proportion of particles are released into the wash water with little to no alteration of the size compared to the primary particle size (as determined by sp-ICP-MS). As seen in our previous work, detergent chemistry plays a significant role in concentrations of Ag released, where those containing oxidizing agents clearly assist in release of Ag particles (but not Au NPs, again inferring the additional chemical reactions specific to Ag). This undertaking of a large matrix of variables makes us confident to make more overarching characterizations since trends can be measured across more variables than in any other nano-composite release study to date.

378

Fate and Bioaccumulation of Nanosilver in a Lake Ecosystem
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There is potential for the release of AgNP into the aquatic environment through discharges of municipal wastewater. AgNPs undergo dissolution in aerobic environments to release silver ion, which is toxic and has potential for bioaccumulation. The stability and transformations of AgNPs in the aquatic environment are affected by environmental factors, so the fate of AgNPs is expected to vary greatly in aquatic ecosystems. In this study, we conducted a comprehensive suite of studies which allowed us to study the released particles, fabric digestion and total metal analysis after each exposure from textiles, we conducted a comprehensive suite of studies which allowed us to study the released particles, fabric digestion and total metal analysis after each exposure, preliminary ICP-MS/ICP-AES data reported a concentration in roots of ~7 mg/L, and ~12 °C. Average discharge rates were a few m³/d but increased to more than 100 m³/d after heavy rain events. The total suspended solids (TSS) were mostly between 2–20 mg/L. One liter of runoff was filtered through 0.2 μm polycarbonate filters and acid digested using a microwave assisted acid digestion system. The digest was diluted 1:50 with DDW and Ti was measured on an ICP-OES. The Ti concentrations were ~10 μg/L, expect after one heavy rain event, when more than 150 μg/L Ti was measured in the runoff. This exceptionally high Ti concentration was paralleled with TSS content of more than 70 mg/L. For electron microscopy analysis, runoff was either filtered (0.4 μm) or the supernatant of centrifuged samples (5 min, 700 g) was directly centrifuged on TiO2 grids. About 1% of all particles (> 10'000) detected using automated electron microscopy was identified as TiO2 particles. The particle number of all particles and also of the TiO2 particles showed an exponential increase with decreasing particle diameter down to 0.5 μm, corresponding to the detection limit resulting from the applied microscope settings. More detailed analysis of individual particles from the supernatant in the electron microscope revealed individual TiO2 particles in the nano-range. Single particle ICP-MS analysis revealed a total number concentration of TiO2 particles (< 500 nm) in the order of 10⁸+ / m³. These results indicate that individual TiO2 particles are released from landfills. Although the amount of TiO2 particles released from landfills is currently orders of magnitudes smaller than the natural background of TiO2 in surface waters, the predicted increase in the use of NMs in building materials, consumer products, electronic devices etc. calls for a more detailed analysis of the release mechanisms of NMs from landfill sites.
μg/L. Titanium was found in most surface waters that are used for drinking water production. None of the fullerenes were detected in the other aqueous samples. In air, concentrations of nano-sized metals (PM 0.1) increase with traffic density, however only a few mass percent is present as nanoparticles. C_{60} was found at one location (5 pg/m^3).

380 Innovative combination of spectroscopic techniques to reveal nanoparticle fate in a crop plant
C. Lanao, CNRS / Ecolab Laboratoire déécologie fonctionnelle et environnemental; H. Gardel, CEA/ESRF / ID; R.J. Stein, Ruhr University Bochum / Plant Physiologie Department. B. Fayard, Novitom; E. Pouyet, J. Villanova, ESRF / ID; V. Magnin, ISTerre; N. Tercera, SOLEIL / LUCIA; S. Legros, CEA Grenoble / LITEN; S. Sorieul, CENBG / AIRFRA; G. Sarret, ISTerre / Geochemistry Nanotechnologies are developing fast and in a number of application fields like materials, sustainable development or plant-based products. These new applications also imply an increased dissemination of nanoparticles (NPs) in the environment. Still, their fate is largely unknown mainly because of the lack of adapted techniques to assess their behavior in complex matrices (soil, plant or animal). In this study, we report the use of complementary spectroscopic techniques to determine the fate of two different NPs (TiO_2 and Ag) in a crop plant: lettuce (Lactuca sativa). The behavior of NPs was elucidated by studying a crop plant using its micro-X-ray fluorescence (μXRF), their in situ speciation by X-ray absorption spectroscopy with a micro-focused beam (μXAS) or in full-field mode and their local quantification by micro-particle induced X-ray emission (μPIXE) and Rutherford backscattering spectroscopy (RBS). After a 7-day exposure, preliminary ICP-MS/ICP-AES data reported a concentration in roots about 6 times higher for Ti than for Ag. μXRF analysis of root cross-sections revealed that the high Ti value was mainly due to the formation of an envelope of Ti around the root. Indeed, metal concentration studied with a laterally resolved technique proved that Ag is more internalized in the roots than Ti and thus potentially more translocated to edible organs. This technique also highlighted the different behavior of those two NPs; Ti was detected mainly as hot spots in the tissue and Ag had a more diffuse and homogeneous signal. This difference was substantiated by XAS analysis. Ag in the root was detected under two oxidation states from 100% Ag^0 to 100% Ag^+ and in association with different ligands regardless of plant tissue. The predominant ligand identified by linear combination fitting was a thiol-containing molecule (glutathione), known to be implied in plant detoxification mechanisms. Inversely, Ti was detected unaltered with a TiO_2 anatase crystalline form. New information were also obtained on the effects of NP exposure on the ionome. Both NPs affected the ionome in a similar way, but on the case of Ti only for high concentrations. NPs reduced Cu, Zn and Mn concentrations in the parenchyma and vascular cylinder of the plants. All together and with an appropriate statistical analysis, those techniques permitted to deepen our knowledge about NP fate in plants and to obtain new and original data about their impact on plant ionome.

381 Assessing the Paris city contribution to CeO2 and TiO2 nanoparticles concentrations in the waters of the Seine River by spICPMS and FEG-SEM imaging
K. Song, Institut de Physique du Globe de Paris; Y. Siivy, IPGP - VAT FR79195753282; H. Pauwels, BRGM; A. Gelabert, L. Cordier, IPGP; G. Wille, X. Bourrat, BRGM; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; M.F. Benedetti, Université Paris Diderot. Engineered nanoparticles (ENPs) are of great interest because of their specific properties which make them useful especially in industrial area. For instance, cerium dioxide NPs (CeO_2NPs) can be found in the automobile domain and titanium dioxide NPs (TiO_2NPs) are used in cosmetics as an UV blocking filter. Nevertheless, these ENPs are expected to be found in the environment at modeled concentration of ppb or ppt levels (1). In order to detect those ENPs at relevant concentrations and among natural NPs, innovative analytical tools are required. A field investigation was done along the Seine River on May, 2015 from upstream to downstream Paris city. single particle Inductively Coupled Plasma-Mass Spectroscopy (spICPMS) (2) and Field Gun Emission - Scanning Electron Microscopy (FEG-SEM) are applied on the river water samples to detect and characterize CoO_NPs and TiO_2NPs. Moreover, three tributaries (Orge, Marne and Ourcq rivers) are studied in order to see if there is a contribution of these rivers on the Seine River. Particle number concentration in the Seine River increases from upstream to downstream of Paris city for Ce and Ti. Indeed, 500,000 Ce-bearing part mL^{-1} and up to 1,350,000 Ti-bearing part mL^{-1} are measured at Marnay-sur-Seine, 96 km upstream Paris city. The highest particle concentration was found in the Marne River, a tributary of the Seine River, with 1,400,000 part mL^{-1}. This has an immediate downstream the confluence between the Seine and the Marne rivers where the particle number concentration increases near Paris sampling point (1,400,000 part mL^{-1} for Ce and 4,700,000 part mL^{-1} for Ti). The next increase was observed near a water treatment plant (Bourgival) with 1,200,000, sucré-developmental or plant protection part mL^{-1}, after. Aragenteuil sampling point (11 km downstream of Paris city). FEG-SEM imaging shows that particles containing Ce and Ti can be found in the suspended matter of the Seine River water. The size of detected particles varies from 200 nm to 5 µm. To further investigate the origins of NPs, elemental ratios of Ce/La and Ti/Al also need to be studied. 1. Gottschalk et al. 2013; 2. Miranow et al. 2012

Wildlife ecotoxicology: from food chain exposure to population effects
N. Vallverdu-Coill, IREC; Wildlife Toxicology; C. Castaño, INIA National Institute for Agricultural and Food Research and Technology; F. Mougeot, C.S.I.C; M.E. Ortiz-Santalestra, J.J. López-Perea, IREC; J. Rodríguez-Estival, University of Calgary / Ecosystem and Public Health; J. Santiago-Moreno, INIA National Institute for Agricultural and Food Research and Technology; R. Mateo, U.C.L.M.-C.S.I.C. / Instituto de Investigacion en Recursos Cinegéticos Lead (Pb) poisoning by the ingestion of shot pellets, bullet fragments or fishing weights is a frequent cause of death in wild birds, but also have a wide range of sublethal effects. Here we report on the effects that a sublethal Pb exposure during the prelaying period have on the breeding performance of red-legged partridges (Alectoris rufa). We studied separately the effects of exposing males or females on egg properties, laying performance, reproductive success, levels of dietary antioxidants and carotenoid-based coloration. We also studied the effects of male exposure on sperm quality and the relationship between sperm quality, carotenoid-based ornaments and antioxidant levels. We show that the prelaying Pb exposure induced the production of heavier and larger eggs, heavier chicks and reduced hatching success when females, but not males, were exposed. Fecundation rate and other laying performance parameters were unaffected. In males, Pb exposure decreased acrosomal integrity and sperm motility, and increased sperm vigour, but did not affect sperm viability, concentration or overall progress. Moreover, clutch size was increased in pairs in which the male had been exposed to Pb in comparison to unexposed pairs. Pb exposure increased levels of circulating antioxidants in males, whereas the percentage of eye-ring area pigmented by carotenoids decreased in exposed females. Overall, the sublethal Pb doses used here did not induce spermatozoa death or infertility in males, but rather caused an increase in reproductive investment. Pb exposed females also exhibited increased investment in reproduction, laying larger and heavier eggs and chicks, but had reduced carotenoid-based coloration and hatching rate. Several sperm parameters showed positive relationships with carotenoid-based coloration and levels of antioxidants that were influenced by Pb exposure, suggesting that redder males may be more capable to preserve sperm from oxidative stress.

383 Mercury trends in seabird eggs from Pacific Canada, 1968-2012, and relationships with stable isotopes
K.H. Elliott, McGill University; J.E. Elliott, Environment Canada / Science Technology Branch Seabird eggs are used to monitor environmental mercury because they are relatively easy to collect and integrate a signal from the entire food web. Trends in mercury from seabird eggs, however, may represent variation in diet rather than variation in mercury availability, due to biomagnification, seabirds switching to feed at a higher trophic level will usually have higher mercury levels. We measured mercury concentrations in eggs from six seabird species in Pacific Canada during the period 1968-2012. In contrast to expectation, storm-petrels feeding partially on invertebrates had the highest mercury burden while herons feeding on large fish had the lowest mercury burden. Rather than correlating with trophic level (δ^{15}N), mercury levels correlated with δ^{2}S (R^2 = 0.86). For corromorts, the only group showing a significant temporal mercury trend, both mercury and δ^{2}S decreased over time. Sulfate-rich environments (high δ^{2}S) are occupied by sulfate-reducing bacteria that produce methylmercury, and we hypothesize that variation in mercury within and among seabirds is associated with variation in methylmercury production by sulfate-reducing bacteria at the base of the food web. Variation in mercury levels in seabirds across space and time were associated with the origin of sulfur in the diet.

384 Contamination of four ivory gull (Pagophila eburnea) colonies in Svalbard in link with their trophic behaviour
M. Lucía, H. Stroem, Norwegian Polar Institute; P. Bustamante, Université de La Rochelle / Littoral ENvironnement et Sociétés LIENSS; D. Herzke, Norwegian Institute for Air Research NILU; G.W. Gabrielsen, Norwegian Polar Institute The ivory gull is a high Arctic species that spends the entire year closely associated with sea ice. This species is considered endangered in most parts of its breeding range. Ivory gulls must indeed cope with not only the reduction of sea ice cover triggered by climate change but also increasing contaminant loads due to changes in global contaminant pathways and the release of previously stored pollutants from melting snow and ice. In this context, the main objective of this study was to assess the trophic behaviour of this species in five colonies located
Dynamics 
Quantitatively document the hazard of brodifacoum to non-target birds of prey. 

An exposed kestrel as being anemic, toxicity reference values (TRVs) were determined and plasma was frozen for clotting time assays. Birds were euthanized, tract, evidence of bleeding) and some microscopic hemorrhages were apparent at (Stringiformes), its toxicity in other raptors (Falconiformes) in controlled models are small mammals, a raptor the barn owl, and cadmium (Cd), a persistent 

Wildlife Research Center / USGS; J.J. López-Perea, Spanis Institute of Game and Development of Sublethal Thresholds and Toxicity Reference Values to Contaminants, as the Cd, affect the survival and the reproduction abilities of most of the PFASs, -nonachlor, PCB (polychlorobiphenyl) and PFOS, its diet. Contaminants, encompassing a number of organochlorines (OCs), brominated flame retardants (BFRs), and perfluorinated alkyl substances (PFASs), were determined in the blood (plasma and whole blood) of ivory gulls collected over several years. In parallel, carbon and nitrogen stable isotopes were determined in different tissues (feathers, plasma and red blood cells, or whole blood) to infer the trophic level (δ13N) and feeding habitat (δ15C) during both the breeding and the molting periods. The most quantitatively abundant contaminants found in the ivory gull were p,p-DDE (dichlorodiphenylchlorodiphenyl ether), 2PBC (polychlorobiphenyl) and PFOS (perfluorooctane sulfonate). These contaminants display changes in the diet between the breeding and the molting periods. The molting period was characterized by enriched δ13C and δ15N signatures which suggest a more oceanic foraging location and a higher trophic position. This study also highlighted the same feeding habitats and strategies amongst ivory gull’s breeding sites. The lack of differences in δ13C and δ15N signatures between Sciences and Rurideth, either that birds from Barentsya mainly come from the same migrating routes, or that they maintain the same diet independently of their wintering area. Several compounds including most of the PFASs, trans-nonachlor, cis-nonachlor, and BDE-28 demonstrated their biomagnification potential in the ivory gull food web. Overall, the levels of OCs, BFRs and PFASs did not suggest direct lethal exposure to these compounds but their potential synergetic or additive sublethal effects warrant continued monitoring.

Development of Sublethal Thresholds and Toxicity Reference Values to Examine the Risk of Brodifacoum to Raptors B.A. Ratter, USGS-Patuxent Wildlife Research Ctr / Beltsville Lab; K. Horak; J. Lankton, USGS-National Wildlife Health Center; R. Lazarus, USGS Patuxent Wildlife Research Center / USGS; J.J. López-Perea, Spanis Institute of Game and Wildlife Research / CSIC/ULMCM; T.G. Bean, University of Maryland / Department of Environmental Science and Technology. On a global scale, brodifacoum continues to be one of the most widely used anticoagulant rodenticides for control of vertebrate pest species. Its toxicity and risk to non-target raptorial and scavenging birds is well-documented. While numerous exposure and toxicity studies have been conducted in owls (Strigiformes), its toxicity in other raptors (Falconiformes) in controlled exposure studies is less-well described. American kestrels (Falco sparverius) were provided daily access to two 25 g meathalls (Nebraska Bird of Prey diet) containing vehicle (control) or brodifacoum at nominal concentrations of 0.3, 1 or 3 ppm wet weight for 7 days (N=5 birds/group). These nominal brodifacoum concentrations were analytically verified (90.3–101.0% recovery), and are similar or less than carcass and liver concentrations (i.e., ~3 μg/g and ~18 μg/g, respectively) found in target rodents following eradication operations. Un eaten food scraps were collected daily, kestrels were observed twice each day, and weighed and examined at various intervals. After 7 days of exposure, a jugular blood sample was drawn into a syringe containing sodium citrate. Hematocrit was determined and plasma was frozen for clotting time assays. Birds were euthanized, necropsied and various tissues were fixed in phosphate-buffered formalin for histopathological evaluation. Neither food consumption nor body weight differed significantly among groups. Over signs of intoxication (bruxis on featherless tract, evidence of bleeding) and some microscopic hemorrhages were apparent at discrete concentrations of 1 and 3 ppm. In all cases, hematocrit was reduced (P<0.05) by ingestion of 1 and 3 ppm brodifacoum, with some birds being classified as anemic (hematocrit < 30). Prothrombin time and Russell’s viper venom time were both prolonged (P<0.05) in all groups receiving brodifacoum. Using data on daily brodifacoum consumption and classification of an exposed kestrel as being anemic, toxicity reference values (TRVs) were generated. The dietary-based TRV at which 50% of exposed kestrels exhibited anemia was estimated to be 246 μg brodifacoum consumed/kg kestrel body weight-day and 2.62 mg brodifacoum consumed/kg kestrel body weight-week. These TRVs are below environmental concentrations that might be encountered by free-ranging raptors consuming rodent following an eradication effort, and quantitatively document the hazard of brodifacoum to non-target birds of prey.

Balance between ecological interactions and chemical exposure on food-chain dynamics V. Baudot, Université Bourgogne Franche-Comté; C. Fritsch, CNRS / UMR Chronoenvironnement; A. Perasso, Université de Franche Comté UMR CNRS; M. Banerjee, IIT Kanpur / Department of Mathematics and Statistics; F. Raoul, University of Franche-Comté / UMR UFCCNRS. In this study, we develop an ecotoxicological model based on a system of differential equations to predict patterns of concentration dynamics of a terrestrial one-prey one-predator food chain exposed to metal contaminants. Biological models are small mammals, a raptor the barn owl, and cadmium (Cd), a persistent contaminant. We use our model to test different exposure scenarios of the predator according to prey trophic position (herbivorous, carnivorous or omnivorous). Contaminants, are known to affect survival and reproduction of exposed individual prey and predator, and therefore change the classical predator-prey dynamics. Analytical results show a general pattern where cyclic dynamics are observed at low concentration of Cd in soil, and then, the increase of Cd stabilizes populations dynamics. When the concentration of Cd increases, the model may have two stable steady points meaning that a subtle change in the initial conditions (initial prey and predator densities) implies totally different steady states (predator-prey coexistence or extirpation of the predator). At higher contaminant concentration in soil, the biomagnification process extrapolates the predator population what releases the predation pressure on preys and allows their increase. Finally, at high concentration, both prey and predator species collapse. The comparison of several prey species differentiated by their diet from herbivorous to carnivorous, allows a different pattern of contamination pressure, and therefore change the dynamics of the entire food-chain. The results obtained from this food chain model illustrates the importance of cascades effects, that is indirect effect modulated by trophic relationships. For instance, the model reveals the occurrence and position of tipping point (bifurcation) where a subtle change in contaminations causes a sudden change in the ecological system. The exploration of the tipping point behaviors with a mathematical bifurcation analysis allows to measure the impact of a change in prey resources (bottom-up cascade effect) or in the apex predator abundance (top-down cascade effect). As a consequence, this simple food chain model can help to identify populations that are critical for transferring adverse effects of contaminants across trophic levels. The direct next step of this work is the incorporation of multi preys though the use of multi-species functional responses to model the prey-selection behavior of the top predator.

Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (II) BEEHAVE model evaluation according to Good Modelling Practice F. Stresiess, EPSA / Pesticides Unit; M. Arena, EPSA - European Food Safety Authority / Pesticides; J. Boesten, Altaira / ERA team; F. Hatjina, Division of Apiculture, Inst. of Animal Science, Hell. Agr. Org.DEMETER; A. Rortais, EPSA / Scientific Committee and Emerging Risks Unit Department of Risk Assessment; F. Sgoilstra, Dipartimento di Scienze Agrarie, Università di Bologna; R. Smith, School of Applied Sciences, University of Huddersfield; C. Snellings, EPSA - European Food Safety Authority / Pesticides Unit; C.J. Topping, Aarhus University / Department of Bioscience. Rapid scientific progress has been made in recent years in ecological modelling. Workshops such as Lemtow and MEMORISK have explored the use of ecological models in pesticide risk assessment. Industry has submitted models to address specific risk assessment questions. However, the lack of guidance on the evaluation of models has been a major hurdle for using them in a regulatory context. In order to facilitate the evaluation (and the development) of regulatory ecological models EPSA issued an opinion on good modelling practice [1] and GMP. The GMP approach was first applied in the EPSA PPR panel statement [2] to evaluate the model BEEHAVE [3], a comprehensive individual based model simulating honeybee colonies that was published in 2014 (http://beehave-model.net/). The BEEHAVE model fulfilled most of the criteria outlined in the GMP opinion [1]. It was concluded that the model is not yet usable in a regulatory context or to address the risk from multiple stressors at the landscape level. Recommendations for further developing the model in order to address these questions are, for example the development of a pesticide module, the introduction of ecological agents such as infectious agents, pests and predators and interactions between those agents and the landscape (including critical beekeeping practices, climate, weather and landscape characteristics). The supporting data and default parameter values should be further evaluated and justified. The PPR Panel recommends adopting the model as the basis for modelling the impact on honeybee colonies of pesticides and other stressors. Further development might require a different programming language, for example a standard object-oriented language.

The honeybee model BEEHAVE: current status and future developments V. Grimm, Helmholz centre for environmental research - UFZ / Department of Ecological Modeling; M.A. Becker, The University of Exeter / Environment Sustainability Institute; J. Horn, Helmholtz centre for environmental research - UFZ, P.J. Kennedy, J.C. Rumkee, J.L. Osborne, The University of Exeter / Environment Sustainability Institute. The honeybee model BEEHAVE was developed to explore the influence of various stressors on colony health and survival. The main stressors represented are limitations in forage, diseases transmitted by varroa mites, and pesticide-induced mortality of in-hive bees or foragers. An expert working group at EPSA evaluated in great detail the suitability of BEEHAVE for regulatory risk assessment. Because of this potential of BEEHAVE in BEEHAVE, we here summarize the state of the art regarding BEEHAVE and outline possible future developments. BEEHAVE was used to (1) explore the effects of forage gaps and (2) pesticide-induced mortality on colony survival. Forage gaps of two weeks in June or July are most detrimental. Increased mortality of adults affected colony survival.
Mechanistic effect models (MEMs) are useful tools for ecological risk assessment of chemicals. The most important recommendations of the SETAC MODELING workshop are that the models should be as realistic as possible for a specific risk assessment question, and the level of conservatism required for a specific risk assessment should be reached by designing appropriately conservative environmental and exposure scenarios. As for aquatic environments, phytoplankton algae have to be protected at the population level by considering their abundance/biomass in edge-of-field surface waters (EFSA AGD). Especially for the ecological recovery option (ERO), all relevant processes that determine population viability and the propagation of effects on the community-, ecosystem- and landscape-level must be considered. As defined by the EFSA AGD, the reference tier for algae are micro-/mesocosms including competition, predation and natural stressors. According to these requirements, aquatic algae should be modelled as populations within an ecosystem modelling framework. The main question to be addressed is the level of complexity which is needed for a realistic and tractable description of algal dynamics. In the present presentation, we will introduce an ecosystem model framework for quantifying responses to stress at different levels of ecological complexity for freshwater algae in standing waters. We use a complex biogeochemical lake model (StoLaM) to predict population dynamics of phytoplankton species under realistic field conditions taking into consideration growth rate inhibition as the toxic mode of action. StoLaM’s modular structure enables us to simulate a wide range of ecological scenarios from laboratory conditions (one microbial species, constant temperature and light conditions) to high ecosystem complexity with several competitors, trophic levels and dynamic physico-chemical and weather conditions. Our aim is to develop ecological scenarios of increasing ecosystem complexity using StoLaM, and to evaluate these scenarios under different exposure and climatic conditions with respect to the sensitivity of the phytoplankton.

393 Building improved in-vitro exposure assessment capability: Towards the development and implementation of enhanced QIVIVE tools
T. Goum, Unilever / Safety and Environmental Assurance Centre; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical Environmental Science; M.R. Embry, ILSI Health & Environmental Sciences Institute (HE Risk assessment is generally divided into human health risk assessment (HRA) and environmental risk assessment (ERA)). For instance, scientific research, policy discussions, and regulatory instruments for HRA and ERA are addressed by individuals with different sets of expertise and knowledge, oftentimes at different institutes, research organizations, and government agencies. Integration of HRA and ERA, however, could provide substantial benefits, particularly by providing a more efficient framework on which to address emerging problems and questions that have the potential to impact both the environment and human society. Society is facing a variety of challenges; growing concerns about the effects of multiple

SETAC Europe 26th Annual Meeting Abstract Book 89
stressors (both chemical and non-chemical); risks associated with exposure to complex mixtures; and demands to quantify local site-specific risks. Integration of HRA and ERA could thus lead to more scientifically sound assessments by combining cross-discipline expertise and data, lowering costs and reducing the time needed to improve quantification of risks. A key challenge, however, in advancing our understanding and between toxicological and chemical partitioning theory is the need for improved tools to measure and control the freely dissolved concentration of a chemical, particularly in an in vitro test system. Current practice typically relates results obtained from an in vitro test system to a nominal concentration, which acts as a surrogate in establishing exposure-response relationships. However, depending on the physicochemical properties of a chemical, substantial variability between the nominal and the freely dissolved concentration might exist. Characterizing uncertainty related to the difference between the nominal and freely dissolved concentration is necessary to expand our understanding of integrating hazard and exposure information.

278 BPA and the other OEDs that can then be compared against exposure intake rates for risk-based partitioning theory to examine differences in the dissolved chemical concentration and in vivo conditions.

279 There is a paucity of measured toxicity data compared to the large number of chemicals and endpoints required for chemical hazard assessment. Emerging alternative test methods are being developed and evaluated to reduce and eliminate unnecessary animal testing. Cell-free and cell-based in vitro systems are being developed and applied for hazard and risk assessment. For human health assessment nominal concentrations from these in vitro assays are commonly assumed to be equivalent to steady-state blood concentrations in vivo. These steady-state blood concentrations are then used to calculate oral equivalent doses (OEDs) that can then be compared against exposure intake rates for risk-based prioritization. Here we use in vitro mass balance models and equilibrium partitioning theory to examine differences in the dissolved chemical concentration corresponding to the assumed nominal (administered, unmeasured) chemical concentration from an in vitro assay for a range of chemical partitioning properties and in vitro assay conditions. We compare the in vitro concentrations to in vivo blood concentrations and illustrate how the data can be interpreted differently. Finally, we apply the chemical activity approach in the data analysis as a means to clarify data interpretation and translation across systems (i.e., in vitro to in vivo). Implications for hazard and risk-based ranking are discussed.

395 Bioavailability of organic micropollutants in cell-based biosassays
F.C. Fischer, Helmholtz Centre for Environmental Research - UFZ / Cell Toxicology; K. Gouza, Helmholtz Centre for Environmental Research - UFZ / Analytical Environmental Chemistry; A. Jahnke, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology; M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; B.I. Escher, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology

In vitro biosassays are generally complex heterogeneous systems, and differences in chemical distribution are recognized as important issues concerning in vitro bioavailability. The freely dissolved concentration is generally considered as responsible for biouptake and can be reduced by sorption to medium compartments, biomaterial and plate material as well as evaporation and degradation. Consequently, the nominal concentration is often not an adequate metric to describe exposure and characterize toxicity. Our approach is to apply and experimentally validate a full mass balance model to different cell-based biosassays covering various toxicity pathways and a selection of non-volatile priority chemicals. We will then use the model to link observed toxicity directly to mass balances and bioavailability of chemicals. Next, we will use equilibrium partitioning constants, with kinetic processes, such as degradation and diffusion into plate material excluded. We collected partition constants from the literature or used Quantitative Structure-Activity Relationships and other models for prediction. Bovine serum albumin and liposomes (membrane lipid bilayer vesicles) served as models for proteins and lipids in the test medium and cells. The protein and lipid concentrations in the medium and cells were measured. Chemicals with low hydrophobicity were predicted to be predominantly freely dissolved and thus bioavailable. With increasing hydrophobicity, the fraction of chemicals bound to proteins and lipids increased strongly, with fractions of freely dissolved chemicals <0.1% for chemicals with a log k

\[ k_{olv} \] > 6. These results demonstrate the potential challenge of evaluating the nominal concentration of toxicity, in particular for hydrophobic chemicals, whereas correcting effect concentrations by modelled freely dissolved and cellular concentrations can lead to higher significance and comparability of toxicity data. We aim to improve the accuracy of the model in the near future. For this purpose, partition constants for chemicals with diverse physicochemical properties to medium compartments and cells will be measured. We will optimize and generalize the biosassays to which the model will be applied and produce empirical data on medium composition and structure of the respective cell lines. Furthermore, time-dependent processes like diffusion in plate materials, degradation, cell-uptake and growth dilution will be considered.

396 Getting biotransformation kinetic parameters as a bonus out of bioaccumulation experiments
D. Kuo, City University of Hong Kong / Architecture and Civil Engineering; C.C. Chen, City University of Hong Kong / Department of Architecture and Civil Engineering

Biotransformation is one of the important processes that can influence the fate of contaminants in living organisms. To properly understand the contribution of biotransformation, the in vivo metabolite rate constant, k\textsubscript{M}, needs to be determined. Despite its importance, k\textsubscript{M} has remained one of the least understood (in terms of modelling) and one of the most hard-to-get parameters. Two major approaches are currently used and commonly used to determine k\textsubscript{M} by difference and by experiment. The difference approach derives k\textsubscript{M} by subtracting the contributions from growth (k\textsubscript{g}), egestion (k\textsubscript{e}), and out-going transport from the biota (i.e., k\textsubscript{t}) from the total depuration constant (k\textsubscript{d}). While this approach has been successfully applied, leading to the development of k\textsubscript{M} database (e.g., Arnot et al. 2008 ET&RC 27:341-351), it has the disadvantage of producing negative k\textsubscript{M} values. The experiment approach, while preferred in principle over the difference method, suffers from a lack of mathematical tools to properly delineate k\textsubscript{M} from the observable quantities. Furthermore, it is often complicated with experimental constraints intrinsic to standard bioaccumulation procedures or practices. We proposed a generic protocol, developed a set of mathematical models, and devised a scenario map to address the need for determining k\textsubscript{M} experimentally. Equations were constructed from first principle following the established first-order kinetic framework for different exposure and accumulation scenarios. The developed approach allows k\textsubscript{M} of the parent compound (PC) to be determined from the kinetic measurements of its metabolites (MBs). The protocol and the scenario map were applied to and successfully validated using data from studies where PC and MB kinetic data were reported. This study ended with recommendations on how bioaccumulation of contaminants in living organisms may be experimentally investigated to reduce resource, effort, and data-reduction complexity while getting accurate toxicokinetic properties of the contaminant. Our message is that k\textsubscript{M} can be easily determined as a “bonus” if bioaccumulation experiments and data reduction are done right.
The protein and lipid concentrations in the medium and cells were measured. Bioassays covering various toxicity pathways and a selection of non-volatile exposure-response relationships. However, depending on the physicochemical metric to describe exposure and characterize toxicity. Our approach is to apply degradation. Consequently, the nominal concentration is often not an adequate Centre for Environmental Research UFZ / Cell Toxicology blood concentrations and illustrate how the data can be interpreted differently. Transition partitioning theory to examine differences in the dissolved chemical concentration prioritization. Here we use in vitro mass balance models and equilibrium assessing nominal concentrations from these in vitro assays are commonly being developed and applied for hazard and risk assessment. For human health chemical-by-chemical basis has been successful. However, changes in emphasis -

399 Integration of Epigenomics in Systems Toxicology J. Navarro-Martin, Institute of Environmental Assessment and Water Research / Department ofEnvironmental Chemistry; E. Ortiz, M. Casado, L. Herrera-Nogareda, Institute of Environmental Assessment and Water Research IDAEA-CSIC / Department of Environmental Chemistry; L. Peñarrubia, J. Viñas, Universitat de Girona; R. Tauler, Institute of Environmental Assessment and Water Research IDAEA-CSIC; B. Pina, IDAEA-CSIC / Department of Environmental Chemistry Increasing evidence demonstrates that environmental insults occurring during early development may have life-long effects, indicating that epigenetic mechanisms could be involved. However, epigenetic approaches in ecotoxicology are still lacking. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining transcriptomics and epigenomics. Applying Next Generation Sequencing (NGS) technologies, we aimed to: 1) recognize target genes regulated by EDC exposures at the transcriptomic and epigenomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate transcriptomic and epigenomic platforms to identify whole genome epigenetic footprints characteristic of EDCs exposures. Due to its similarities in epigenetic regulatory machinery to mammals, the zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. In this study, we studied the differences in epigenetic mechanisms that could be involved in the development of the organism.

400 Genetic and epigenetic modifications following parental exposure to the herbicide diuron: The GIMEPEC project F. Alecha, IFREMER / Laboratory of Ecotoxicology; A. Baulenger, IFREMER / Laboratory of Ecotoxicology Laboratory of Genetics and Pathology of Marine Molluscs; E. Bachere, IFREMER / UMR IBPE; A. Benabdellouma, IFREMER / Laboratory of Genetics and Pathology of Marine Molluscs; J. Rouzel, IFREMER; R. Rondon Sallan, IFREMER / UMR IBPE; C. Cosseau, C. Granato, Université de Lille, UMR IBPE / Biologie et Genétique des Mollusques; L. Peñarrubia, J. Viñas, Universitat de Girona; R. Tauler, Institute of Environmental Assessment and Water Research IDAEA-CSIC / Department of Environmental Chemistry Increasing evidence demonstrates that environmental insults occurring during early development may have life-long effects, indicating that epigenetic mechanisms could be involved. However, epigenetic approaches in ecotoxicology are still lacking. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining transcriptomics and epigenomics. Applying Next Generation Sequencing (NGS) technologies, we aimed to: 1) recognize target genes regulated by EDC exposures at the transcriptomic and epigenomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate transcriptomic and epigenomic platforms to identify whole genome epigenetic footprints characteristic of EDCs exposures. Due to its similarities in epigenetic regulatory machinery to mammals, the zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. In this study, we studied the differences in epigenetic mechanisms that could be involved in the development of the organism.

401 Genetic and epigenetic modifications following parental exposure of the Pacific oyster, Crassostrea gigas, to the herbicide diuron: The GIMEPEC project F. Alecha, IFREMER / Laboratory of Ecotoxicology; A. Baulenger, IFREMER / Laboratory of Ecotoxicology Laboratory of Genetics and Pathology of Marine Molluscs; E. Bachere, IFREMER / UMR IBPE; A. Benabdellouma, IFREMER / Laboratory of Genetics and Pathology of Marine Molluscs; J. Rouzel, IFREMER; R. Rondon Sallan, IFREMER / UMR IBPE; C. Cosseau, C. Granato, Université de Lille, UMR IBPE / Biologie et Genétique des Mollusques; L. Peñarrubia, J. Viñas, Universitat de Girona; R. Tauler, Institute of Environmental Assessment and Water Research IDAEA-CSIC / Department of Environmental Chemistry Increasing evidence demonstrates that environmental insults occurring during early development may have life-long effects, indicating that epigenetic mechanisms could be involved. However, epigenetic approaches in ecotoxicology are still lacking. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining transcriptomics and epigenomics. Applying Next Generation Sequencing (NGS) technologies, we aimed to: 1) recognize target genes regulated by EDC exposures at the transcriptomic and epigenomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate transcriptomic and epigenomic platforms to identify whole genome epigenetic footprints characteristic of EDCs exposures. Due to its similarities in epigenetic regulatory machinery to mammals, the zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. In this study, we studied the differences in epigenetic mechanisms that could be involved in the development of the organism.

402 Differential DNA methylation following monoo2-ethylhexyl)phthalate and 5-azacytidine exposure in zebrafish. J. Kamstra, NMBU / BaSam. L. Bastos Sales, VU University Amsterdam / Institute for Environmental Studies IVM; P. Cenijn, Vrije Universiteit Amsterdam / Institute for Environmental Studies: M. van der Broeck, J. Konings, VU University Amsterdam / Institute for Environmental Studies IVM; M. Aleström, Norwegian University of Life Sciences / Basic sciences and aquatic medicine. J. Legler, Vrije Universiteit Amsterdam / Institute for Environmental Studies Recent rodent studies demonstrating environmentally induced transgenerational epigenetic effects linked to increased disease risk have drawn much attention. Some of those studies observed changes in the DNA methylene indicating that effects can be inherited via DNA methylation at high concentrations of these exposures. As these studies used high concentrations and some results cannot be replicated in follow up studies. Clearly, more research is needed to understand the basic mechanisms encompassing transgenerational epigenetics. Here, we used a transgenerational set up using zebrafish as a vertebrate model organism to investigate the acute, latent and transgenerational effects on gene expression and DNA methylation of early life exposure to the putative obesogenic phthalate metabolite mono(2-ethylhexyl)phthalate (MEHP) and the DNA methylation inhibitor 5-azacytidine (5AC). We exposed zebrafish from 0 to 6 days post fertilization (dpf) at concentrations, which did not cause embroyotoxicity (30 and 10 μM for MEHP and 5AC, respectively). We analyzed the expression of a panel of selected genes, performed global (hydroxymethylation analysis and genome wide DNA methylation analysis (reduction represented bisulfite sequencing) in F0 dpf larvae. Analysis of gene expression and global methylation in F0 larvae (15 dpf) and adults (brain, gonads) as well as F1 and F2 embryos is currently underway. In addition, we performed bisulifte sequencing on 10 differentially methylated regions (DMRs) discovered by the genome wide methylation analysis. Our results show a high number of genes (18 out of 41) were differentially expressed at 6 dpf, but only two of those genes were differentially expressed at 15 dpf following MEHP exposure. Subsequent analysis is ongoing. Genome wide DNA methylation analysis revealed 148 and 311 DMRs for MEHP and 5AC, respectively. The results reveal a considerable change in DNA methylation following exposures during early life in zebrafish to MEHP and 5AC at nontoxic concentrations. This study will provide new knowledge about transgenerational epigenetics in zebrafish.
Field evidence of reproduction impairment through sperm DNA damage in the fish nose (Chondrostoma nasus) in anthropized hydrosystems A. DEVAUX, INRA-CNRS / UMR LEHNA USC INRA IGH ENTP; S. BONY, INRA-CNRS; S. PLENET, LEHNA CNRS; P. SAGNES, ONEMA; S. SEGURA, LEHNA CNRS; R. SANTOS, CEFEE; M. NOVAK, LEHNA CNRS; A. GILLES, CNRS; J. OLIVIER, LEHNA CNRS

A clear trend for a worldwide decline of fish populations has been documented over the last decades in freshwater ecosystems. Among numerous potential causes, pollution pressure due to industrial, agricultural and urban activities is considered as one of the main factors responsible for this decline. Contaminants can affect fish reproduction through different mechanisms leading to endocrine disrupting effects (ED), i.e., modification of embryo and larval development. Previous studies have shown a link between the integrity of sperm DNA and the quality of offspring development in fish and invertebrates. Present work aims to explore this relationship in the field as a possible cause of the decline through reproduction impairment of a feral fish population. Mature males (Chondrostoma nasus) were caught during the breeding season in three locations (A, B and C) of the Rhône River basin and gametes were collected by stripping. Sampling locations were chosen according to the following gradient of contamination due to human activities on the watershed: A>B>C. Samples of a pool of collected oocytes were fertilized with the sperm of individual males and then incubated individually back to the lab to study embryo-larval development as well as sperm sample DNA integrity. Genetic analysis clearly showed the absence of a difference in genetic structure between the three studied fish populations from the Rhône basin. Sperm DNA integrity was significantly lower in males from station C compared to other males when sperm biochemical characteristics and fertilization rate remained globally unchanged whatever the station. Mortality and hatching rates measured at both hatch and at the end of yolk sac resorption stages followed the same trend than the sperm DNA damage, demonstrating an impact of river water quality on nase fitness through a loss of sperm DNA integrity. Since the level of both abnormalities and mortality measured in offspring of fish caught in the most contaminated area reached high values up to 15% and 80% respectively, the hypothesis of an observed nase decline in Rhône River stemming through selection forces can be put forward. Possible modifications in habitat and food resources threatened by river regulation and climate change, and immunosuppression triggered by high level of chemical pollution should be kept in mind for a valuable assessment of human activities consequences on aquatic ecosystem functioning.

Mechanistic links between molecular alterations and transgenerational effects of depleted uranium and radionuclides in Daphnia magna F. Alonsoz, D. Praire, F. Parison, C. Adam-Guillermin, IRSN; M. Triant, IRSN/PRP-EN/LCOC / PRPENSVSILCOC; J. Garnier-Laplace, IRSN / PRPENSVSIRIS

Understanding how toxic contaminants affect species at sub-cellular, histological, physiological, organism, population, levels of biological organization is a major research goal in both ecotoxicology and radiobiology. Mechanistic links among the different levels of living processes are necessary for the understanding of survival, growth and reproduction which are critical for population dynamics. Time scales at which such links are studied are rarely relevant for natural biota. With a small size and short life cycle, the cladoceran crustacean Daphnia magna is suitable for studying contaminants effects over several generations. Multigenerational toxicity studies are much more relevant to natural biota for which exposure can involve many successive generations. Multigenerational studies of toxic effects were conducted under controlled conditions in D. magna exposed to depleted uranium (U), americium-241 (Am-241) and cesium-137 (Cs-137), representing respectively a dominantly chemotoxic metal, an alpha internal contamination and a gamma external radiation. Results showed in all cases that toxic effects on physiology and life history (survival, body size, fecundity) increased in severity across generations. These observations demonstrated that measured effects in one generation might not be representative of toxicity in the following offspring generations, and ultimately of the population response. Reduction in somatic growth and reproduction induced by depleted U were analyzed using a DEBatox approach (dynamic energy budget applied to toxicology). Modelling results suggested that depleted U primarily affects assimilation. This metabolic mode of action was confirmed by measurements of assimilation reduction and observations of histological alteration of the digestive epithelium. However the mechanisms involved in the transgenerational increased effects remain unknown. Recent studies investigating DNA damage in daphnids exposed to depleted U and Cs-137 demonstrated that molecular alterations were accumulated in females over the course of exposure and transmitted to their progeny. Such alterations were interpreted as the underlying mechanism causing the increase in effect severity over generations and will be investigated, as a future perspective, during exposure to Am-241. DEBatox models considering the accumulation and transmission of genetic damage were used to analyze toxic effects of depleted U, Cs-137 and Am-241 in daphnids exposed over successive generations.

Evolution of Lymnaea stagnalis inbreeding depression under pesticide chronic exposure A. Duval, INRA Agrocampus Ouest; M. Collinet, M. Coke, INRA; M. Coutellec, INRA / Ecology and Ecosystem Health UMR INRA Agrocampus Ouest

Ecolical and genetic factors may modulate the toxicity of chemicals to natural populations. In particular, inbred populations may be more sensitive to pollutants than their outbred conspecific counterparts, due to high inbreeding coefficient, loss of genetic diversity, or increased genetic load. On the other hand, strong inbreeding, such as expected in highly selfing hermaphrodites, may have a positive effect on population response to chronic (multigeneration) exposure to toxicants. First, selfing may facilitate the response to the selection exerted by a toxicant, through a transmission of coadapted gene complexes more efficient than under random mating. This is due to the fact that selfing reduces effective recombination. Furthermore, evolutionary theory predicts that spontaneous deleterious mutations can be preferentially purged under selfing, whereas they are maintained for a longer time within outcrossing populations, as masked in heterozygotes. Thus, high and longlasting inbreeding may have positive effects on fitness, and possibly on the ability to face chronic environmental stress. We tested these hypotheses in the freshwater gastropod Lymnaea stagnalis, using a three-generation exposure to the pro-oxidant herbicide diquat. A total of 16 lines derived from a highly diverse genetic pool were either exposed to diquat at early stages or maintained under control conditions. Chemical exposure was crossed with the mating system, i.e., in each treatment (control, diquat), four lineages were forced to self-fertilize and four other lineages were allowed to outcross. After three generations, inbreeding depression, the fitness decrease of selfed progeny relative to the outcrossed one, was compared across treatments. Inbreeding depression increased under diquat exposure, reflecting an aggravation of the toxic effects under inbreeding. In other words, selfing did not improve the response to selection by diquat. However, selfed lines also showed a higher response to selection, especially under diquat exposure. Some inbred lines performed better than some outcrossed lines, suggesting that the response to selection might depend on lineage genetic composition. In terms of toxicity testing, the use of strains maintained for generations in the laboratory may be questionable, due to high inbreeding and deleterious genetic variability. To circumvent this drawback and improve ecological risk assessment, we recommend the use of at least two distinct original populations.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (II)

Thyroid disruption in zebrafish (Danio rerio) larvae: different molecular initiating events leading to impaired eye development and visual functions L.A. Baumann, University of Bern / Centre for Fish and Wildlife Health; A. Ros, University of Bern / Centre for Fish and Wildlife Health; K. Rehberger, University of Bern / FiWi; S.C. Neuhäus, University of Zurich / Institute of Molecular Life Sciences; H. Segner, University of Bern / Centre for Fish and Wildlife Health

The vertebrate thyroid hormone system is important for multiple developmental processes, including eye development. Thus, its environmentally induced disruption may impact important fitness-related parameters like visual capacities and behavior. The present study investigated the relation between initiating molecular events of thyroid disruption and morphological and physiological changes of eye development of zebrafish (Danio rerio). Two test compounds representing different molecular modes of thyroid disruption were used: propylthiouracil (PTU) as enzyme-inhibitor within thyroid hormone synthesis, and tetramethylbenzophenol A (TBBPA) as ligand of thyroid hormone receptors. Both test chemicals significantly altered transcript levels of thyroid-related genes in a compound-specific way. Despite differing molecular response patterns, both treatments resulted in similar pathological alterations of the eyes such as reduced size and pigmentation, which were concentration-dependent. The morphological changes translated into impairment of swimming activity and visual performance of the larvae: the optokinetic response was significantly and concentration-dependently decreased in both treatments, together with a significant increase of light preference of PTU-treated larvae. This study provides first evidence that thyroid disrupting compounds do not only impair morphological but also functional eye development in fish early life stages, and that the phenotypic outcomes appear to be similar for different modes of molecular action of the thyroid disruptors.

Obesogenic effects in Daphnia magna. An emerging endocrine disrupting effect C. Barata, CSIC / Environmental Chemistry; R. Jordao, CESAM & University of Aveiro / Biology; B. Campos, IDAEA-CSIC / Environmental Chemistry; F. Casas, CSIC/GAC; E. Garreta, CSICDIAAE; F. Lemos, Instituto Politécnico
Assessment of endocrine disrupters has been largely focused on vertebrates and on disruption of the hypothalamic-pituitary–gonadal/thyroidal axes of laboratory animals. There are, however, many compounds able to disrupt other nuclear receptor signaling pathways. An important group of endocrine disrupters are obesogens that disrupt the retinoic X receptor and PPAR and cause obesity in vertebrates. There is, however, little evidence of obesogenic effects in non vertebrates. A recent study showed that tributyltin (TBT) activated the ecystoidsteroid, juvenile hormone and retinoic X receptor signaling pathways, and disrupted the development of the sex-specific lipids in the crustacean Daphnia magna, impairing the transfer of triacylglycerols to eggs and hence promoting their accumulation into lipid droplets in post-spawning females. Tributyltin disruptive effects translated into a lower fitness for offspring and adults. The present study aims to addresses the disruptive effects of existing compounds alone and in mixture on the development and reproduction in non vertebrates and their health effects. D. magna individuals were exposed 12 chemicals that included vertebrate obesogens (TBT, triphenylenyl, bisphenol A (BPA), 4-nonylphenol (NP), di-2-ethylhexyl phthalate), other contaminants known to affect arthropods (pyrrolxoyphenyl (PP), fenamidone (FEN), methoprene (MET), emamectin benzoate (EM and fluoxetine (FX)), as well as the natural hormones methyl farnesioate (MF), 20-Hydroxyecdysone (20E)). Reproductive effects were assessed by Life History analysis methods. Quantitative changes in storage lipids were studied using Nile red staining and ultra-performance liquid chromatography coupled to a time-of-flight mass spectrometry. Ten compounds disrupted storage lipid in a concentration related manner enhancing (TBT, MF, PP, BPA, 20E) or decreasing (NEO, EM, NP, FX) lipid contents in post-spawning females. Joint binary mixture effects indicated that the studied compounds acted on storage lipids additively and non-additively disrupting the signaling pathways of ecysodine, methyl farnesioate and retinoic X receptors. In eight compounds disruptive effects translated into detrimental effects in growth in and or reproduction. Acknowledgement - This work was funded by the Spanish Ministry of Science and Innovation project (CTM2011-30471-C02-01, CTM2014-51985-R).}

### 407 Occurrence of alkylphenols, volatiles, and metals in tap water in households with epoxy coated water pipes

**J. Ruijssenaars**, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; **M. Pernica**, Masaryk University / Research centre for toxic compounds in the environment; **J. Kuta**, L. Blaha, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; **Z. Simek**, Masaryk University / Research centre for toxic compounds in the environment; **L. Blaha**, Masaryk University, Faculty of Science / Research centre for toxic compounds in the environment RECETOX

Organic chemicals in tap water originate from several sources. Organic pipe materials are a potential source of contaminants. Compared to communal distribution system small water pipes in households have potentially greater impact on water due to higher surface-to-volume ratio. Organic pipe materials include pipe inner surface lining materials such as epoxy resins. Epoxy lining has been used as a cost-effective alternative to renovate old water pipes. Most common component in epoxy resins is bisphenol A (BPA). Leaching of chemicals such as the endocrine disrupter BPA has been shown to occur from epoxy linings in pipes [2]. In addition, epoxy liners of food and beverage cans has been shown to leach BPA to the contents [3]. Consequently, long-term durability and safety of drinking water pipe epoxy linings has been a topic of discussion. In this study drinking water from apartment houses with different age epoxy linings were studied. Samples were collected in summer 2015 from 6 houses with epoxy lined drinking water pipes and a drinking water treatment plant (DWTP) in Helsinki, Finland. Tap samples were collected after about 8 hours of discontinued water usage and after 2 min flowing, both on cold and hot lines. For comparison further sampling were done in December 2015 at 5 other sites with different age and material pipes. Samples were analyzed for alkylphenols BPA, bisphenol F (BPF), 4-carboxyphenol (CP), 4-nonylphenol (NP) with HPLC-MS [4], and for volatile organic compounds, and metals (IPC-MS). Total (anti)estrogenic and (anti)androgenic potency and BPA-like activity will also be tested with yeast-cell-based assays [5, 6]. Bisphenol A was frequently detected in water of houses with epoxy lined pipes. DWTP samples had no detectable BPA. Incoming water had cold water concentrations up to 33 ng/L. Hot water had clearly higher levels of BPA in two sites, even up to 1.65 μg/L, whereas in three sites BPA levels were below 60 ng/L. BPF, NP, and OP were detected only in few samples below 2; 1.4; and 22 ng/L, respectively. BPA in tap water samples mainly originates from the epoxy lining, but the contribution of contamination in col-lining water values up to 15 % and 80 % respectively, the hypothesis of an observed nase on storage lipids additively and non-additively disruption the signaling pathways of ecysodine, methyl farnesioate and retinoic X receptors. In eight compounds disruptive effects translated into detrimental effects in growth in and or reproduction. Acknowledgement - This work was funded by the Spanish Ministry of Science and Innovation project (CTM2011-30471-C02-01, CTM2014-51985-R).
from the pesticide formulation. An important target of various ED chemicals is steriodogenesis, i.e., the synthesis of steroids such as progestogens, sex steroids (androgens and estrogens), and corticosteroids, which control the complex physiological processes associated with growth, reproduction, and pregnancy. A group of pesticides shown to disrupt steriodogenesis are azole fungicides. In this project we analyzed in vitro the disruption of human steriodogenesis by the chiral azole fungicide imazalil in dependence of the enantiomeric form. As model system we used the human adrenocortical carcinoma cell line H295R, which expresses all important human steroids. Pure enantiomers of imazalil were isolated with enantioselective HPLC and the absolute configuration of the enantiomers was identified with circular dichromism. H295R cells were exposed for 48 h to racemic imazalil as well as to the individual enantiomers in concentrations ranging from 0.001 – 8 µM. After exposure 17 steroids were quantified in the cell medium using HPLC-MSMS. Racemic imazalil inhibited the synthesis of several steroids belonging to androgens, estrogens and corticosteroids with EC50's in the range of 0.005 – 1.1 µM. Also 1 µM of R-imazalil decreased estradiol production approx. 7 times stronger than R-imazalil, whereas progesterone production was stimulated by R-imazalil to a 3 times higher extent than by S-imazalil. These results indicate that the disruption of steriodogenesis by azole fungicides is enantiospecific in vitro. Which significance our findings have for the role of endocrine related diseases has to be tested in future in vivo studies.

Science based strategies for the environmental assessment and management of pharmaceuticals and veterinary medicines

411 Industry Approach to Managing Potential Risks from Active Pharmaceutical Ingredients in Manufacturing Effluent
F. Mastracco, Pfizer, Inc. / Department of Environment Health Safety; R. Kappler, Johnson & Johnson / Environmental Engineer; D.J. Caldwell, Johnson & Johnson

To address stakeholder concerns with pharmaceuticals in the environment (PiE), the pharmaceutical industry has developed a proactive product stewardship approach entitled, 'Eco-Pharmaco-Stewardship' (EPS) consisting of three ‘pillars’, namely, enhanced environmental risk assessment (eERA), extension of the scientific knowledge base (IPIE), and manufacturing effluent management, the focus of this presentation. Through the application of a risk-based approach, the industry is seeking to ensure that manufacturing effluent discharge is effectively managed to minimize environmental risk across the supply chain. The development and application of step-by-step guidance by several manufacturers was described, as well as, steps taken to ensure all in the supply chain are aware of practices that can be deployed to ensure that risks from manufacturing effluent are low. The use of a maturity ladder concept to establish current program sophistication level and gauge advancement will also be presented.

412 The need for targeted testing to improve the regulatory environmental risk assessment of veterinary medicines used in aquaculture
A. Lillicrap, A. Macken, NIVA / Ecotoxicology and Risk Assessment; K.V. Thomas, NIVA / Norwegian Institute for Water Research / Product Metabolism

The use of pesticides in aquaculture has increased significantly over the recent years. Controlling salmon lice infestations is not simply an economic goal, but also it is a requirement that fish farms maintain levels below 0.1 lice per fish to limit transmission to wild pulmonates of salmonids. With the emergence of more resistant strains of salmon lice, control strategies are becoming more aggressive and the consequence to non-target organisms is currently receiving a great deal of media publicity. For some pesticides used to treat salmonids, standard acute toxicity tests may be sufficient to predict environmental effects on non-target organisms exposed to veterinary medicines in aquaculture. However, standard acute toxicity tests are not sufficiently powerful to estimate possible chronic effects of substances that have a very specific mode of action, for example chitin synthesis inhibitors. Therefore, more targeted assessment strategies using non-standardised test methods aimed at the specific mode of action is necessary to capture the possible long term effects of these pesticides. This presentation describes some of the pesticides that are currently being used to treat salmon lice and the possible long term effects of these pesticides. In this project we analyzed in vitro the disruption of human steriodogenesis by the chiral azole fungicide imazalil in dependence of the enantiomeric form. As model system we used the human adrenocortical carcinoma cell line H295R, which expresses all important human steroids. Pure enantiomers of imazalil were isolated with enantioselective HPLC and the absolute configuration of the enantiomers was identified with circular dichromism. H295R cells were exposed for 48 h to racemic imazalil as well as to the individual enantiomers in concentrations ranging from 0.001 – 8 µM. After exposure 17 steroids were quantified in the cell medium using HPLC-MSMS. Racemic imazalil inhibited the synthesis of several steroids belonging to androgens, estrogens and corticosteroids with EC50's in the range of 0.005 – 1.1 µM. Also 1 µM of R-imazalil decreased estradiol production approx. 7 times stronger than R-imazalil, whereas progesterone production was stimulated by R-imazalil to a 3 times higher extent than by S-imazalil. These results indicate that the disruption of steriodogenesis by azole fungicides is enantiospecific in vitro. Which significance our findings have for the role of endocrine related diseases has to be tested in future in vivo studies.

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413 Refined exposure estimation to support an Environmental Assessment for a veterinary medicine

The U.S. Food and Drug Administration, Center for Veterinary Medicine, evaluates whether significant environmental impacts would occur with the approval of new animal drugs pursuant to the National Environmental Policy Act. The relationship between predicted no-effect concentrations (PNECs) derived from an enhanced environmental risk assessment (EA), which contains sections addressing problem formulation, environmental fate, exposure, effects and risk characterization. Using a recent EA as a framework, this presentation will focus on the refinement of environmental exposure estimates using spatial techniques to identify representative and protective environmental concentrations linked to exposure models commonly used in the U.S. Environmental Protection Agency (USEPA) pesticide registration process. A Geographic Information System (GIS) was used to identify regions of high exposure potential across the US based on beef cattle characteristics and climatic conditions. From within each region, a single vulnerable watershed was selected to characterize a chiral meso-scale modelling following USEPA Tier-2 pesticide exposure approaches. Three potential sources of chemical were modelled: feedlots, agricultural fields applied with manure collected from the feedlots, and pasture. Using PRZM and EXAMS models, runoff and erosion inputs to surface water from these sources were assessed over a 30-year timeframe to produce final PECs suitable for use in the effects portion of the EA. The results of the national vulnerability assessment identified five regions with diverse intensive-use characteristics. From within these, a single intense-use watershed was selected and modelled. Loadings from each of the land covers were combined on a daily basis and transported to the receiving water body, from which daily PECs were calculated. Based on the aggregate aquatic exposure, no significant effect was identified and a finding of No Significant Impact (FONSI) was determined. The process presented here discusses the development of refined methods to estimate exposure using spatial techniques to identify representative and protective environmental scenarios. It linked these scenarios to accepted EPA exposure models which addressed all potential sources of chemical loading and predicted a series of suitable risk management options. This approach is a robust and viable methodology incorporating real world information but maintains inherent safety assumptions from USEPA Tier-2 pesticide framework.

414 Use of Acute and Chronic Ecotoxicity Data in Environmental Risk Assessment of Pharmaceuticals
J. Vestel, Merck & Co., Inc.; D.J. Caldwell, Johnson & Johnson; L.A. Constantin, Pfizer, Inc. / Pharmacokinetics Dynamics and Metabolism; V. D’Aco, Quantum Management Group, Inc.; T. Davidson, Bristol-Myers Squibb / EHS; D.G. Dolan, S. Millard, Probability Statistics and Information; R. Murray-Smith, Richard Murray-Smith; N. Parke, Eli Lilly and Company / Global HSE Environmental Affairs; J. Ryan, GlaxoSmithKline; J. Straub, F.Hoffmann-La Roche Ltd / Roche Group Safety Health Environmental Protection; P.W. Wilson, Sanofi U.S., Inc. / Health Safety and Environment

For many older pharmaceuticals, chronic aquatic toxicity data are limited. To assess risk during development, scale-up and manufacturing processes, acute data was calculated. Based on the aggregate aquatic exposure, no significant effect was identified and a finding of No Significant Impact (FONSI) was determined. The process presented here discusses the development of refined methods to estimate exposure using spatial techniques to identify representative and protective environmental scenarios. It linked these scenarios to accepted EPA exposure models which addressed all potential sources of chemical loading and predicted a series of suitable risk management options. This approach is a robust and viable methodology incorporating real world information but maintains inherent safety assumptions from USEPA Tier-2 pesticide framework.

415 Evaluating the Risk of Pharmaceuticals in the Terrestrial Food Web
L. Carter, University of York / Environment Department; T.G. Bean, University of Maryland / Department of Environmental Science and Technology; A. Boxall, University of York / Environment Department

This study investigated the risk to bird species consuming earthworms contaminated with active pharmaceutical ingredients (APIs) using a combination of previously published earthworm bioconcentration factors (BCFs) and new experimental results. Food chain transfer scenarios were subsequently compared between soil - earthworm - bird using a range of pharmaceuticals. In total, an exposure assessment was carried out to evaluate food web transfer of selected APIs from the environment into earthworms, suitable for risk characterization. The most important field scenarios, taking into account the effect of soil type and species traits on
earthworm API uptake. API residues were predicted in two earthworm species, namely Eisenia fetida and Lumbricus terrestris from experimentally determined BCFs and environmentally relevant soil concentrations. Using a combination of calculated API residues in earthworms together with known consumption data of earthworms for the selected bird species, the daily intake of each API by song thrush, rook, starling, lapwing and common gull was modelled. Based on these exposure scenarios the resulting earthworm concentrations reached a maximum of 2462.03 µg/g. Subsequent analysis demonstrated that the food web transfer of selected pharmaceuticals reached a maximum daily intake of 459.8 µg per bird. Ultimately, a range of parameters can influence the bioavailable fraction of the API in the soil with a range of daily intake values. Sandy soils typically have higher bioavailability than clay soils which can influence earthworm API uptake and thus bird exposure. In general, the larger BCFs for L. terrestris resulted in a higher uptake in comparison to E. fetida, given a fixed soil concentration, and thus a greater intake by the bird species on a per g basis. To evaluate any potential long term toxicity by birds ingesting APIs contaminated earthworms the bird elimination half-life required to accumulate the human therapeutic dose (HTD) in 90 days was subsequently calculated. The birds included in this analysis would require elimination half-lives in the range of 1 to 3 orders of magnitude longer than humans to accumulate the HTD. Therefore, as birds typically have faster metabolic rates than humans, and so are likely to eliminate APIs quicker than it is unlikely that the calculated elimination half-lives required to accumulate the HTD would be observed.

416 Post-approval environmental management of human medicinal products: An extended environmental risk assessment (eERA) framework
J. Snape, AstraZeneca UK Ltd.; AstraZeneca Global Environment; J. Erickson, Pfizer Inc.; Environmental Sciences PDM; T. Davidson, Bristol-Myers Squibb; EHS; R.D. Meyerhoff, El Lilly and Company / Ltl Health Safety And Environmental; J. Ryan, GlaxoSmithKline / Corporate Environment Health Safety Sustainability; R. Laenge, Bayer Pharma AG / Dept Experimental Toxicology; J. Straub, F.Hoffmann-La Roche Ltd / Roche Group Safety Health Environmental Protection; R. Journel, SANOFI; S. Eskola, Eli Lilly; K. Nedog, EGA; M. Moussa, AESGP

The Association of European Self-Medication Industry (AESGP), European Federation of Pharmaceutical Industry Associations (EFPIA) and the European Generics Association (EGA) are currently looking to implement a holistic approach to the environmental management of human medicines products called EcoPharmaStewardship (EPS). Within this EPS initiative, AESGP, EFPIA and EGA propose to extend the ERA paradigm into the post-approval phase (specifically for new APIs to their usage figures based on total sales). Evaluation of environmental impact, fate, exposure, effects and risk characterization. Using a recent EA as a case study the development of refined exposure estimation to support an Environmental Assessment for a tier-2 pesticide exposure approaches. Three potential sources of chemical were determined. The process presented here discusses the development of refined exposure estimates using spatial techniques to identify representative and environmentally relevant endpoints.

418 Synergies and divergence between LCA human toxicity assessment and Risk Assessment approaches
M. CORNELIUS; R.K. Rosenstein, National Research Institute of Science and Technology for Environment and Agriculture - Isetra / UMR ITAP, S. Causse, EVEA; V. Grammont, A. Toise, INERIS; J. Garcia, P. OSSET, SCORE LCA 1. Introduction Beyond the assessment of toxic effects and risks within the framework of European regulations, such as REACH and CLP, other assessment methods for sanitary risks exist but differ from one country to another. Methodologies are different but always based on the founding principles of dose-response relationships and exposure scenarios describing the source-to-target vector. The complementarity of Life Cycle Assessment (LCA) and Chemical Risk Assessment (RA) may yield a more accurate and exhaustive approach to assess human toxicity. 2. Materials and methods 2.1. Panorama of methods assessing human toxicity Various approaches to assess human toxicity risks exist and allow to supply toxicity data. The classifications, such as those established by the European Union, allow to identify hazards of substances. Risk and hazard based approaches typically apply (reasonable) worst-case assumptions for modelling and data selection. In contrast, LCA toxicity assessment methods apply the concept of best-estimates. Another important difference is that RA is site-specific whereas LCA is site-generic. A number of relevant methods for human toxicity assessment within the LCA and RA frameworks have been analysed in order to allow for a mapping of the methods. The results and the divergences identified between the methods are going to be presented in a condensed way. 2.2. Complementarities The methods have been analysed according to many criteria. This talk will underline the similarities and differences as well as the advantages and the associated drawbacks linked to each method. To support this analysis, a comparison based on a case study applying both LCA and RA methods was performed for the human toxicity assessment of a paraben-free cosmetic formula. A detergent was also analysed with different methods. Throughout the study, independent scientific experts have been associated to assure a peer-review of this study. 3. Results and discussion Propositions for the correct interpretation of results as well as their limitations and research needs were identified. Methodological issues will be discussed. Moreover, guidelines have been suggested for tailoring the LCA toxicity assessment will be proposed, detailing which method serves which purpose, where they overlap and where they complement one another. This study invites experts to work together to find solutions to the current issues in human toxicity assessment.

419 Addressing fresh water ecotoxicity impact category under the EU Product Environmental Footprint (PEF)
E. Saouter, EU Commission JRC / Sustainable Assessment Unit; M. Secchi, University of Milano Bicocca, / Department of environmental and earth science; S. Salsi, European Commission - Joint Research Centre / Sustainability Assessment unit; R. Pant, European Commission / Institute for Environment and Sustainability; K. Aschberger, European Commission - Joint Research Centre / Institute for Health and Consumer Protection IHCP; S. Bopp, European Commission - Joint Research Centre / Systems Toxicology Unit

SETAC Europe 26th Annual Meeting Abstract Book 95
This paper aims at highlighting the main areas where modifications could be made has conducted an in-depth evaluation of the model and data used to calculate CFs. The limitations of both attempts are the lack of normalization and weighting. The new LCIA methodology provide additional and complementary insights which cannot be achieved with traditional LCA-methodologies. Thus, the dispersion are sensitive to the differences in the acoustical characterises of the tires. Thus the outomes of the calculation (i.e characterisation factors (CFs)). The lack of transparency as well as complexity of using the model to calculate new characterisation factors were identified as a limitation for further use. The data (physico-chemical, half-life and toxicity) that have been used to calculate the CFs provided with the model (about 3000) seems to be a source of significant differences, controversies and criticisms. For many substances, especially fated and effect data may not be suitable as currently used in USEtox. Most PEF Pilots recommended not to use the model further before agreement is reached on the selection of input data. After this workshop, the Joint Research Centre (JRC-Ipsra) has conducted an in-depth evaluation of the model and data used to calculate CFs. This paper aims at highlighting the main areas where modifications could be made to improve the reliability and acceptance of the CFs.

420 Analysis of the different technique to include noise damage in life cycle assessment. A case study for car tires.
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WHO (2011) reported very comprehensively on the burden of disease from environmental, noise, highlighting noise as a threat to public health problem. Despite this need, the integration of traffic noise damages on human health in life cycle assessment (LCA) is still debated. Although several methodological attempts were advanced to fill in this gap, none of them has become consensus so far. The objective of this study is threefold: i) to analyse how the different methods for noise damage assessment are sensitive to different input noise levels; ii) to compare the results obtained from the three methods and iii) to evaluate the importance of noise damages on human health as compared to other burdens contributing to the DALYS. To this aim, three methods for noise damage assessment are investigated. A case study on car tires is considered as seminal example of functional unit to highlight and discuss the differences between the results obtained from the three methods. For our case study, three different tires are considered: one reference tire, and two tires (Tire 1 and Tire 2) with improved acoustical characteristics provided by a tire manufacturer. The complete LCA of the car tires (functional unit: a tire used over one kilometre) was also conducted using the ReCiPe method following the hierarchical perspective and average weighting set (B). The results show that all the noise characterization methods are sensitive to the differences in the acoustical characterises of the tires. Thus they allow reflecting in a quantitative way the improved sound level performance of the tire into clear impact differentials expressed in DALYS. ii) The dispersion of the results obtained from the methods show the need for further refinement of the assessment methodology to improve the accuracy and consistency of the integration of noise impact in LCA. iii) Despite the variation between the methods used, noise impact represents at least 90% of the total impact of the tires on human health. It highlights the importance of taking into account noise impact in LCA.

421 The development of an operational LCIA-methodology with impact categories based on the control variables in the Planetary Boundaries framework
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This study presents a first attempt at an operational LCIA-methodology basing the definition of the impact categories on the control variables as defined in the Planetary Boundaries (PB) framework. The PB-framework introduced a set of biophysical thresholds for pressures and drivers which should be respected for Earth to remain in the Holocene state. The concept is attracting a strong interest from industry as companies seek to assess and communicate the environmental performance of their products relative to the PBs. The PB-framework has previously been attempted included in LCA as part of normative life cycle assessment (LCA). The limitations of lack of spatial differentiation for spatially differentiated PBs and the requirement for harmonizing the control variables with indicators already used in life-cycle impact assessment (LCIA). A way to overcome these limitations is to directly use the control variables in the PB-framework as impact categories in LCIA, which is also the objective of this study. This work defines a mathematical framework for a LCIA-methodology where Characterisation Factors (CFs) are included for all Earth system processes in the PB-framework, for all substances contributing to effects on the Earth system processes and expressed in the units of the control variables. Except for novel entities and biophase integrity which are currently excluded from the LCIA-methodology because the latter is lacking a planetary boundary metric while a full understanding of the cause-effect chain is missing for the former. The CFs were estimated identifying the most important models needed to model the control variables of the PB-framework and adapting these to fit the LCIA-framework. This work provides a full set of CFs for all the Earth system processes in the PB-framework. The new LCIA-methodology provide additional and complementary insights which cannot be achieved with traditional LCA-methodologies. These new insights can be used for communicating the product’s environmental performance and to support definitions of absolute reduction targets relative to the PBs.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (II)

422 Passive sampling and toxicity profiling: a case study in Rivers Meuse and Rhine, The Netherlands
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The TIPTOP project hypothesizes that time-integrative passive sampling followed by toxicity profiling is a toxicologically and ecologically more relevant and more protective approach for chemical water quality assessment than the assessment based on a comparison between concentrations of predefined individual compounds to their Environmental Quality Standard (EQS). To test this hypothesis, a demonstration study was designed at well-defined WFD sampling sites in the Dutch delta. Time-integrative sampling was performed using partitioning-based and adsorption-based passive samplers. Toxicity profiling was performed using a test battery consisting of in vitro and in vivo bioassays. Currently, all chemistry and toxicity profiles are available. Several approaches are followed to interpret these profiles in a toxicological and ecological context. First of all, the chemical data are used to make a traditional comparison between water concentrations of individual contaminants and their environmental quality standard concentration defined within the WFD. Moreover, the chemical analyses are used to determine the contribution of the analyzed compounds to the observed toxicity and to calculate the potentially affected fraction of species by exposure to these multiple substances (msPAF). Moreover, molar concentrations in the passive samples are used to estimate to what extent the critical body burden for narcotic compounds is reached and to estimate the msPAF for narcotics. Bioassay results are used to benchmark toxicity profiles from river sampling locations to WWTP effluents. In vitro bioassay results are further used to compare to newly derived trigger values, i.e. bioassay responses below which no ecological risk is to be expected. In vivo bioassay results for organisms belonging to six different taxonomic classes are used to derive a species sensitivity distribution (SSD) for each sampling location, which can be used to determine a msPAF value based on bioassay responses measured after exposure to the whole sample, rather than on a limited set of chemical data. Finally, calculated and measured msPAF values are compared to the actual biological monitoring data obtained at the very same sampling locations. The outcome of TIPTOP should ultimately lead to a proposal for a cost-effective approach for chemical water quality assessment.

423 Investigating the time-integrative properties of passive sampling for chemical monitoring and application in bioassays
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Regular chemical water quality assessment of surface water is performed by single grab sampling of the water in compliance to Environmental Quality Standard (EQS). A disadvantage is that a grab sample gives only a snap shot of the water quality and may not be representative for compounds with an irregular emission pattern. Application of (time-integrative) passive sampling might be a better strategy, because water is sampled over a longer period and a higher water volume can be sampled. After deployment of the samplers and extraction in the laboratory, these extracts can be used for chemical analysis in the same way as in
the regular monitoring and/or used in bioassays to perform toxicity profiling. Uptake of passive samplers depend on deployment time and water turbulence or flow. The time-integrative properties of sampling was investigated within the TIPTOP project for partitioning-based and adsorption-based passive samplers. Silicone-rubber (SR) samplers and adsorption based Speedisk (SD) samplers were deployed in the water for short and long periods overlapping with each other. In After deployment the samplers were extracted and analysed on targeted chemical analysis with LC-MS and GC-MS, including PAHs and a range of plant protection substances. In addition all extracts were subjected to toxicity profiling. The release of Performance Reference Compounds dosed prior to deployment was used to establish the sampling recovery. In the current study, LC-MS was also used to estimate the water volume sampled by the Speedisk. In the presentation the time-integrative properties of the samplers is evaluated as well as the evolution of the results from toxicity profiling.

424 In silico prediction of sampling rates for polar organic chemical integrative samplers (POCIS)
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The modelling and prediction of polar organic chemical integrative sampler sampling was performed for compounds using artificial neural networks is presented for the first time. Molecular descriptors were chosen based on previous retention descriptor models and also a genetic algorithm which shortlisted 24 descriptors from a total of 201 that covered topological, geometrical and physicochemical properties. Each network was validated by testing the predictive ability for an external validation dataset (n = 6) of benzo[a]pyrene with the best network based on chromatographic retention molecular descriptors. The genetic selected descriptor network showed an average absolute predictive error of 0.0437 ± 0.02 L.d⁻¹ which contrasted to the retention descriptor network error of 0.0145 ± 0.008 L.d⁻¹. The sum squared errors for the training, verification and test subset were 0.092, 0.062 and 0.121, respectively. The average predicted error for the verification (n = 11) and test (n = 11) was set was 0.03 ± 0.02 L.d⁻¹ of the experimentally determined R, value. The model was built in triplicate to assess the variability of predictions across networks and in comparison to experimental variance which gave better or similar variance to the measured values. These novel findings indicate the potential of in silico predictive tools for R, determination which represents significantly more economical approach than laborious laboratory based calibrations.

425 Performances of the Continuous Flow Integrative Sampler (CFIS) for monitoring organic pollutants in surface waters
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Passive sampling has recently emerged as an efficient alternative to conventional active sampling for monitoring organic pollutants in water. The technique consists in exposing the passive sampler in the water for days to weeks, depending on the sampler, to accumulate the target pollutants. Average concentrations integrated on the exposure period are then determined from the mass of pollutants sequestered in the sampler. The concentration of the target pollutants in the receiving phase allows reaching lower quantification limits than conventional sampling techniques. Moreover, this technique reduces dramatically analytical costs, as it provides realistic average concentrations with a single analysis. A passive sampler is typically composed of a receiving phase and a membrane that separates the receiving phase from the medium. Several passive samplers have been developed for targeting pollutants of different physical chemical properties, and especially different polarities. The Semi-permeable Membrane Device (SPMD) has been developed for non-polar organic pollutants such as polychlorobiphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) in water whereas the Polar Organic Chemical Integrative Sampler (POCIS) targets polar organic pollutants such as pharmaceuticals and personal care products. On the other hand, on the passive samplers, the performances of these passive samplers may depend on the exposure conditions, and especially on flow velocity. We have recently developed an automated, fully submersible, integrative sampling device to monitor both non-polar and polar organic pollutants in different types of water, the continuous flow integrative sampler (CFIS). Unlike conventional passive samplers, CFIS are independent from flow velocity. The performances of the CFIS were assessed and compared with those of spot sampling for monitoring hormones, pharmaceuticals, PCB, PAH, hydrophobic pesticides and plasticizers in the influent and the outflow waters of a fish farm in Ardtoe (Scotland). Exposure campaigns of 10 days have been started in July 2015, for one year. For each campaign, the CFIS was exposed for 10 days, while spot samples were collected on the first and the last days of the campaign. Analysis results showed better sensitivity of the CFIS and good agreement between the results of CFIS and spot sampling. Therefore, the CFIS proved to be an efficient, sensitive and cost-effective alternative for monitoring organic pollutants in surface waters.

426 Application of miniaturized cell-based bioassays for high-throughput effect-directed analysis of passive sampler extracts using microfractionation
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Routine monitoring of drinking water sources for endocrine disrupting chemicals is commonly limited to the determination of known priority substances through chemical analysis. With the ever changing composition of the mixtures of compounds in the environment, other potentially hazardous chemicals remain undetected. Efforts to keep track of the release of potential endocrine disruptors into the environment and to monitor the processes of drinking water purification depend on the ability to determine the presence of these compounds in surface, ground and effluent water. The advent of cell-based reporter bioassays allowed detection of active toxicants in mixtures based on their biological activity. The application of these bioassays together with analytical identification methods in effect-directed analysis (EDA) provides a strategy to detect and identify novel endocrine disrupting chemicals for risk assessment of e.g. water samples. EDA suffers from lack of throughput and is regarded as a laborious, time consuming method. To achieve higher-resolution separation and faster analysis on multiple end-points, a high throughput-EDA (HT-EDA) method was developed through application of UPLC-based microfractionation in combination with a battery of miniaturized 384-well plate cell-based reporter assays performed in parallel. The applicability of this approach will be demonstrated by analysis of selected extracts from partitioning based silicone rubber (SR) passive samplers and adsorption based Speedisk passive samplers. The application of high-resolution microfractionation together with miniaturized bioassays will open the way to HT-EDA and will emphasize the advantages of the unification of chemical analysis and bioassays in the analysis of environmental samples, including drinking water.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health

427 Co-selection for antibiotic resistance by biocides in the aquatic environment
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Antibiotic resistance is a growing problem in the treatment of clinical infections, leading to predictions that we are about to enter a post-antibiotic era where many bacterial infections are untreatable. Increasing attention is being placed on the environment as a reservoir of antibiotic resistant bacteria and in the potential for antibiotic resistors and other compounds to select or co-select for antibiotic resistance. Quaternary ammonium compounds are used as biocides and are found in a wide range of personal care products, reaching mg/L concentrations in waste water. Work by Gaze et al. (2005) was the first to demonstrate co-selection by quaternary ammonium compounds (QACs) for antibiotic resistance in the environment. Recent work has shown that high QAC exposure selects for QAC resistance mechanisms that have also been implicated in resistance to antibiotics. Functional metagenomic libraries were screened for resistance to biocides, and transposon mutagenesis was used to identify and sequence novel biocide resistance genes. Current work focuses on selection for QAC resistance and co-selection for AMR in experimental microcosms, to determine the minimal selective concentration of QACs that co-select for antibiotic resistance. Data suggests QAC concentrations in highly polluted waste streams are in the same order of magnitude as the MSC.

428 Selection for antibiotic resistance in the environment
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Antibiotic resistant bacteria are a significant threat to both human health and the global economy. As few new antibiotics are currently in development, antimicrobial stewardship is currently the best strategy to avoid progression into a ‘post-antibiotic era’, a world in which most modern medical and farming practices will be unviavle, and routine infections could once again become deadly. Most
mitigation strategies implemented thus far have focused primarily on intervention at the clinical level, and the role of the natural environment has been largely overlooked. However, it is known that the environment is not only a reservoir of clinical resistance genes and that these can be mobilised into human pathogens, but that resistance genes and antibiotics are constantly being introduced into the environment via several sources including waste water treatment plant effluent and run off from agricultural land. A key study by Gulberg et al. (2011) demonstrated that selection for resistance can occur at extremely low (potentially environmentally relevant) antibiotic concentrations, and therefore the environment may even be a platform for selection for resistance in situ. However, there are currently no standardised methods in practice which can estimate the effect concentrations of antibiotics in the environment, in terms of selecting for, maintaining, or mobilising resistance genes. Therefore mitigation strategies have been unable to be designed. In this study, natural, complex communities were exposed to varying concentrations of antibiotics and resistance gene prevalence quantified by real-time PCR. Antibiotic concentrations were chemically quantified and antibiotic degradation curves generated to determine the optimal culturing conditions to emulate continuous exposure at clinical and subclinical concentrations. The primary aim of this research is to understand how resistance may be selected for in the environment, and to design an assay which can be used for environmental risk assessment.

429 Impact of dairy manure pre-application treatment on dynamics of antibiotic-resistance genes in crop production systems

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Antibiotics are widely used in livestock and poultry production for growth promotion, prophylaxis and treatment of illness. There is a widespread concern that this practice promotes resistance and erodes the efficacy of antibiotics used in human medicine, a crucially important public health challenge. The recycling of antimicrobial and poultry litter as crop production inputs can provide valuable nutrients and improve soil quality is widely practiced. However, a reservoir of antibiotic resistance genes in the environment that is made larger through contamination with agricultural wastes may represent an enhanced threat to human health. In the present study, we sought to determine if pre-treatment of dairy manure through composting, dewatering, or anaerobic digestion would alter the loading rates of antibiotic resistance genes, their persistence in soil following application, and the burden of antibiotic resistance genes on crops at harvest. Experiments were undertaken during the 2014 growing season on the Agriculture and Agri-Food Canada research farm in London, Ontario, Canada. Manure for applications in the spring of 2014 was obtained from two local dairy farms. One farm supplied the raw manure slurry, and anaerobically digested manure digestate. The second farm supplied the mechanically dewatered, and the composted manures. Replicated field plots received no manure (ie. untreated control), raw, composted, dewatered or anaerobically digested dairy manure. Plants were planted to carrots, radish, or lettuce. DNA was extracted from manures, soils throughout the growing season and vegetables at harvest. These were variously analyzed by straight PCR, quantitative PCR, or high throughput sequencing to establish the loading rates, soil persistence, and exposure of vegetables to selected gene targets associated with antibiotic resistance or gene mobility. Composted manure had the lowest abundance of gene targets, whereas anaerobic digestate was the highest. These differences in Manure composition are consistent with the gene targets in soil following manure application. Referenced to vegetables grown in ground without manure there was no consistent increase in gene target abundance on vegetables grown in manured ground.

430 Fate of antibiotics into agricultural soils amended or not with organic waste products, and combined effects of contaminants

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Antimicrobial (ATB) resistance is a concern of human and veterinary medicine, are for the most part extected. Waste water treatments do not eliminate them completely. Consequently, spreading organic waste products (OWP) issued from sewage sludge or liquid and solid manures may disseminate ATB in soils with unknown risks for human health and environment. In addition, these OWP contain other contaminants such as trace metals (TM), which tend to accumulate in soils submitted to repeated spreadings. Interactions between contaminants may affect the fate or and the potential effects of ATB. Our objectives were to investigate the impact of soil type and combinations of ATB and TM on the degradation of two ATB in soils, ciprofloxacin (CIP) and N-acetyl-sulfamethoxazole (N-SMX). Cu and Zn were chosen as TM. Soil incubations of 14C labelled ATB under laboratory conditions were used to quantify over time their fate and distribution in the soil (mineralized fractions, easily and hardly extractable fractions, and non-extractable fraction). The influence of the type of soils was studied from applications of N-SMX (0.02 mg/kg) or CIP (0.15 mg/kg), on three agricultural soils likely to harbour a microflora more or less adapted to degradation of ATB: a conventionally managed soil and two soils amended every two years since 1998 respectively with a compost of sewage sludge/green waste and with farmyard manure. Results showed that the N-SMX mineralization was low, in the order of 10% after 150 days, whereas mineralization of the CIP was negligible. The majority of the radioactivity was concentrated in the hardly extractable and non-extractable fractions. The fate of these ATB has been barely affected by soil type in the impact of combined application of N-SMX and CIP. The mineralization of N-SMX was studied through applications of N-SMX alone (0.02 mg/kg) in the conventionally managed soil, or in a solution of copper and zinc, at realistic doses (respectively 20 and 30 mg/kg) and at five times this dose (respectively 100 and 150 mg/kg). The presence of metals provided at the highest dose resulted in a decrease in mineralization and an increase in adsorption, attributed to a direct effect of metal (complexation with N-SMX) and inhibition (copper and zinc) of activity of certain microorganisms and to an indirect effect via a decrease in pH.

431 Adsorption and ecotoxicity of three pharmaceuticals: cocktail effect

R. Leitg, Champagne-Ardenne; S. Sayen, University of Reims Champagne-Ardenne; G. Emmanuel, Université de Reims Champagne Ardenne; FR. SERET, Centre Universitaire JeanFrançois Champollion Albi France Pharmaceuticals are a class of emerging contaminants whose production has increased for many years and which are emitted in large amounts to the environment via different pathways (wastewater treatment plants effluents, sludges, manure...). They are continuously detected in the environment because their rate of removal is compensated by the daily input of new molecules, and they present a risk of toxicity for non-target organisms and human health. In the environment, they are present as mixtures of compounds and they coexist with other pollutants such as, for example, metalic trace elements. Nowadays, there is a lack of information about when, where, at what concentration and about the potential interactions between the different compounds (cocktail effect). Indeed, metals can for example interact with pharmaceuticals in particular through the formation of complexes which may affect their behaviour in the environment. Among the processes governing the fate of pharmaceuticals, adsorption processes play a key role controlling their mobility and bioavailability to organisms in soil and water compartments. It is thus necessary to bring knowledge about their behaviour at solid/water interfaces and their toxicity towards organisms considering the cocktail effect in order to design more realistic scenarios. The aim of this work is to bring knowledge about the behaviour in the environment of three pharmaceuticals considering their cocktail effect: sotalol (a beta-blocker), furosemide (a diuretic) and sulfamethoxazole (an antibiotic). We present in this study (i) their adsorption behaviour on a selected soil as a function of concentrations, which is needed to define the mobility of the compounds and to precise at which extent they are available to organisms, and (ii) their toxicity on two aquatic organisms (Vibrio fischeri, and Daphnia magna) at different concentrations. In each case, we studied the behaviour of each molecule taken separately and of mixtures containing the three pharmaceuticals. In addition, the influence of the presence of copper(II), an ubiquitous metallic cation, was also considered. Keywords: adsorption, toxicity, pharmaceuticales, metals.

432 Occurrence and environmental risk assessment of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya

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In this study, we investigated the occurrence of three antibiotics (sulfamethoxazole, trimethoprim and ciprofloxacin) and three antiretroviral (lamivudine, nevirapine and zidovudine) drugs in Nairobi River Basin, Kenya. The analytical procedure involved extraction using solid phase extraction followed by liquid chromatography-electrospray ionization tandem mass spectrometry (SPE-LC-ESI-MS/MS). In the study, 40 sites were selected for sampling, including 38 sites along the rivers and 2 wastewater treatment effluents sites. All the studied compounds were detected with sulfamethoxazole having the highest detection frequency of 97.5% and ciprofloxacin had the lowest at 60%. The results showed that the concentration of the drugs increased in highly populated regions especially within the informal settlements. The maximum (median) concentration in the river waters for sulfamethoxazole, trimethoprim, ciprofloxacin, lamivudine, nevirapine and zidovudine in mg/L were 13800 (1800), 2650 (327), 509 (129), 5430 (1000), 4860 (769), and 7680 (660), respectively. The maximum concentrations in the river waters were generally higher than those of the wastewater treatment plant effluents signifying that the rivers are substantially contaminated by domestic wastewater. The environmental risk was evaluated by calculating the risk quotients (RQs) for algae, daphnia and fish based on the maximum and median concentrations of the analytes in the river basin and was expressed as the ratio of measured environmental concentrations (MEC) to predicted no effect concentrations (PNEC). The RQs ranged from 0–507.8 and apart from lamivudine that had a low RQ, all the other analytes had RQs>1 at maximum and median measured concentrations for at least one taxonomic group. The high RQs are indicative of possible adverse ecological effects and calls for corrective and mitigation strategies. Key words: Antibiotics, antiretrovirals, occurrence, risk.
433 Changes in the metabolome of Mytilus galloprovincialis exposed to fullerene aggregates under environmentally relevant conditions
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The experimental study of nanomaterials has been a thriving topic during the last years because of their high number of potential emission sources; unique transport and distribution patterns, different to those of the conventional micropollutants; and the high uncertainty around their ecological effects. Among them, fullerenes have received a particular attention and their presence in rivers [1,2], soils [3], wastewater effluents [4] and air particulate [5] has been recently characterized. Because of their particular colloidal behaviour the properties and toxic effects of fullerenes in experiments at lab-scale are highly influenced by the dispersion method and physico-chemical properties of the medium. Therefore, conducting experiments simulating conditions similar to those observed in the real environment is crucial in order to create aggregates with realistic toxic effects and results that are translatable to the real environment. In the present work fullerenes were dispersed and aged in artificial estuary water (with controlled ionic strength, pH and humic acid content) by means of long time stirring with air and sunlight exposure. The short and long-term changes were characterized by means of scanning electron microscope analyses and nanoparticles tracking analysis. The obtained suspensions were employed in metabolic experiments involving Mediterranean mussels (Mytilus galloprovincialis). The exposition was carried out in marine mesocosms of 450 l with pristine seawater at sub-pb concentration levels. The metabolomic profile of the exposed mussels was analyzed by LC-ESI-HRMS with hybrid quadrupole-Orbitrap analysers in data-dependent scan acquisition mode. Several time-dependent changes were observed in the tested organisms, including changes in the lipid composition, changes in the aminoacid profile and the appearance of oxidative stress biomarkers, such as ophthalmic acid. Overall, the results are consistent with an oxidative stress response, which is observed even at sub-pb concentration levels and that can raise questions about the chronic toxicity of these emerging contaminants. [1] J. Sanchí et al. (2014) Analytical and bioanalytical chemistry, 407(15), 4261-4275. [2] A. Astefaniei et al. (2014) Journal of Chromatography A 1365, 61-71. [3] J. Sanchí et al. (2015) Science of the Total Environment, 505, 172-179. [4] M. Farti et al. (2010) Journal of hydrology, 383(1), 44-51. [5] J. Sanchí et al. (2011) Environmental science & technology, 46(3), 1335-1343

434 Assessing the heteroaggregation of manufactured nanoparticles with naturally occurring colloids in a typical surface water
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To assess the risk posed by nanotechnology-enabled products, the likelihood of engineered nanoparticle (NP) exposure through aqueous media must be considered, as it is a receptacle of these materials throughout their lifecycle. The predicted concentrations of engineered NPs in surface water systems are expected in the µg/L level for nano-TiO₂, and consequently, in real aquatic systems, the probability that NPs interact with each other may be lower relative to their collision frequency with naturally occurring colloids present at substantially higher concentrations (mg/L to g/L). Due to the high specific surface area and reactivity of colloids, they may strongly affect the fate and transport of NPs via heterogeneous aggregation processes. Thus, fate models aimed at predicting NP behaviour and concentration profiles must account for this heteroaggregation. A NP-colloid sticking efficiency, θ_{hetero}, is well suited as an input for such fate models, but remains a challenge to determine experimentally. Here, we present a novel method for determining θ_{hetero} at environmentally relevant NP concentrations by using a combination of laser diffractometry measurements and aggregation modeling based on the Smoluchowski equation. Interactions between TiO₂ NPs (15 nm) and different types of larger mineral colloids (i.e., silica microspheres, smectite clay, and natural riverine suspended particulate matter) were used to demonstrate this new approach. Studies were conducted at low NP concentrations (0.1 to 4 mg/L) with regard to the colloid occlusion (100 mg/L) to develop realistic fate scenarios for surface in situ systems. The NP-colloid number ratio was found to be a key component in the heteroaggregation mechanism and the effects of ionic strength, pH, and natural organic matter on NP heteroaggregation were also explored. Our data show that at relevant concentrations, NP behaviour is mainly driven by heteroaggregation with colloids, while homoaggregation remains negligible. The dimensionless θ_{hetero} value is a key parameter needed to feed environmental fate models that are of high importance in the field of risk assessment of engineered NPs, as they contribute to better predict the exposure aspect in aqueous systems with continuously increasing relevance. Work funded by the French National Research Agency as NANOHETER program under the frame of SHIN, the ERA-NET, and the EREF. France – U.S. bridge funded by the PUF Program. http://nanoheter.cerefe.eu

435 Effect of surface coating on nanoparticle stability and fate in high strength electrolyte solutions in silver nanowires in marine water
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Much of the early work on the characterisation of nanomaterials focused on pristine nanomaterials in simple matrices such as ultrapure or freshwater. Characterisation of nanoparticles in more complex matrices such as physiological fluids has not had the same level of attention paid to it and analysing nanoparticles in such matrices remains a significant challenge. While some methods for the extraction of nanoparticles from confounding matrices have been brought to bear, they are relatively harsh leading to the nanoparticles or their (bio)surface coating being modified as a result of the extraction process. Hence, the characteristics of extracted nanoparticles that are ultimately determined are not the same as when they were originally in the matrix. This has a critical impact on toxicity studies as these extracted nanomaterials are ultimately only proxies for the original biological identity of the nanomaterial that would have been encountered by living organisms. To investigate if the bio-corona coating is preserved, we have studied bovine serum albumin-coated silver nanoparticles as a proxy for probing the impact of a bio-coating on nanoparticle behaviour and fate in high strength electrolyte solutions. We have found that bovine serum albumin (BSA) stabilizes silver nanoparticles in high strength electrolytes, with the stabilisation effect increasing with both ionic strength and BSA concentration. The ion release kinetics of the dissolution of silver nanoparticles is significantly lower in the presence of BSA and may be related to BSA suppressing ion release or acting as a store for silver ions and hence the measured quantity of ions released in solution is misleading. Thus understanding the impact of a bio-corona on nanoparticle behaviour in complex matrices is a key preliminary factor that must be considered when modelling nanoparticle fate and investigating their toxic potential in real life scenarios.

436 Extrapolated long-term stability of titanium dioxide nanoparticles and multi-walled carbon nanotubes in artificial freshwater
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The potential environmental release and the resulting exposure to engineered nanomaterials (ENM) along their life cycle are expected to constantly increase (Nowack et al., 2012; Pettit and Lead 2013). Based on environmental exposure scenarios, water is one of the main compartments where ENMs can be released (Nowack et al., 2014). Shah et al. (2014) showed that in the marine environment, intrinsic physico-chemical properties of ENMs as well as the specific environmental conditions mainly determine fate and behaviour of ENMs (Hartmann et al., 2014). Therefore, a comprehensive characterization of all the processes affecting the ENMs system stability is needed. Among all, transformation processes, such as surface modification, homo- and hetero-aggregation, and transport processes, i.e. advection and sedimentation, have been identified as key factors to affect fate and behavior of ENMs in the aquatic environment (Quik et al., 2014). In this work, long-term stability of ENMs, i.e. the inorganic n-TiO₂, P25 and the organic Multi-Walled Carbon Nanotubes (MWCNTs) NC7000, dispersed in artificial freshwater (500 mg-1), was investigated by extrapolation from short-term stability experiments. Hydrodynamic diameter and ζ-pot, calculated by means of Dynamic and Electrotheoretic Light Scattering, respectively, qualitatively indicated a general ENMs dispersions instability over 1 h time. Sedimentation results, obtained by centrifugation, allowed to estimate the quantitative long-term (over 30 days) stability of ENMs. Settling data fitted satisfactorily with a first order kinetic equation (R² in the range of 0.918-0.989). The extrapolated settling rate constant k values at gravity spanned one order of magnitude, i.e. from 7.21·10⁻⁶ to 4.12·10⁻², and increased with the increasing of initial ENMs concentration. Sedimentation velocities were in good agreement with those reported in complex matrix (7.92·10⁻² - 1.7·10⁻¹ m·d⁻¹ vs. 5·10⁻³ - 3·10⁻¹ m·d⁻¹ for n-TiO₂ and 5.9·10⁻² - 3.4·10⁻¹ m·d⁻¹ vs. 2·10⁻¹ - 1.2·10⁻² m·d⁻¹ for MWCNTs). n-TiO₂ showed a higher long-term stability with respect to MWCNTs (average: 1·10⁻³±3.4·10⁻³ m·d⁻¹ instead of 1·10⁻¹±1·10⁻¹ m·d⁻¹, respectively).

437
Assessing the detection limits of multi-isotopically labelled CdSe/ZnS quantum dots in natural and biological environments

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The vast majorities of NPs-related studies presented in the literature are carried out in conditions far from those in real environmental medium [1] (water, soil, sediment). In particular, the high concentrations (>1 mg/L) of nanoparticles (NPs) used in experimental studies are related to the difficulty of detecting nanoparticles in complex and "noisy" natural and biological environments. However, changes in NPs concentration can affect its physicochemical behavior (ex: solubility, aggregation) and thus the final interpretation and understanding of results. In order to overcome analytical barriers while working at representative realistic concentration, innovative tools such as HR-ICP-MS and non-traditional stable isotopes (isotopically modified NPs [2] or "spiked") have been used. 7 nm sized isotopically labelled quantum dots (QDs), CdSe/ZnS core-shell structure were synthesized, enriched in 68Zn, 77Se and 111Cd. They were disseminated at very low concentration using a protocol described by Dybowska et al. [3]. Preliminary results allowed to assess the detection limits of spiked QDs in complex matrices such as river water or saliva and plasma. The feasibility of isotopic labeling at very low concentrations has been demonstrated: spiked Zn, Cd and Se issued from QDs were quantifiable at 10, 3 and 20 ng/L respectively in a media not containing the sampling matrix (water or saliva) and equivalent with 1 ng/L of spiked Zn in Seine river water. The results obtained in this experimental work are applicable for studying QD's fate and behavior in most aquatic and biological media.

Fish model species in environmental toxicology

438 Addressing whetherroach in English rivers have adapted to the harmful effects of exposure to oestrogenic wastewater treatment work effluents

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Wastewater treatment work (WWTW) effluents comprise a large proportion of the flow of lowland rivers across the world. Many WWTW effluents are oestrogenic and induce a range of feminised phenotypes in wild male fish including the intersex condition – the presence of developing eggs in the testes of otherwise male fish. In UK rivers, the reproductive success of the most feminised male roach (Rutilus rutilus) has been reduced to 76%. However, population genetic analyses have identified some populations of roach that have been largely resistant to the effects of oestrogenic effluent over multiple generations. This raises the question of whether roach can, and have, adapted to the harmful effects of exposure to oestrogens. To investigate this, we are searching for footprints of selection by analysing single nucleotide polymorphisms (SNPs) in wild roach populations; in tandem with an experimental approach, to examine differences in oestrogen sensitivity. We assembled a transcriptome for roach and used it to identify SNPs in a suite of oestrogen-responsive genes including aromatases, oestrogen receptors and vitellogenins. Genotyping wild roach populations derived from four effluent contaminated rivers (Lee, Foss, Aire and Mole) and five other river stretches that receive little or no effluent using this SNP panel revealed variation in oestrogen-responsive genes, and we are currently investigating patterns of selection in these genes. Analysis of SNPs derived from restriction-site associated DNA (RAD) tag genotyping of roach from the polluted River Lee and nearby clean river Cuffley Brook identified diverse genes with signatures of selection including several that are related to regulation of cell growth and DNA repair in humans. Functional annotation in DAVID identified two overrepresented GO terms: metal ion binding and exposure to organic substances, both consistent with the view that exposure to chemical pollution has acted as a selective pressure on roach in the River Lee. We have also conducted a 2 year exposure to 1.8 mg/L ethylenedioctoetradiol (ED2) to compare sensitivities of the offspring of roach from different rivers. Exposure resulted in a range of gonadal phenotypes in male fish; from fish with normally developed gonads to those with both male and female gametes (intersex) and an ovarian cavity. We are now comparing feminised responses between fish from different rivers.

439 Integrating biotic factors in toxicokinetic modelling of metal accumulation in fish

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Fish are exposed to both pollutants and parasites. Parasites might affect the toxicity of pollutants like metals. So it is important to consider parasitism in modelling metal accumulation in fish. Toxicokinetic (one- or multi-compartment) models have been used for simulating metal accumulation in aquatic organisms. The multi-compartment model allows for predicting tissue-specific concentrations, but requires a larger number of parameters. We developed a one-compartment model and a multi-compartment model (covering blood, storage, gills, kidney, liver, intestine, and gallbladder) to simulate metal accumulation in the chub Squalius cephalus unreinfected and infected with the acanthocephalan Pomphorhynchus laevis. In models for the uninfected chub, uptake toxicokinetics was related to species weight and metal properties while the absorption efficiency and elimination rate constants were parameterised based on published data. For the infected chub, physiological parameters were parameterised from the value for the uninfected. Tissue-blood partitioning in the multi-compartment model was calibrated using published data on 210Pb accumulation over time, but not validated. The one-compartment model was validated using data set on Pb concentrations in the whole fish at the end of exposure experiments. Predicted concentrations of Pb in the fish-parasite system by the one-compartment model were significant related to the measured. The estimates were generally within one order of magnitude of the measurements. Moreover, the predictions of Pb accumulation in the system were more sensitive to the absorption efficiency than to the elimination rate constant. For the multi-compartment model, preliminary calibration results show high tissue-blood partitioning in intestine and gallbladder but both their rates of elimination. Effects of parasites on the tissue-blood partitioning in liver and intestine. Modelled concentrations of 210Pb in all fish organs were generally within one order of magnitude of the measured. Stable concentrations in storage and increasing concentrations in intestine and gallbladder of the uninfected chub could be explained by the model. The stability in 210Pb accumulation in different fish according to gender and treatments. This work will give a better understanding of the significance of these data and point towards further improvements in these models.

Ecotoxicological impacts of cyanobacteria on fish: effects of chronic exposure of medaka fish to cyanobacteria-dominated conditions

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The interactive effects of eutrophication and climate change may promote cyanobacterial blooms in continental aquatic ecosystems, which pose potential risks to fish ecology and ecosystem sustainability. To date, more than 600 cyanobacterial compounds potentially toxic have been described from various taxa. These cyanotoxins can be released by cyanobacteria into the water leading to potential toxic effects to aquatic organisms. Among the various families of cyanotoxins, two classes of metabolites have attracted more attention due to their toxicity: the hepatotoxins such as microcystin (MC) and the neurotoxins. Although their presence has been documented in fish, the effects of cyanobacteria on fish physiology and tissue-blood partitioning in liver and intestine. Modelled concentrations of MC in all fish organs were generally within one order of magnitude of the measured. Stable concentrations in storage and increasing concentrations in intestine and gallbladder of the uninfected chub could be explained by the model. The stability in MC accumulation in different fish according to gender and treatments. This work will give a better understanding of the significance of these data and point towards further improvements in these models.
and complex mixtures

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There are over 100,000 chemicals on the market, many of which end up in the environment as complex mixtures. The current chemical-by-chemical approach to environmental quality assessment has significant limitations, as only a limited number of chemicals can be assessed. Testing or monitoring single chemicals also does not address the complexity associated with mixtures. There is growing public concern about the risks associated with low levels of potent chemicals such as drugs, hormones and pharmaceuticals which end up in the environment via waste streams. Therefore, other sensitive methods are needed to assess the risk of chemical mixtures certainly when they are presented in complex mixtures, like in the environment. Here, we aim to provide a new sensitive genomics-based tool for safety assessment of chemicals and complex mixtures. Therefore we developed ZF-Tox-Array, a combination of state of the art multiplex qPCR and zebrafish (Danio rerio) toxicity testing, as a new cost-effective, sensitive effect-based approach to screen chemicals and environmental samples for their toxicity. With the ZF-Tox-Array, multiple toxic modes of action (MoA) can be assessed in parallel compared to standard applied in vitro assays which are mostly specific for only one MoA. Our new system allows to screen up to 42 target genes, covering several toxicity pathways in parallel. Based on literature, target genes were selected known to be involved in commonly assessed toxicity pathways was mainly focused on DNA covering, apoptosis, stress response, metabolism, metabolism phase II/III, endocrine disruption, adipogenesis, insulin signaling and neurotoxicity. A great advantage of our ZF-Tox-Array system is the flexibility as the list of target genes can be adapted to a variety of MoA of interest. The ZF-Tox-Array system was first validated and optimized using model substances. In the next step we applied our new system to screen our environmental pollutants for their neurotoxic potential using a special set of neurotox target genes. In an additional study we selected 42 target genes covering different MoAs that are commonly studied with water risk assessment and screened different drinking, surface and waste water samples for their toxicity. Our study shows that even at concentrations where no visual malformation can be seen strong gene expression changes can be observed and that new toxic insights can be gained by using ZF-Tox-arrays.

442

Stickleback FET test with gene expression analysis can detect endocrine disrupting chemicals

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The three-spined stickleback (Gasterosteus aculeatus) is a useful sentinel species in chemical risk assessment, particularly for detecting endocrine disruptors (EDs). As EDs are known to be toxic to fish at low concentrations, using stickleback embryos can be developed as an alternative to adult fish test in compliance with animal welfare. Gene expression analysis following FET test can provide an insight on the signalling pathway responding to chemicals and as such can provide an early warning for downstream effects. To understand the differences in transcriptional response to chemicals between adult fish and embryos, we performed global gene expression analysis of the embryos using microarrays (Agilent-029767) following FET test. In the FET test, we exposed fertilized stickleback eggs to the 6 single chemicals (cadmium, dibutyl phthalate, fluoxetine, ibuprofen, 17α-ethinylestradiol(E2) and levonorgestrel) and 6 mixtures, each consisting of 5 chemicals for 10 days. The results revealed that for the stickleback embryo to reach the self-feeding stage). The microarray results were compared with those obtained from adult fish liver after 4 days of exposure. Our overall aim was to investigate if the fish embryos were capable of expressing at least partly the signalling pathway from molecular initiating event to adverse outcome and evaluate the potential relevance of adverse endpoints statistically. No significantly expressed in 6 single chemicals from controls was as follows: E2 = 3.5, levonorgestrel = 10, DBP = 875, Cd = 940, Ibuprofen = 1589, Fluoxetine = 0. Similar response was found after Cd, ibuprofen, and DBP exposure, suggesting typical chemical stress response (e.g. oxidative stress). For E2, estrogen related genes (vitellogenin, chorion) were upregulated as was previously found in adult fish. For levonorgestrel, only single gene was upregulated, which was not observed in the liver of adult fish (spiggin is expressed only in the kidney of adult fish). Levonorgestrel antagonised the action of E2 by significantly reducing the expression of vitellogenins and chorionins, whereas E2 antagonised the action of levonorgestrel by reducing the expression levels of spiggin. Both findings were identical to those observed using adult fish. These results indicate that the stickleback FET test is a useful screening assay for EDs, providing clear responses to both estrogenic and androgenic chemicals.

MetaOMics in ecotoxicology: evaluation of alterations in the structure and functions of ecosystems

443

Next-Generation Sequencing to highlight community changes in river biofilms linked to pharmaceuticals from a wastewater treatment plant

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444

Structure of microbial communities impacted by hydrocarbons in oxic/anoxic interface in coastal marine sediments

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Northern marine sediments constitute unique functional ecosystems constantly subjected to oxic/anoxic oscillations according to tide cycles, bioturbation activities by macrofauna and other mechanical processes. Due to their location they are exposed to pollution injuries such as oil spills. Microbial communities play critical roles in global biogeochemical cycles in marine sediments, particularly in response to the presence of hydrocarbons. Thus, understanding the response of these key microorganisms exposed to hydrocarbons under oxic/anoxic conditions provides pivotal information for estimating the resilience capacities of the ecosystem. Recently, we showed that the organization of hydrocarbon degrading bacterial communities was driven by sediment reworking activities (Staufert et al., 2013). Indeed, three different microbial communities were obtained applying various reworking strategies but their overall degradation efficiency was similar highlighting the fundamental redundancy involved in hydrocarbons degradation. In order to further characterize the microbial assemblages, two microbial communities were incubated in different oxygen regimes (oxic, anoxic and oscillating conditions) in bioreactors. Both Bacteria and Archaea 16S rRNA transcript amplicons were analysed by high-throughput sequencing. Although PICRUSt functional analyses (Langille et al., 2013) revealed that the three Archaea communities were different, they were not affected by the oxygenation conditions imposed in the bioreactors. In contrast, Bacteria communities were structured according to the oxygenation conditions. Correlation analysis showed that Archaea/Bacteria interactions, particularly the methanogens/sulfate-reducing bacteria relationships, are of primarily importance in the microbial communities assemblages in presence of hydrocarbons including the hydrocarbon degradation capacity. We thus suggest to carefully examine the Archaea/Bacteria interactions in microbial ecotoxicology studies to evaluate the

**445** Large-scale shotgun metagenomics of periphyton communities exposed to triclosan
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High-throughput DNA sequencing has a great potential to detect effects from toxicants in natural communities. However, its potential in community ecotoxicology has so far not been realized. In this study we test if high-throughput Illumina shotgun sequencing can identify effects of the bactericide triclosan (TCS) in natural marine periphyton (biofilm) communities. We performed a long-term (17 days) flow-through microcosm experiment with natural marine periphyton. These communities are highly diverse and a large sequencing effort is needed to access the community, its good soil controls and 8 TCS exposure levels, covering a concentration range of 0.32-1000 nM with and triplicates of the 3.16 nM, 31.6 nM and 316 nM treatments, was included. Paired-end sequencing libraries was prepared with the TruSeq PCR-free kit and sequencing was done using the HiSeq 2500 Illumina sequencing platform. We generated 475 Gbases (18 billion DNA read pairs) of metagenomic sequence data, which after quality control summed up to ~ 3.5 billion sequences. After assembly we obtained 1,284,307 scaffolds with an average N50 value among the samples of 3662 bp. The composition of these protein-encoding gene families different database. The salinity stressor has an important impact into the community that drive the variation in the community and the adaptation to the different temperatures might alter these effects. Using climate chambers, we investigated effects of TCS in community structure, and to compare shotgun and amplicon sequencing approaches. Bioinformatic analyses are ongoing, but first annotation results using TIGRFAM identified genes from 3415 functional classes, of which 656 were differentially abundant in unexposed control communities vs. communities exposed to 316 nM TCS. The composition of these protein-encoding gene families in control communities and communities exposed to 316 nM TCS was clearly different. TIGRFAMS that were found to be significantly more abundant in exposed communities included, for example, those for protein and energy metabolism, metal resistance and various transporters (including ABC-transporters). TIGRFAMs significantly more abundant in unexposed controls included, for example, many genes encoding photosynthesis proteins. We will also present further analyses of TCS effects on functional and taxonomic composition, as well as analyses of proteins correlated to TCS tolerance. To our knowledge, this is the first large-scale metagenomics study of toxicant effects on microbial communities.

**446** Characterization of environmental metallothioneins: a new metallothionein family isolated from soil eukaryotic metatranscriptome.
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the presence of sulphide ligands in the Cd-EMT complexes, and the presence of aromatic amino acids. But they have unusual features too: an “important” l

The risk posed by plant protection chemicals to amphibians shall be evaluated applicable insights into the toxicity of contaminants and, instead, introducing changes in the experimental setup, all also depending on the studied species. This risk assessment procedures rely on surrogate species (e.g. fish and birds) to cover the variation in the community and the adaptation to the exposure to pollution and to abiotic changes in the environment. The SBD-foil method for protein extraction has increased the amount of protein recovered as well as the biodiversity. This method was offering the highest values for reproducibility among biological replicates. Our results indicate that two step in the data analysis pipeline are crucial to implement this methodology for environmental assessment: the selection of the database or the utilization of an in-house database for protein identification, and the comparison between search engines. We compare our results from two database: Cameraad NCBI non-redundant repository database and the LC-MS (2)-spectra were searched using a combination of two different search engines: Unipet and MEGAN against different database. The salinity stressor has an important impact into the community response. Our results indicated that the amount of microbial community responses could be correlated to both changes in the molecular responses of the communities, interspecies interaction and therefore interpret and predict the ecosystem response to stressors or exposure to pollutants. Furthermore, we can explore the prediction capabilities of these methodologies to develop a metaproteomics-based environmental assessment.

**447** DVMICO, metaproteomic analysis of environmental microbicide dynamics as response to pollutants and stressors
J. Kuruvilla, Linköping University / Clinical and Experimental Medicine; G. Danielsson, Stockholm University / Biochemistry and Biophysics; S. Cristobal, Linköping University / Department of CI and Exp Medicine Cell Biology Metabolomics analysis could provide a functional overview from several levels of complexity: at the community level, how the communities respond to the exposure or stress; at the molecular level, which protein families have been modified their level of expression; and at the biochemical level, which catalytic reaction with relevant role to maintain the community in equilibrium. We presented the metaproteomic analysis of aquatic sediments from Baltic Sea exposure to propranolol and salinity changes for 6 weeks. We tested if a metaproteomics-based assessment can estimate: i) variation in the abundance of protein families, ii) changes in biodiversity, and iii) what are the key players in the community that drive the variation in the community and the adaptation to the exposure to pollution and to abiotic changes in the environment. The SBD-foil method for protein extraction has increased the amount of protein recovered as well as the biodiversity. This method was offering the highest values for reproducibility among biological replicates. Our results indicate that two step in the data analysis pipeline are crucial to implement this methodology for environmental assessment: the selection of the database or the utilization of an in-house database for protein identification, and the comparison between search engines. We compare our results from two database: Cameraad NCBI non-redundant repository database and the LC-MS (2)-spectra were searched using a combination of two different search engines: Unipet and MEGAN against different database. The salinity stressor has an important impact into the community response. Our results indicated that the amount of microbial community responses could be correlated to both changes in the molecular responses of the communities, interspecies interaction and therefore interpret and predict the ecosystem response to stressors or exposure to pollutants. Furthermore, we can explore the prediction capabilities of these methodologies to develop a metaproteomics-based environmental assessment.

**Pollutant risks to amphibians and reptiles: how much we know and what we need**

**448** Compared ecotoxicity of raw and oxidised carbon-based nanoparticles on Xenopus laevis larvae
L. Lagier, S. Cadarsi, ECOLAB UMR5245 CNRS UPS INPT; A. Mottier, ECOALB UMR5245 CNRS; C. SARRIEU, E. Filaht, CIRIMAT CNRS 5085 UPS INPT; E. Pinelli, F. Mouchet, L. GAUTHIER, ECOLAB UMR5245 CNRS UPS INPT Carbon-based nanoparticles (CNPs) such as carbon nanotubes or graphene have numerous attractive properties leaching to their use in many application fields whether they are raw or functionalised, such as oxidised. More and more produced, these nanoparticles may finally contaminate the aquatic environment, which is a major recepacte of pollutants. This work consists in assessing the ecotoxicological risks induced by a range (from 0.05 to 50 mg.L-1) of double-walled carbon nanotubes (DWCNTs) and few layer graphene (FLG) on the amphibian Xenopus laevis. We studied the influence of surface chemistry by comparing raw materials with their functionalised counterparts testing different oxidation levels and thus different nanoparticles: oxidised DWCNTs...
449 Effects of a glyphosate-based herbicide and temperature on the development of Common toads (Bufo bufo, L.; Amphibia: Anura) 

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Herbicides based on the active ingredient glyphosate are increasingly applied in agriculture, horticulture and private gardens all over the world. Recently, leaching of glyphosate or its metabolite into water bodies inhabited by amphibians has been reported. However, very little is known about non-target effects of these herbicides on amphibians and even less is known to what extent different temperatures might alter these effects. Using climate chambers, we investigated the effects of the glyphosate-based herbicide Roundup PowerFlex® (480 L g⁻¹ glyphosate, formulated as 588 L g⁻¹ potassium salt) on the larval development of Common toads (Bufo bufo, L.; Amphibia: Anura) under different temperature regimes (15°C vs. 20°C). We established five herbicide concentrations: 0, 1, 3, 3, 4 mg acid equivalent L⁻¹ and a 4 mg a.e. L⁻¹ pulse treatment (totally three applications of 1, 1.5 and another 1 mg a.e. L⁻¹) at each temperature in a full-factorial design. Each treatment combination was replicated 5 times; the experiment ran for 24 days. Results showed no effect of herbicide concentration on body length and body width but a highly significant effect of temperature on these growth parameters. Moreover, highly significant interactions between herbicide and temperature on body length and body width were observed suggesting that herbicides had different effects on different temperatures. In conclusion, although Roundup PowerFlex® at the tested concentrations appeared to have no acute toxicity to larvae of Common toads, the observed effects on tadpole growth will potentially affect competitive interactions in spawning ponds of amphibians. Our findings of herbicide x temperature interactions might become more prevalent when human-induced climate change will lead to more extreme temperatures.

450 Standardize or diversify conditions in experimental ecotoxicology? A case study on herbicide toxicity to larvae of two anuran amphibians


As a part of biodiversity crisis, amphibian species are experiencing population declines and extinctions throughout the world. One of the major causes of these declines is increasing pesticide use. Even though there is a steeply increasing number of ecotoxicological studies treating the effects of pesticides on non-target organisms, studies assessing the adequacy of different experimental approaches have been remarkably scarce. We scrutinized effects of a glyphosate-based herbicide on larvae of two European anuran amphibians by estimating species-specific LC50 values. To assess how an additional factor may influence outcomes of a standard toxicity test, we investigated whether predation threat exacerbated the effects of the pesticide. Finally, to study how repeatable the measured effects are across experiments, we repeated experiments several months later under identical experimental conditions (Rana dalmatina) and common toad (Bufo bufo) tadpole exposed to two predator treatments (no predator, dragonfly larvae) combined with seven (0, 0.009, 0.03, 0.24, 1, 2, 6.5 mg a.e. / L glyphosate) and nine (0, 1, 2, 3, 4, 5, 6, 7, 8 mg a.e./ L glyphosate) herbicide concentrations in a full factorial design and repeated the experiment with a subset of the experimental treatments and partly with slight modifications on a different number of animals. Our results indicated that the herbicide was moderately toxic to the studied animals. The presence of chemical cues on predation threat increased the toxicity of the herbicide in case of agile frog tadpoles, but, surprisingly, made the herbicide less lethal for common toad tadpoles. The repeatability of the experimental results across experiments differed between the two species: the estimated sensitivity of agile frog tadpoles varied considerably, while in case of common toad tadpoles, it remained very similar across experiments. Our results support the hypothesis that the effects of pesticides can be significantly influenced by additional stress factors and by slight changes in the experimental setup, all also depending on the studied species. This draws attention to the suggestion that strict standardization may not deliver widely applicable insights into the toxicity of contaminants and, instead, introducing some variation into the design of future ecotoxicological experiments may prove highly beneficial.

451 New histological and molecular biomarkers for developmental reproductive toxicity in Xenopus tropicalis

M. Säfholm, E. Jansson, Uppsala University / Dept of Environmental Toxicology; C. Berg, Dept. of Environmental Toxicology / Dept of Environmental Toxicology

The risk posed by plant protection chemicals to amphibians shall be evaluated according to the EU Plant Protection Product Regulation. To accomplish this, amphibian test methods for investigating adverse effects of chemicals need to be developed. The present study aimed to develop the Xenopus tropicalis test system for developmental reproductive toxicity by characterising molecular and histological features of sexual development. The ontogenetic development of the Müllerian ducts (precursors of the female reproductive tract) was characterized histologically. In addition, the mRNA expression of amh (anti-Müllerian hormone), amh2 (amh receptor 2), intracellular and membrane progesterone receptors (ipgr and mpgr beta) and cytochrome P450 19a1 (cyp19a1) were determined in the urogenital complex (composed of kidney tissue, gonads and sex ducts). amh expression was evaluated as a molecular biomarker for phenotypic sex. The animals were sampled for mRNA analysis during sex differentiation at Nieuwkoop and Faber (NF) stage 51 and 56, and at 4 weeks post-metamorphosis. Gonadal and Müllerian duct development were characterized histologically at 4 weeks post-metamorphosis. As X. tropicalis displays a high variability in larval developmental rate, the sampling scheme was designed to obtain both age- and stage matched groups. The results show that the amh mRNA expression levels were higher in individuals with low cyp19a1 expression (ovarian marker) and vice versa. The sexually dimorphic expression profile was more distinct for amh than for cyp19a1, supporting our hypothesis that amh expression is useful as a testicular biomarker during gonadal differentiation in X. tropicalis. The pgrs expression levels increased over the studied period and showed no sex differences. The cyp19a2 expression level was higher in females than in males at NF 56 and at 4 weeks post-metamorphosis. The histological evaluation showed that folliculogenesis had initiated and that the Müllerian ducts were larger in females than in males. The proportion of follicular oocytes in the ovary at 4 weeks post-metamorphosis increased with increasing time for the individual to complete metamorphosis, emphasizing the importance of having study groups that are both age- and stage matched in toxicity studies of larval amphibians. This new knowledge on sexual development in X. tropicalis is useful in the development of early life-stage endpoints for developmental reproductive toxicity.

452 Acute oral toxicity in terrestrial life stages of amphibians: comparisons to birds and mammals

L. Weltje, BASF SE / Crop Protection Ecotoxicology; M. Crane, AG-HERA; M.C. Finnegan, Syngenta; S. Kosmala-Grzechien, M. Gross, WCA Environment; J. Wheeler, Dow AgroSciences

Amphibians are currently the most threatened and rapidly declining group of vertebrates and this has raised concerns about their potential sensitivity and exposure to plant protection products and other chemicals. Current environmental risk assessment procedures rely on surrogate species (e.g. fish and birds) to cover the risk to aquatic and terrestrial life stages of amphibians, respectively. Whilst a recent meta-analysis has indicated that most cases of impacts to terrestrial amphibian life stages that are available for acute chemical oral exposure when compared with mammals and birds. Are there correlations between oral toxicity data for amphibians and data for mammals or birds?”. Identifying a relation between these data may help to avoid additional vertebrate testing. Acute lethal oral amphibian toxicity data collected from the scientific literature and ecotoxicological databases were compared with toxicity data for mammals and birds. It was found that toxicity data for amphibian life stages are less sensitive to chemicals than fish, little research has been conducted on the comparative sensitivity of terrestrial amphibian life stages. Therefore, in this paper we address the questions “What is the relative sensitivity of terrestrial amphibian life stages to acute chemical oral exposure when compared with mammals and birds?” and “Are there correlations between oral toxicity data for amphibians and data for mammals or birds?”.

SETAC Europe 26th Annual Meeting Abstract Book 103
Do developmental stage and route of exposure determine pesticide effects on lizards?

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Reptiles are an understudied group in ecotoxicology and the risk of pesticides on them is supposed to be covered by bird and mammal toxicitity data derived from oral routes. Exposure of juveniles through dermal uptake and/or overspray of pesticides once hatchlings leave the nest could constitute relevant exposure ways for reptiles. We conducted a two-stage exposure of wall lizard (Podarcis muralis) eggs and hatchlings to three pesticides in order to analyse the effects of pesticide exposure on each developmental stage and to compare the relative importance of dermal and oral exposure of juveniles through different exposure routes. Eggs were incubated in soils previously sprayed with a pesticide solution corresponding to 40% of the field application rate (to consider relevant crop interception) of formulations of either glufosinate ammonium (herbicide), folpet (fungicide) or fenpyroximate (insecticide). Eggs exposed to folpet hatched earlier and with a lower body mass than controls, whereas fenpyroximate reduced body size at hatching. Upon hatching, animals were assigned to a juvenile treatment consisting in the exposure through spiked soil (dermal) or food (oral) in a factorial block design considering both in-ovo and hatching exposure. Concentrations during the hatching exposure corresponded to 25% of the application rate. Interestingly, egg exposure but not hatching exposure determined the body mass gain during the post-hatch stage of lizards exposed to glufosinate ammonium or fenpyroximate. Oxidative stress biomarkers showed different trends as a function of life stage; the activity of glutathione peroxidase was increased after egg exposure to folpet and glufosinate ammonium and also after juvenile exposure to any pesticide. However, in animals exposed to folpet as eggs, and further exposure to the fungicide as juveniles reduced the activity of this enzyme, which could be interpreted as a hormetic response consequence of a saturation of the enzyme synthesis under prolonged exposures to folpet. Effects of pesticides on juveniles exposed either dermally or orally were very similar, which could be interpreted as a hormetic response consequence of a different trends as a function of life stage; the activity of glutathione peroxidase (GSH-Px) showed a hormetic response in juvenile lizards exposed to folpet and fenpyroximate, whereas glutathione-S-transferase (GST) only showed a trend towards a hormetic response in juvenile lizards exposed to glufosinate ammonium.

The WFD aims to provide a holistic approach to managing the water environment in Europe, and brings together objectives to protect the water environment from the effects of chemical pollution and broader ecological objectives, designed to protect the structure and function of aquatic ecosystems themselves. Under the WFD, the overall environmental status of a waterbody (be it river, lake, estuary or coastal) is determined by deriving an overall ecological quality status (EQS) for its ecological receptor of concern (ERC). Chemical status is based on the comparison of the measured concentrations of ‘Priority Substances’ with their substance-specific Environmental Quality Standard (EQS), which represents the concentration below which effects on populations of organisms are not expected to occur. The list of ‘Priority Substances’ regulated under the WFD is updated on a 4-5 year cycle, and currently comprises around 40 substances or groups of substances. While this set of substances is considered (at least under the auspices of the WFD) to represent those with the potential to cause the greatest harm to the environment, it clearly does not comprise all substances with demonstrated adverse effects on aquatic organisms. In fact, there are even all substances with demonstrated adverse effects on European waterbodies, so we need a more flexible and comprehensive approach to forward the sustainable use of chemicals.

Does evidence from field population studies of aquatic wildlife suggest that the current risk assessment of chemicals underestimate toxic stress in Europe?

A. Johnson, CEH Wallingford / Wallingford

Does evidence from field population studies of aquatic wildlife suggest that the current risk assessment of chemicals underestimate toxic stress in Europe?

Does the current risk assessment of chemicals underestimate toxic stress in Europe?

P. Vos, Ohe UBA / Federal Environment Agency / IV Pharmaceuticals

There is evidence that anthropogenic chemicals can have profound local and regional effects on aquatic communities, while the overall relevance of chemicals featuring larger spatial scales remains mostly unknown. The reason for this are both the lack of comprehensive monitoring datasets as well as the availability of respective ecotoxic-data. Here we present the first risk assessment of organic chemicals on a continental scale. The study is based on regulatory monitoring data for a total of 4,000 monitoring sites available from the European Environmental Agency for the years 2008-2012. For the 223 organic substances monitored, the available experimental ecotox-data was collated and supplemented by QSAR predictions in case of data gaps. Results showed that organic chemicals are likely to cause acute lethal and sublethal effects on populations of their chemical status, and aquatic life, or algae species in 14% and 42% of the sites, respectively. Pesticides, brominated flame retardants, and triptylin were the major contributors to the overall chemical risk. Their presence was related to agricultural and urban areas in the upstream catchment. Moreover, the risk of potential acute lethal and chronic long-term effects increased with the number of ecotoxically relevant chemicals that have been analyzed at each site. However, no such monitoring programs considered in this study often included only a subset of these chemicals, our assessment is likely to underestimate the actual risk. Ultimately, we analyzed whether the observed chemical concentrations had any effects on aquatic communities. For that reason, we used a sub-set of sites in France where many of these toxic chemicals’ ecological hazard was analyzed and corresponded to high concentrations. We found that an increasing chemical risk was associated with...
increased deterioration in water quality indices for fish and invertebrates in communities. Our results therefore clearly indicate that chemical pollution is a large-scale environmental problem that requires far-reaching, holistic mitigation measures to preserve and restore ecosystem health. It also suggests that current risk assessment approaches on a substance by substance bases are likely not protective for the environment. This is especially so when considering that the large and chemically diverse group of pharmaceuticals has not been included in this study, due to a lack of monitoring data as well as appropriate ecotoxic data that considers the specific effects of these substances in wildlife. Examples for the latter include the feminization of the male roach (Rutilus rutilus), a widespread spawners-spawning fish in English rivers, due to waterborne estrogens, or the population decline of the eastern white-backed vulture (OVWB; Gyps bengalensis), which was associated with renal failure and visceral gout after feeding on prey treated with anti-inflammatory drugs.

457 Forwarding sustainable use of chemicals via ecological impact analyses: the eco-epidemiological approach

D. DeZwart, DdZ-Ecotox; S.D. Dyer, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; M. Hujibregts, Radboud University Nijmegen / Department of Environmental Science; A.M. Schipper, A. Piliere, K.E. Kapo, C.M. Holmes, Waterborne Environmental, Inc.; A. Burton, University of Michigan / School of Natural Resources Environment; L. Posthuma, RIVM / Centre for Sustainability Environment and Health Monitoring Data have developed us only fragmented insights in chemical exposures of biota (man and species in ecosystems) and – in fact – very little insights in non-integrated data. With applied in-ovo their chemicals present for a long time by man, exposing millions of species, the question is whether we are on the right track in the scientific enterprises underpinning chemical safety regulations. The tome ‘Chemicals without harm’ (Geiser, 2015, reviewed in Science, DOI 10.1126/science.aac9931) collates some visionary approaches towards a safe and sustainable chemical economy. Prioritizing the design of novel chemicals and products being a key step. But logically, we also need to fill the gap between the low percentage of chemicals being considered by regulations and the plethora of chemicals and mixtures out there. Eco-epidemiological analyses have been proposed already in 1984 to diagnose impacts of chemicals and their mixtures in ecosystems. It is only in the last decade that this diagnostic approach has gained further attention, enabled by the large growth of monitoring data as well as analytical modelling power. This contribution describes the eco-epidemiological approach and how preventive regulatory action on various chemical groups together with field impact assessments have brought the field forward. The approach starts by ranking sites where ecological impacts are highest and where this is likely to be attributable to chemical mixtures, then in ranking contributions of specific chemical(s) (groups) in those effects. This enables a solution-focused approach to abatement to be chosen. Examples are presented from a decade of eco-epidemiological diagnostic analyses, in the context of solution-focused approach to forward the sustainable use of chemicals.

458 Does evidence from field population studies of aquatic wildlife suggest laboratory ecotoxicology information is misleading?

A. Johnson, CEH Wallingford / Wallingford

With endocrine disruption from aquaculture and the evidence from aquarium tests together with the inspection of wild fish anatomy pointed to a threat to fish populations living in proximity to sewage effluents. This issue has in part led the EU for the first time to put three ‘pharmaceuticals’, ethinylestradiol, estradiol and diclofenac, on a watch list potentially leading to them being priority substances needing control. Despite these concerns and enthusiasm for control, we still don’t know if fish populations are actually being harmed by pharmaceuticals or estrogens in sewage (wastewater) effluent. It is important to distinguish here between harm to individuals (for which evidence certainly exists for estrogens) and harm to populations. Harm to individuals is a matter of regret, whilst harm to populations is a potential catastrophe. Recent evidence implies that we may not be facing a disaster. Compilation of fish catch data caught for recreation in the UK has been on an upward trend since the 1970s. We have recently compared the abundance of wild fish populations in the UK with wastewater exposure and over the past 10 years and there was no relationship. Looking at annual trends in abundance of different fish species it is not showing declines in response to years of high wastewater exposure. In some cases roach (Rutilus rutilus) populations have grown in response to years of higher effluent exposure. Similarly, macroinvertebrate families do not seem to behave as we expect. Thus, whilst diversity is reduced with wastewater exposure, the trends in individual families are not so predictable. These populations may be able to adapt and grow in years with high wastewater exposure occurring. The prevalence of sensitive families was not linked to levels of wastewater exposure. Nevertheless, some would argue that the precautionary principle requires us to act where a concern exists, even though the evidence may be insubstantial. However, governments still require some calculation of proportionality to be carried out. In other words, are the costs of a remedy proportional to the benefits regulators in a dilemma, since the key evidence of harm to fish populations is lacking. Scientists are not the decision makers and regulators may still proceed on the evidence of harm to individual wildlife. But evidence on the state of populations in response to the challenge of chemicals in effluent still remains a great unknown in the debate.

459 Discussion

A. Johnson, CEH Wallingford / Wallingford

Consensus building in life cycle impact assessment: experiences, achievements and challenges

460 General description and introduction

R. Frischknecht, Procter & Gamble

Improving life cycle impact assessment models to be applied in the integrated environmental assessment of products is crucial. Several efforts are ongoing to address this need of improvement. To answer this, the UNEP—SETAC Life Cycle Initiative (2012—2017) has launched a flagship project to provide global guidance and build consensus on environmental LCIA indicators (see http://www.lifecycleinitiative.org/). The flagship project is focusing on building Improving life cycle impact assessment models to be applied in the integrated environmental assessment of products is crucial. Several efforts are ongoing to address this need of improvement. To answer this, the UNEP-SETAC Life Cycle Initiative (2012—2017) has launched a flagship project to provide global guidance and build consensus on environmental LCIA indicators (see http://www.lifecycleinitiative.org/). The flagship project is focusing on building consensus for different impact categories. A Pellston workshop (to be held in January 2016) will be devoted to find consensus on a first set of impact categories, namely: land use impact on biodiversity, water depletion, respiratory inorganic compounds, climate change. The overall LCIA framework as well as several cross cutting issues will be also discussed. A common case study is being developed and serves to test and evaluate the harmonised impact indicators and to ensure their practicality. The aim of the special session is to report the result of the Pellston workshop and to discuss the way forward, e.g. in policy and business related contexts. Regarding the European policy context, the European Commission is aiming at a similar process covering land use related impact at midpoint, water related impact, respiratory inorganic compounds and air/soil depletion to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session. Acceptability and applicability of the methods will be discussed aiming at improving the use of the methods in practice and the interpretation of the results. The session will also place some emphasis on the balance between continued methodological development and the need for stability and consensus for more efficient use of life cycle approaches in policy and private sector, supporting decision-making and communication.

461 The WULCA consensus for water scarcity footprints: Assessing impacts of water consumption based on human and ecosystem demands

A. Boulay, CIRAG / Ecole Polytechnique de Montréal / Chemical engineering department

The need for consensus-developed and recommended methods for water use impact assessment is clear in order to perform a water scarcity footprint consistently with ISO 14064-2014 and for consistently assessing water consumption impacts in LCA. This challenge was undertaken by the WULCA working group, of the UNEP-SETAC Life Cycle Initiative in 2013. Including method developers and experts from different fields, the group developed a consensus-based indicator to assess impacts from water consumption at the midpoint level, complying with the requirements of the ISO document. This work presents the recommended methodology for performing a water scarcity footprint including a case study application. The process started with the identification of the question to answer and three proposals which emerged from the three expert workshops held in Zurich, San Francisco and Tsukuba in 2014. The group then selected criteria used to evaluate the proposals which led to a preliminary recommendation presented in 2015. From this recommendation and the testing phase that followed, additional specific and influential modeling choices were identified, analyzed and adjusted accordingly, and sensitivity analysis were performed on the most uncertain aspects. These choices and analysis are presented, which include the span of the indicator, the different choices of spatio-temporal aggregation and their meaning when the native resolution of the indicator cannot be used, and the sensitivity of the environmental water requirement (EWR) parameter. The resulting and consensus-based single metric, covering the entire globe, modelled at various temporal and spatial scales for application in LCA, is presented in details along with the interpretation and application on the rice case study. The group is proposing the result of its work and new consensus-based indicator with the expectation that it will be adopted widely and hence decrease disparity and confusion when it comes to applying the new ISO standard on water footprinting, by providing an internationally approved,
robust and simple indicator for assessment of potential impacts from water consumption.

462 Characterisation of water scarcity impact on human health – development of a census-based model within WULCA

M. MOTOSHITA, National Institute of Advanced Industrial Sci. and Human health is one of the impacted area of protection from water scarcity. There are several characterisation models to assess the impacts on human health caused by agricultural/domestic water scarcity. However, these models characterise the impacts on human health in different ways even though they focus on the same impact pathways. A recommended characterisation model has been developed based on consensus among method developers and stakeholders in WULCA working group of UNEP/SETAC Life Cycle Initiative. As a first step for the development, sensitivity of parameters used in effect factors of previously developed models on agricultural water scarcity (Pieters et al. 2009; Barz et al. 2011; Motoshita et al. 2014) and domestic water scarcity (Motoshita et al. 2011; Bouly et al. 2011) were analysed and reviewed to identify critical parameters in characterisation model. The results of sensitivity analysis indicated the significance of health response factor to food/household water deficit and adaptation capacity to potential health damage. In order to test the validity of different types of health response factors and adaptation capacity, health damage due to agricultural/household water deficit was estimated based on those factors and compared with malnutrition/diarrhoea damage reported by WHO. According to the comparison of estimated and reported damage, health damage per calorie/water in deficit and inequality adjusted adaptation capacity (HDI-base) showed closer estimation of health damage to WHO report. Regarding agricultural water scarcity, food trade effects also showed high influence on the effect factor. The trade effect factor is composed of food supply dependency on domestic and imported food, as well as of the adaptation capacity through trade. Critical parameters of trade effect are identified through sensitivity analysis. The outcome of these discussions and the rice case study allowed the group to build a recommended methodology integrating the optimal options for each of these modelling choices. This recommended model is presented as an output of this consensus building within the UNEP/SETAC LCI flagship project of environmental life cycle impact assessment indicators and we expect it to improve the results of water consumption impacts in LCA and water footprinting.

463 Biodiversity impacts of land use

A. Assumpcio, IRITA; L. Mila / Canals, UNEP

Land use and land use change are main drivers for biodiversity loss and degradation of a broad range of ecosystem services. Despite substantial contributions to address biodiversity in LCA, no clear consensus exists on the use of specific impact indicator(s) to quantify land use impacts on biodiversity. This lack of consensus not only limits the application of existing models, but also imposes constraints on the comparability of results of different studies evaluating land use impacts based on applying different models. This TF aims at global guidance and consensus regarding indicators and methods for the assessment of biodiversity impacts from land use in LCA. In order to identify models of particular promise for future application and development, Land use Task force has performed a review of existing indicators in and out of the field of LCA. 30 models were selected. Based on the approach used by the European Commission within the International Reference Life Cycle Data System, we grouped sets of evaluation criteria under the following categories: completeness of scope; biodiversity representation; impact pathway coverage; scientific quality; model transparency and applicability, and stakeholder acceptance. In addition, two expert workshops were organized during 2014 (San Francisco, USA, 7/11 and Brussels, BE, 18-19/11). The events included discussions centred on four key topics: (a) concept of biodiversity and modelling strategies, (b) data availability and feasibility, (c) desired characteristics of indicators, usability and consensus and (d) concerns and limitations about using biodiversity indicators in LCA. Based on outcome of expert workshops and revision conducted we could summarize that there is clearly a need to model characterisation factors in terms of both (i) local damage factor for direct land use, and (ii) regional “state and pressure” weight to reflect broader biodiversity patterns and processes surrounding the location of land use. For reasons of data availability, species richness is an obvious candidate for both local, and regional damage. However, species richness is insufficient to depict the complexity of biodiversity and ecological processes. One pragmatic way of building consensus would be to use a combination of available indicators from the reviewed models for both local and regional biodiversity damage. A rice case study is developed to test different options.

464 Health effects from indoor and outdoor exposure to fine particulate matter in life cycle perspective

T.E. McKone, University of California / School of Public Health; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment Division

Fine particulate matter (PM2.5) pollution has been estimated to contribute more than 7% to the total global human disease burden from 1990 to 2013 (http://healthdata.org/gbd). Ambient (outdoor) and household indoor PM2.5 exposures are reported to account for 41% and 58% of this impact, respectively, emphasizing the need to include both, outdoor and indoor exposure into overall estimates of health burdens in life cycle impact assessment. However, lacking clear guidance on how to consistently include health effects from exposure to PM2.5 in life cycle perspective, practitioners fail to report related life cycle impacts. To address this gap, a global initiative has worked on building a coupled indoor-outdoor intake fraction framework combining exposure to PM2.5 emitted indoors and outdoors with exposure to PM2.5 formed indoors and outdoors from combustion emissions. An Exposure Response model (ERMOD) for PM2.5 concentrations is consistently combined with exposures from indoor and outdoor sources. All factors are systematically built into a model parameterized for different archetypal outdoor and indoor settings, such as specific residential and occupational settings and different urban area sizes. Model and parameters are tested in a case study on the indoor and outdoor exposure and processing of rice in three distinct scenarios covering urban China, rural India and U.S.-Europe. Recommendations are to use this coupled, generic framework whenever emission locations are unknown and to apply spatial models whenever emission locations are known. Our study constitutes a first step towards providing guidance on how to include health effects from PM2.5 indoor air exposures in product-oriented impact assessment.

465 Improving global warming impact assessment: From recent developments in climate science to LCA practice

F. Verones, NTNU / Department of Energy and Process Engineering

In life cycle assessment (LCA), global warming impacts are usually assessed using Global Warming Potentials (GWP) for a 100-year time horizon as published by the Intergovernmental Panel on Climate Change (IPCC). In the recent past years, concerns have been raised regarding the use of appropriate modeling choices and alternative metrics have been proposed. The Global Warming Task Force of the project entitled Global Guidance on Environmental LCIA Indicators let by the UNEP/SETAC Life Cycle Initiative has performed an extensive critical review of current knowledge and limitations regarding climate metrics. Topics such as the consideration of near-term climate forcers, the inclusion of carbon-cycle and climate feedbacks in GWPs, or the consideration of biophysical climate forcings from land use and land cover changes have been discussed. Special focus has been set toward new findings presented in the fifth IPCC assessment report, Working Group I, Chapter 8. The pros and cons of each modeling choices have been identified and recommendations have been drafted. The main line of thought is to first use more than one indicator (e.g. different time horizons, with and without carbon-cycle and climate feedbacks) to test the sensitivity of global warming LCA results to the different metric choices. If conclusions are unchanged, LCA results are robust. If they change from one metric to another, the range of results should be used to communicate about the sensitivity of LCA results to the metric choice. Metrics using different modeling choices have then been applied to a case study about the consumption of rice in three regions of the world. It has shown that LCA results may be particularly sensitive to the time horizon selected, and that the consideration of near-term climate forcers implies uncertainty and inventory data availability issues.

466 Reaching consensus on cross-cutting issues

F. Verones, NTNU / Department of Energy and Process Engineering

Consistency across impact categories is important, in order to facilitate and enable comparisons across impact pathways. There are multiple issues that need to be dealt with in a cross-cutting manner and not all of them can be resolved in a simple manner. The focus of last year’s work of the cross-cutting issues task force has focused on spatial aspects, normalization, uncertainty, reference states and impact category indicators for endpoint units and metrics for human health, ecosystem quality and resources. That as well as a greater raise regarding how current life cycle assessment (LCA) can be related to socioeconomic indicators. There is an unanimous consensus to keep DALY (Disability Adjusted Life Years) as endpoint indicator. We acknowledge that this does already contain a weighting, which is however internationally well-accepted. Endpoint indicators for ecosystem quality need to reflect species disappearance at global level. There are different approaches how this can be reached and consensus is required. It is especially important that method developers provide the means to convert different units, such as PDF and PAF (Potentially disappeared/affected fraction of species). This will ensure full consistency between different impact categories. A preliminary consensus was reached on the vulnerable species concept and the related global standards. Future work will be included. Models for doing so within LCA are being developed, but will need further investigation for consensus-finding. Especially important for ecosystem quality is also the discussion of reference states. It is difficult to find one common reference state across all areas of protection or all impact categories within one area of protection. We therefore propose to group impact categories in a meaningful way (e.g. based on the ecosystem type affected). It would be one common reference state. Other sub-tasks, like finding consensus on an optimal...
spatial level, approaches for normalization and weighting, as well as the metric for resources are at the time of writing in discussion, without having reached a final recommendation yet. We would also like to stress that most recommendations are aimed for an immediate use. We acknowledge that LCA is a very dynamic field with many ongoing developments in terms of operational methodologies and refinements and we encourage further development and investigation that in future could lead to adapted recommendations.

467 Mainstreaming life cycle thinking through a consistent approach to footprints
b. Ridoutt, CSIRO

Over recent years, footprints have emerged as an important means of reporting environmental performance. Some individual footprints have become quite sophisticated in their calculation procedures. However, as an overall class of environmental metrics they have been poorly defined, having a variety of conceptual foundations and an unclear relationship to LCA. The variety and sometimes contradictory approaches to quantification have also led to confusing and contradictory messages in the marketplace which have undermined their acceptance by industry and governments. In response, a task force operating under the auspices of the UNEP/SETAC Life Cycle Initiative project on environmental Life Cycle Impact Assessment has been working to develop generic guidance for developers of footprint metrics. The initial work involved forming a consensual position on the difference between footprints and existing LCA impact category indicators. In short, footprints are deemed to have a primary orientation toward society and nontechnical stakeholders and report only on selected topics of concern. On the other hand, LCA impact category indicators have a primary orientation toward technical stakeholders and report in relation to a larger framework designed for comprehensive evaluation of environmental performance and trade-offs. The task force has also developed a universal footprint definition, as an essential prerequisite for the development of more detailed guidance. In parallel to Area of Protection, we introduce Area of Concern. In the same way that LCA uses impact category indicators to assess impacts that follow a common cause-effect pathway toward Areas of Protection, footprint metrics address Areas of Concern. The critical difference is that Areas of Concern are defined by the interests of stakeholders in society rather than the LCA community. In addition, Areas of Concern are stand-alone and not part of a framework intended for comprehensive environmental performance assessment. The Area of Concern paradigm is needed to support the development of footprints in a way that fulfills their distinctly different purpose. Accordingly, footprints are universally defined as metrics used to report life cycle assessment results addressing an Area of Concern. The task group is now actively working on detailed guidance. One priority topic is the acceptable use of aggregation and weighting where the Area of Concern requires the use of multiple impact assessment models.

468 Product environmental footprint (PEF): improving life cycle impact assessment
M. Galatoła, European Commission / DG Environment

Regarding the European policy context, the presentation will continue by presenting the consensus process initiated by the European Commission, aiming at covering land use related impact at midpoint, water related impact, respiratory inorganics and resource depletions to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session.

469 Life cycle impact assessment models for PEF: assessment of models and applicability challenges
S. Sala, European Commission - Joint Research Centre / Sustainability Assessment unit; R. Pant, European Commission / Institute for Environment and Sustainability

Regarding the European policy context, the presentation will continue by presenting the consensus process initiated by the European Commission, aiming at covering land use related impact at midpoint, water related impact, respiratory inorganics and resource depletions to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session. The session will end by a stakeholder presentation from industry and government workshop representatives and general feedback from session participants.

The sustainability of wine production in Europe

470 Ecosystem services and soil biodiversity in French vineyards
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Essential ecosystem services provided by viticultural landscapes result from diverse communities of above- and belowground organisms and their interactions. As a result of management intensification in the last century several ecosystem services were affected leading to high rates of soil erosion, degradation of soil structure, soil fertility, contamination of groundwater and decrease of longevity of vines etc. Here we report how management intensity affects biodiversity of aboveground and belowground biota as well as their impact on ecosystem services in terms of regulating services in the agro-ecosystem, provisioning services and cultural services. Some alternative techniques and conservation measures that have the potential to mitigate the negative effect on biodiversity were introduced in some wine regions, thereby restoring ecosystem services. Furthermore, the impact of landscape structure on biodiversity and ecosystem services in vineyards is currently investigated in the Biodiversa project “VineDivers”.

471 Footprinting the sustainable wine production in Italy
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472 Study of the variability of a vineyard sensitivity to fungus diseases in order to adapt chemical input: a priori zoning of Physiological Behaviour Units (PBU) using precision viticulture techniques
m. raynal, B. DELFOUR, C. DEBORD, M. VERGNES, A. BENNABI, Institut Français de la Vigne et du Vin; M. GEORGES, R. FULCHIC, J. SERVANT, Château Léoville Las Cases

The system analysis of the performance of a vineyard at the scale of a wine-making exploitation is made possible by the use of sensors stemming from so called precision techniques, which allow precise and exhaustive geo-located measures. The aim of our study is to exploit this kind of data and evaluate their information using geographical information systems (GIS) and crossing different layers representing characteristic and independent variables of the production system. The goal is then to elaborate an à priori zoning, likely to explain variations of the physiological development of vines and possible differences of the plants susceptibility to fungus diseases. The study is based on the combination of two maps established on the property of Chateau Léoville Las Cases in the Medoc area of the Bordeaux vineyard (France). These maps represent the behaviour of the two compartments, soil and plant, respectively determined by means of electric resistivity (R) and biomass index (B) measures. Three levels - low, medium, high- are defined for each type of data. The combination of these indicators allows the elaboration of 9 classes of islets, named Physiological Behaviour Units (PBU), whose distribution is bounded by the GIS on the whole vineyard. Six of these nine PBU were selected by exclusion of the medium class of the biomass index. Each PBU is replicated twice, thus establishing an observation device of 12 PBU likely to identify differences in terms of physiological development and disease susceptibility. For this purpose, treated and non-treated zones were delimited for each PBU, and a weekly monitoring of these areas has been performed during the 2014 and 2015 crop years. The first years’ results of the study show that the PBU concept proposed seem to correlate with some of the significant variations observed for physiological and sanitary criterions.

473 Guided discussion: sustainable wine production: opportunities, obstacles and the path forward
S.E. Apitz, SEA Environmental Decisions Ltd

474 Summary, conclusions & next steps
S.E. Apitz, SEA Environmental Decisions Ltd; S. Delrot, University of Bordeaux1; F. Martin-Laurent, INRA / Agroecologie

Biodegradability assessments of organic substances and polymers

475 Chemical analysis and measurement of biomass to enhance interpretation of hydrocarbon solvent biodegradability tests
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Biodegradability is a key parameter for environmental hazard and risk assessment of chemicals. This is typically assessed experimentally following the OECD 301 A-F series of ready biodegradability tests in the first instance. These tests measure the biodegradation of a chemical under stringent conditions, and as such are considered a screening test for readily biodegradable substances. The results of these tests are used extensively both within a regulatory context and to support marketing claims on product biodegradability. However, these methods do have
their limitations, particularly when they are used to assess the biodegradation of complex, volatile and/or poorly water soluble substances. Recent investigations with GTL products have highlighted these issues. In particular, the inclusion of specific chemical analysis in biodegradation studies (not always a guideline requirement) has shown that disappearance of test substances from biodegradation systems is often far more complex than suggested by experiments alone. This is indicative that either the material has been lost from the test system through abiotic processes (e.g. volatilisation), or that it has not been completely mineralised (e.g. it has been used as a carbon source to increase microbial biomass). The OECD 301F test was found to be more suitable for testing such substances because it utilises a sealed system and therefore limits losses due to volatility. In this study a number of hydrocarbon solvents have been tested using OxTop-C test systems, following the OECD 301F test method. In addition to measuring biodegradation by biological oxygen demand (BOD), additional techniques have been incorporated to enhance the interpretation of results. Analysis by TIC/TOC has been conducted to assess the presence of residual test substance and other metabolites, and the increases in microbial biomass have been measured to give an indication of the amount of carbon used by the inoculum to increase its biomass, rather being completely mineralised. In abiotic controls have been run to assess removal from processes other than microbial degradation. Using this combination of additional measures and analyses, it is possible to account for a greater percentage of the total material tested, and therefore to improve the interpretation of these tests.

476 Approaching chemicals’ persistence through a new strategy of use of RBT tests

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Approaching chemicals’ persistence through a new strategy of use of RBT tests

Brillet Françoise1, Maul Arnaud1, DITOC Marie-José1 and Thonand Gérard1
1Université de Nantes, UMR CNRS 6144 GEPEA, IUT Génétique Biologie, La Roche sur Yon, France. 2Université de Lorraine, LIEC-UMR CNRS 7360, Metz, France.gerald.thouand@univ-nantes.com With millions of organic chemicals released every day into our environment, Europe and other continents are confronted with increased health and environmental problems. Even if strict regulations are imposed upon industry to ensure that they prove the harmfulness of their substances during marketing authorization, not all testing procedures are designed to cope with the complexity of the environment. This is especially true for the evaluation of persistence through biodegradability assessment guidelines. Our new approach has been adapted to assess biodegradability assessment to the environmental conditions and model the probability for a biodegradation test to be positive in the form of a logistic function of both the temperature and the cell density. Here, a proof of this new concept is proposed with the establishment of 3D biodegradability profiles of 6 chemicals (including the controversial glyphosate) between 4 to 30 °C and 10 to 100 cells ml-1 as can be environmental compartments in time and space. The results show a significant increase of the predictive power of existing lab scale tests and the creation of new indicators to quantify environmental persistence. Key words: Biodegradability Assessment, Environmental Persistence

477 Screening for Persistence: Tools to Determine Mineralization and Estimate Kinetics in Water, Water-Sediment and Soil

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Standardized biodegradation tests provide data that are required for persistence assessment (e.g. under REACH). In a first step, clearly non-persistent substances are identified within a persistence screening based on results from water-only ready biodegradability tests (RBTs) and QSAR models. However, RBTs provide quantitative data on degradation but not on bioavailability. Hence, predictions of degradation rates and half-lives of organic compounds by QSAR models are mainly based on qualitative experimental biodegradation data related to water-only test systems. Moreover, half-lives related to water are extrapolated to soil and sediment by multiplication with default factors. Within this study, the screening test method to determine biodegradation in water according to OECD 301C (MTL Ministry of International Trade and Industry, Japan) was modified to develop new test systems on the screening test level for water-sediment (WSST, Water-Sediment Screening Tool) and soil (SST, Soil Screening Tool). The tests were applied by selection of observation rates and kinetic parameters. This study shows that mineralization kinetics and screening-test systems WSST and SST proved to be suitable tools to determine reproducible and sound quantitative biodegradation data including biodegradation kinetics for water-sediment and soil, respectively, that could be applied in the regulatory context (e.g. within a persistence screening). The observed substance-specific variation of biodegradability in different environmental compartments provides strong evidence for the extrapolation of half-lives from water to sediment and soil by use of default factors should be avoided. Beyond that, the test systems can be used for building up a database of screening-level biodegradation information across major compound classes, which can serve as reference set for subsequent research into respective in silico models.

478 Identifying limitations of the OECD water-sediment test (OECD 308) and developing suitable alternatives to assess persistence


The OECD guideline 308 describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems and is an integral part of tiered testing strategies in different legislative frameworks for the environmental risk assessment of chemicals. Over the years, several shortcomings of the OECD guideline 308 have been identified and its usefulness for persistence and exposure assessment has been questioned. On October 6, 2015, a JRC stakeholder workshop was held within the Cefic-funded project “LRI ECO18 – An integrated strategy to assess chemical persistence at the water-sediment interface” was held. The goal of this workshop was to summarize stakeholder perspectives on the status quo of both OECD guidelines 308 and 309, and to complement this with a dissemination of the findings of the project LRI ECO18. In this presentation, the main outcomes of the workshop will be presented, i.e., (i) sharing of industry and regulatory experience with OECD 308 and 309 in different regulatory contexts, (ii) identification of major issues and knowledge gaps with respect to OECD 308 and 309, and (iii) presentation of main outcomes and recommendations from LRI ECO18 project [1-3]. [1] Honti M, Fenner K. 2015. Deriving Persistence Indicators from Regulatory Water-sediment Studies – Opportunities and Limitations in OECD 308 Data. Env Sci Technol 49: 5879-5886. [2] Shrestha P, Junker T, Fenner K, Hahn S, Honti M, Balkour R, Diaz C, Hennecke D. 2015. Simulation studies to explore biodegradation at the water-sediment interface – From OECD 308 to OECD 309. In preparation. [3] Honti M, Junker T, Hennecke D, Hahn S, Shrestha P, Fenner K. 2015. Bridging Across OECD 308 and 309 Data in Search of a Robust Transformation Indicator. In preparation.

479 Biodegradation of squame by a Pseudomonas aeruginosa strain and bioavailability considerations

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Very few studies have reported bacterial degradation of the long chain length, acyclic isoprenoid, squame (2,6,10,15,19,23-hexamethylnoracene) although there are several reports on biodegradation of smaller chain length isoprenoids, such as pristan and phytane. The low aqueous solubility and structural complexity limits the bioavailability of branched alkanes to most strains. This study reports squame biodegradation by a Pseudomonas aeruginosa strain RS1 isolated from refinery sludge that could utilize it as sole source of carbon and energy after a long acclimatization period was provided. It exhibited specific growth rate in the range 0.19-0.22 h-1 over the squame concentration range of 250 - 750 mg/L in Bushnell-Haas mineral media. The maximum extent of degradation (67%) was achieved after 96 h for squame concentration 250 mg/L where abiotic loss was less than 20%. Negligible extracellular release of biosurfactants was observed. Contact angle measurements revealed that the cell surface of squame grown cells were moderately hydrophobic, thus, indicating direct interfacial uptake of squame. However, the adherence to n-hexadecane and squame in the bacterial adhesion to hydrocarbon (BATH) assay was unexpectedly low (less than 30%). This apparent anomaly may be explained by the fact that BATH assay is not a true measure of hydrophobicity since it is likely to be influenced by solubility and structure, such as, electrostatic interactions. A similar mechanism was employed by this strain for degrading the aromatic hydrocarbon, pyrene, at relatively high rate compared to other pyrene degraders This is the first study reporting degradation kinetics and bioavailability considerations for squame uptake and thus provides insight on degradation of branched alkanes in petroleum.

Mercury Biogeochemistry and Policy

480 Mercury concentrations in suspended particulate matter, water and mud-sediment up and downstream from old and recent gold mining sites in French Guiana

108 SETAC Europe 26th Annual Meeting Abstract Book
Only the use of mercury (Hg) for gold mining has been officially banned in French Guiana since 2006. The still reasons to be wary of its occurrence in river water and its impact on local populations. In fact, gold-mining generates intense soil erosion and high turbidity in the rivers downstream from mining sites. This contributes to Hg pollution and dissemination both via the remobilization of Hg naturally present in the soils and the release of metallic Hg from the gold recovery process. In the affected rivers, Hg transport and speciation is mainly controlled by the suspended particulate matter (SPM) rather than remaining dissolved. The aim of this study is to understand and quantify the fate of this SPM-associated Hg along the Boulanger River affected by old and new gold mining activities. Furthermore, we provide recent data (2012) essential to evaluate the temporal and spatial efficiency of the control measures implemented in exploited zones to control erosion, river turbidity and SPM transport. Inorganic Hg(II), monomethylmercury (MMHg) and turbidity measurements were measured in water, SPM and mud-sediment collected along the Boulanger River, from the spring to its confluence with the Oropou River. The intensity of gold-mining in this area has been up and down through the last century and presents contrasting conditions with old and recently exploited areas. Dissolved Hg (HgD) levels were low (1-2 ng L⁻¹) and consistent with previous data known for the Amazonian area. Dissolved MMHg concentrations represented up to 8% of HgD which is higher than previous results (around 2%). Some relationships occurred between Hg concentrations in mud-sediment and turbidity values which both decreased downstream. Mining activities, then stabilized until even further down. Hg contents in the mud-sediment show the impact of old and new gold mining practices. These results show that even though Hg use was banned from French Guiana 6 years ago, present gold mining practices still release significant amounts of Hg into the rivers. Survey intensification in French Guiana river systems should provide a more complete data set, essential to change gold mining practices and to reduce SPM and Hg contamination of the aquatic environment.

481 In situ biomonitoring of mercury bioavailability and transfer in a former gold mining area (French Guiana), F. Gingbert, University of Franche-Comté / UMR ChronoEnvironnement; B. Smith, University of Bourgogne Franche-Comté, UMR UFR/CNRS 6249 / UMR ChronoEnvironnement; E. Tessier, LCB-IPREM UMR 5254 CNRS-UPEA; D. Amouroux, Laboratoire de Chimie Analytique Bio Inorganique et Environnement, UMR CNRS IPREM5254, University PaU; N. Bousserhine, Université Paris-Est Créteil / Bioemco; B. Ferrari, Ecotox Centre CH Gold mining activities have a considerable impact on the entire ecosystem, including terrestrial and aquatic compartments. Besides the physical degradation of soils and river network, artisanal small-scale gold mining also leads to the release of mercury (Hg) in the environment. The sources of Hg are both anthropogenic (from fine gold particles amalgamation) and geogenic (from weathering of parent rocks and long-term atmospheric deposition). Mercury contamination of the aquatic food web constitutes both an environmental and sanitary risk since local populations largely feed on topchain predator fishes which exhibit high Hg concentration. Among Hg species, monomethyl-Hg (MMHg) represents the most bioavailable fraction, since it reaches the top of the food chain. The objective of the present study was to investigate the bioavailability and the transfer potential of Hg in a former gold mining area in French Guiana using chironomid larvae, key organisms of the aquatic ecosystem functioning and food chains. Hence, in an active biomonitoring campaign, we investigated (i) inorganic Hg (HgI), and MMHg toxicokinetics in larvae encaged in the field and (ii), by mean of designed exposure devices, the contributions of water and sediments to both HgI and MMHg uptake. The study site was a former goldmine flat of the Combat Creek catchment (French Guiana) exploited until 2010. We used calibrant four instar larvae of Chironomus riparius bred in the laboratory (IRD Cayenne) under controlled conditions. Larvae were exposed to two different types of cages allowing the contribution of water and sediments in HgI and MMHg bioaccumulation to be estimated. Mercury speciation was conducted (GC-ICP-MS) both in unsoluble (exoskeleton, gut content and granules) and soluble (cytosol) fractions. Although HgI did not accumulate, MMHg was bioavailable to control cages, and mainly accumulated in the cytosolic compartment. Moreover, sediments appeared as an important source of MMHg as testified by the higher uptake rate modelled in larvae exposed to both water and sediments compared to water exposure only (0.57 and 0.18 ng g⁻¹ d⁻¹, respectively). Hence, the active biomonitoring using C. riparius larvae allowed to efficiently estimate MMHg bioavailability and its potential to transfer in the trophic web since its concentration in the cytosolic compartment can be considered as trophically available to most, if not all predators.

482 Linking variation in natural solar radiation with seasonal methymercury dynamics in freshwater lake systems, S. Klupstein, Academia University / Earth Environmental Science; S. Ziegler, Memorial University of Newfoundland / Earth Science; D. Fischer, Bayer CropScience LP / RD Environmental Safety; N.J. O'Driscoll, Acadia University / Department of Earth and Environmental Science. Methymercury bioaccumulation in biota is a serious concern for mercury sensitive ecosystems far from direct point sources of pollution. Low buffering capacity in soils and high atmospheric deposition of mercury and acid rain from anthropogenic sources can enhance the solubility and transport of metals into waterways. Plentiful wetland environments and well-mixed lakes make studying the processing controlling methymercury concentrations in water dynamic and complex. While much is know about mercury methylation in freshwater, much less is known about demethylation. The loss of methymercury from the water column of lakes is dependent on several processes including adsorption, deposition to sediments, and demethylation. Microbial demethylation in water columns is very slow, therefore a daytime photodemethylation facilitated by solar radiation can dominate methymercury removal from the water column. To better quantify the photodissipation of methymercury, we used in the laboratory the OECD 301B test and a modified OECD 301F test to determine a) the variation in photo reactive compounds such as dissolved organic matter (DOM) and iron (Fe) and b) the availability of solar radiation with depth in water columns. Freshwater lakes were chosen in Kejimkujik National Park (44.23°N, 64.13°W) to include a wide range in dissolved organic carbon (DOC) and Fe concentrations. Water samples were collected over 3 years and analyzed for ultraviolet (UV)-visible absorbance, DOC, dissolved ions, total mercury, and methymercury concentrations. Floating sensors for UV, photosynthetically active radiation (PAR), and temperature were also installed in two lakes of contrasting DOC concentrations. The depth of 95% UV attenuation was 40-50 cm in the lower carbon lake compared to 10-20 cm in the higher carbon lake. The effect of radiation on UV attenuation was less in the lower carbon lake compared to the higher carbon lake, most likely due to a difference in catchment area. Seasonal alterations to the solar angle of incident radiation strongly controlled the amount of solar radiation entering lake water surfaces and therefore outlined a possible photo reactive season or period within each year. These observations suggest that photodissipation of methymercury in water columns may be limited to a short period of approximately 4 months a year at 44°N. Overall, these field observations provide fine resolution solar radiation data and excellent temporal resolution over 3 years for mercury and carbon cycling.

483 Dietary transfer of Hg from Elodea nuttallii to Gammarus fossarum R. Beauvais-Flueck, Institute Foro Earth and Environmental Sciences; a. chaumot, Irstea / UR MALY Laboratoire Eco-toxicologie; F. Gingbert, University of Franche-Comte / UMR ChronoEnvironment; O. Geffard, Irstea / UR MALY Laboratoire Eco-toxicologie; C. Costa, Geneva University / Institute Foro Earth and Environmental Sciences. In the aquatic environment, the organic form of Hg, methyl-Hg (MMHg) and inorganic Hg (IHg) are both toxic, but MMHg has been shown to biomagnify through food chain while IHg does not. Nonetheless, the precise factors controlling the transfer of Hg from primary producers to heterotroph consumers remains poorly understood. Macrophytes are key organisms of shallow aquatic environment in which MMHg is produced, and have been identified as having a role in Hg transfer to food webs. The objective of this study was therefore to assess how the compartmentation of IHg and MMHg in a representative macrophyte, Elodea nuttallii, affects its bioaccumulation through dietary transfer in Gammarus fossarum chosen as surrogate species for aquatic herbivores and decomposers. Bioaccumulation of Hg in E. nuttallii was higher for cell wall than intracellular in line with previous studies showing an important role of binding of Hg in cell wall in E. nuttallii. Our data suggest no substantial demethylation or methylation during bioaccumulation in E. nuttallii in plants or in water. IHg and MMHg concentrations increased in Gammarids in correlation with concentration found in E. nuttallii. MMHg was more transferable than IHg, based on THg, but a significant part was demethylated during the feeding, while no methylation was observed in the IHg exposure. Cell wall results in higher concentrations than intracellular, suggesting that Hg in cell wall in form of MMHg and IHg was significantly assimilated in Gammarids. Significantly lower fluxes were estimated by the first-order model: uptake fluxes in Gammarids were higher for cell wall than intracellular, while similar uptake rates were observed for IHg and MMHg. In conclusion, G. fossarum is able to feed on E. nuttallii and accumulates Hg from intracellular and cell wall compartments. In line with capacity of fish when looking at THg consumption of MMHg-exposed plants leads to slightly higher accumulation of Hg than IHg-exposed plants, but we observed a significant demethylation of MMHg in Gammarids vs plants. Opposite to literature, we did not observed a significant biomagnification of MMHg. In sum, although the subcelular metal distribution is determinant for many consumers that are known to simulate the GI tract of cell walls, the digestive strategies to handle food of the consumer is also important. Differences in primary producers and consumers species composition may significantly influence the fate of Hg in food webs.

484 Concentrations of mercury in porbeagle shark Lamna nasus E.E. Nicolaus, Cefas Lowestoft Laboratory / Environment and Ecosystems; V.
Bendall, T. Bolam, Cefas Lowestoft Laboratory; T. Maes, Cefas / EQT; J. Ellis, Cefas Lowestoft Laboratory

Concentrations of mercury in three tissues of porbeagle shark Lamna nasus (n = 33) were determined. While all specimens exceeded OSPAR’s Background Assessment Concentrations of 0.035 mg kg⁻¹, Hg concentrations in either the red or white muscle of Porbeagle Euphausia were observed in one-third of specimens. Hg concentration, however, increased with length, and all fish >195 cm had concentrations >1.0 mg kg⁻¹, with a maximum observed value of 2.0 mg kg⁻¹.

485 Spatial and temporal monitoring of total mercury and monomethylmercury in fish from European freshwaters

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For the implementation of the European Water Framework Directive (2000/60/EC) an environmental quality standard (EQS) of 20 µg/kg mercury (Hg) in fresh weight of aquatic organisms was derived (EU Directives 2008/105/EC, 2013/39/EC). Because of their differing chemical behavior and ecotoxicity (e.g., secondary poisoning of predators by MeHg inorganic Hg and organic Hg (mainly monomethylmercury, MeHg) should be evaluated differently. However, in aquatic organisms only determination of total mercury concentrations is required for the WFD compliance monitoring. Here we quantified also the MeHg levels in the fish samples, allowing for risk assessment of the Hg released from previous monitoring program bream (Abramis brama) collected from different European freshwaters were available. In the period 2007-2013 the rivers Tees/UK, Mersey/UK (no sampling 2009-2011), Gótalilv/SE (no sampling 2009-2011), Western Scheldt/NL, and Rhône/FR as well as a lake (Belau/DE) were covered. Pooled muscle tissue samples of 15 fish per site were homogenized and analyzed for total Hg with a Direct Mercury Analyzer as well as for MeHg by SID-GC/ICP-MS (species isoype dilution-gas chromatography - inductively coupled plasma-mass spectrometry). The results of the total Hg analyses of the fish samples from European freshwaters were then compared to the long-term fish monitoring data of the Environmental Specimen Bank operated by the GermanFederal Environment Agency. Data reveal that both for the fish from the European as well as from the German river sites EQS were clearly exceeded. Only data for the one German lake site investigated revealed total Hg levels in the range of the EQS in recent years. Regarding the MeHg fraction of the total Hg literature data could be confirmed which report high MeHg fractions in the range of 70-100 %.

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (I)

486 Oxidative stress response of the aquatic macrophyte Hydrilla verticillata exposed to nanoparticulate and bulk TiO2

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Nanotechnology is a rapidly growing industry with manufactured titanium dioxide nanoparticles (TiO2-NPs) being one of the most commonly employed metal oxide NPs. Due to increasing production volumes concerns about the fate and behaviour of nano-TiO2 in the aquatic environment have arisen, as water reservoirs will be the final destination of TiO2-NPs. Currently, there is emerging research activity concerning the ecotoxicology of TiO2-NPs in aquatic ecosystems. However, biochemical studies investigating the effect of TiO2-NPs on oxidative stress related parameters in aquatic macrophytes are scarce, despite their important role in aquatic habitats. In the present study, oxidative stress effects in Hydrilla verticillata caused by TiO2-NPs of different crystallinity status were investigated and compared to a bulk sized counterpart. Macrophytes were exposed to different concentrations of TiO2-NPs (0, 0.01, 0.1, 1, 10 mg/L) for 24 h, and thus experimental setup included currently predicted levels of nano-TiO2 in surface waters. Additionally, the investigated TiO2-NPs contributed varying crystalline status (anatase, rutile, P25-mixed phase) to assess a potential influence of crystalline phase on oxidative stress responses. As oxidative stress related parameters the level of hydrogen peroxide (H2O2), reduced and oxidized glutathione (GSH and GSSG) and activities of the antioxidant enzymes peroxidase (POD), catalase (CAT) and glutathione reductase (GR) were measured. Whereas POD was not considerable activated in this study, results imply an activation of the defense system, as increased CAT and GR activities were observed. No significant changes in enzyme activities were assessed for the treatments with bulk TiO2 and moreover, such exposed plants exhibited lower enzyme activities at all concentration steps, suggesting a nano-specific influence on antioxidative defense mechanisms in H. verticillata. All TiO2-NP concentrations dropped the GSH/GSSG ratio, indicating a high GSH-dependent metabolic activity to protect against the destructive effects of reactive oxygen species (ROS) generated during nano-TiO2 exposure. Furthermore, the glutathione status seems to be a sensitive marker for changes in the cellular redox state of macrophytes. As the level of H2O2 was solely elevated after exposure to 10 mg/L of P25, adaptive metabolic mechanisms of H. verticillata are probably able to cope with environmentally relevant concentrations of TiO2-NPs.

487 Does a coating matter? Antioxidant enzymes activities in the water flea Daphnia magna exposed to modified copper oxide engineered nanomaterials.

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Production of reactive oxygen species (ROS) has been described as a general pathway of toxicity induced by various metal based engineered nanomaterials (ENMs) and other chemicals leading to oxidative stress. Coating-related changes in three antioxidant enzymes’ activities in the freshwater flea Daphnia magna were assessed, based on information obtained in acute and chronic toxicity tests. Experiments on biochemical responses following exposures to different CuO ENMs were focused on the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-Transferase (GST) after 2, 6 and 24 hours of exposure. To minimise variability in the response to the analysed CuO ENMs, a single laboratory clone of D. magna (clone GG4) cultured at Heriot-Watt University, was selected for this study. Cultured juveniles aged 5-8 days were used for biochemical analysis: 450 for CAT, 450 for SOD, 450 for GST and protein analyses, with three replicates per treatment. The animals were exposed to sublethal CuO ENM concentrations for 48 hours. Enzyme activities obtained after 48 hours of exposure. Enzyme activity responses varied across the ENM panel. SOD, CAT and GST, which are considered the most important antioxidant enzyme systems in invertebrate species, showed different responses across the different surface modifications and time of exposure. After two and six hours of exposure Daphnia juveniles exposed across the ENM panel did not show significant increase in SOD activity. After 24 hours of exposure, SOD activity significantly increased for animals exposed to CuO, CuO-COOH and CuO-NH3 ENMs. After 2 hours no significant differences between treatments were observed for the GST activity across all tested chemicals. The GST activity significantly increased after exposure to CuO-COOH and CuO-NH3 ENMs for 6 hours, and for CuO-PEG after 24 hours exposure. No significant effects on CAT were observed after 2 and 24 hours of exposure; only CuO-PEG ENM showed marginal but not significant increase in CAT activity after 24 hours. Significantly increased CAT activity was observed following exposure to unmodified copper oxide and CuO-NH3 ENMs after 6 hours. The most sensitive endpoints after 6 hours were CAT and GST assessments and SOD, after 24 hours exposure. Based on the above results it is apparent that ROS play an important role in the toxicity pathway observed, and the pattern observed depends on the CuO ENMs surface modification and time of exposure.

488 Secreted protein eco-corona mediates uptake and impact of 'next-generation nanoparticles' on Daphnia magna

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NanoParticles (NPs) have at least one external dimension between 1-100nm. The small size of NPs gives them a large surface area to volume ratio providing them with exclusive properties that differ from bulk material of the same chemical composition. As a result, these unique materials have found numerous applications in industrial fields leading to their inevitable release into the environment during use or at the end of their life cycle. NPs are known to adsorb molecules such as proteins from their environment, to form an eco-corona, which ultimately changes the NP identity and how it interacts with organisms. Secretion of biomolecules is a well-established component of both intra-species communication and predator-prey response in aquatic food-chains and therefore the impact of such secretions on NP uptake and ecotoxicity must be investigated. Here we report some initial studies, using model spherical polystyrene NPs which mimic plastics that degrade slowly in the environment, resulting in build-up of nano-plastic in fresh water systems. We also look at various shaped and charged ‘next-generation NPs’ including gold NPs with different morphologies whose release into the environment is increasingly likely due to the heightened use of these NPs in medical systems. Finally, we look at several novel perovskite NPs, which are increasingly used in solar cells. For all NPs, the impact of the secretion of biomolecules by Daphnia magna on the NP uptake, retention and toxicity was assessed. Interestingly, in all cases studied to date, a significantly increased uptake from D. magna secreted biomolecules resulted in enhanced NP toxicity, related to enhanced uptake and retention of the particles, and in some cases to enhanced oxidative stress.

489 Comparative evaluation of acute and chronic ecotoxicity of Copper Oxide nanoparticles on the pond snail Lymnaea stagnalis
Nanotechnology is a rapidly developing field in the 21st century, and commercial use of nanoparticles for novel applications is increasing explosively. Copper oxide nanoparticles (CuO NPs) are frequently employed for their antimicrobial properties in antifouling paints. Their extensive use can contaminate aquatic ecosystems. The objective of this study was to evaluate and compare the oxidative stress of CuO NPs through acute and chronic toxicity tests with different life stages and the small size of nanoparticles, a major concern of organisms in the benthic ecosystem. Acute waterborne exposure was focused on the evaluation of the acute lethal toxicity of CuO NPs to juveniles (7-9 day old) of the snail L. stagnalis exposed for 96h at 20°C in a static experiment, either in the nano form of CuO NPs or ionic form, as CuSO4·5H2O. The LC50 value estimated in tests with CuO NPs (LC50CuO) was 400 μg L−1 Cu, while the threshold obtained for the tests with CuO NPs (LC50CuO, n = 2500 μg L−1 Cu). Chronic toxicity tests aimed to investigate the effects of exposure CuO NPs on the reproduction to L. stagnalis. Young adult snails (22±2 mm) were exposed to Cu as CuO NPs at 20°C for 30 days in a semi-static experiment. Endpoints such as: mortality, growth and behaviour alteration were also evaluated along with the reproduction parameters. LC10BellandLC50Bell values estimated were respectively, 230 μg L−1 Cu and 480 μg L−1 Cu, indicating a 5 fold higher toxicity than the acute test. Additionally, exposure to CuO NPs showed significant effects (p<0.001) on the growth and reproduction parameters relative to the control. Behavioural changes, such as respiration behaviour, were also observed in the Cu treatments. The experiments’ results demonstrate a time related increasing toxicity of CuO NPs on L. stagnalis, emphasizing the need for more chronic study to accurately evaluate the impact of nanomaterials in the real environment. Furthermore, long-term experiments using juveniles L. stagnalis exposed to CuO NPs are ongoing, evaluating growth and time-related expression profiles of antioxidant enzymes and heat shock proteins response to CuO NPs, in L. stagnalis. Acute and chronic tests assessing the toxicity of safe-by-design CuO NPs and their fragmented products (FP) on L. stagnalis will be performed, applying the same experimental design used for evaluate the toxicity of the pristine CuO NPs. This research project is funded by the European FP7 project SUN “Sustainable Nanotechnologies”.

490 Bioavailability of metal nanoparticles in a sediment dwelling organism: a study of transdermal and oral routes of uptake

R. Cieres, C. Tyler, Biosciences College of Life and Environmental Sciences; T.S. Galloway, University of Exeter / Biosciences Department

Metal engineered nanomaterials (ENMs) are an emerging pollutant considered to be of risk to aquatic environments due to their inherent reactivity and high global production volumes. The behaviour of metal ENMs in aquatic sediment systems is dominated by transformations including aggregation, complexation with organic matter and in some cases dissolution of metal ions. Investigating ENM behaviour in sediments requires novel combinations of separation and microscopy techniques. This will allow us to correctly interpret the results from studies into the bioaccumulation of ENMs in benthic species. Using a combination of centrifugal ultrafiltration and Asymmetric flow Field Flow Fractionation (AF4) the size distribution and dissolution of both ceria (CeO2NPs) and silver (AgNPs) nanoparticles was followed in a model sediment system over 6 days. The aim was to compare uptake through transdermal or oral routes. Two commercially relevant Ag NPs were chosen as test materials: one stabilised sterically with PEG (mono mPEG phosphonic acid ester), the other with an electrostatic stabiliser, citrate. The NPs had a primary particle size of 4-10 nm. A 5 day bioaccumulation exposurewas conducted using the sediment dwelling oligochaete worm, Lumbriculus variegatus. Organisms were either actively feeding (uptake through transdermal and oral ingestion) or non-feeding, achieved by utilising the species’ natural mode of reproduction by clonal fragmentation, to yield non-feeding clones. Centrifugal ultrafiltration examined partitioning of CeO2 and AgNPs between the solute and liquid phases of the sediment following AF4 was used to investigate the size distribution and preferential heteroaggregation between the CeO2 and AgNPs and other natural colloids present in the sediment pore waters. Results demonstrate that for CeO2 NPs, dissolution does not occur and there was no uptake of nanoparticles across transdermal pathways. Coating type and extent also made no difference to bioavailability of these nanoparticles through ingestion. All three NP treatments were significantly more bioavailable than either the geometric Ce present naturally in the sediments or micron sized CeO2. Results for Ag NPs are ongoing and will be reported in full during the presentation. Results will be discussed in the context of the transformations that the nanoparticles have undergone and their interactions with other natural colloidal materials in the sediments.

491 Effects of iron nanoparticles on primary cultures of human bronchial epithelial cells

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Airways constitute the major route of exposure to atmospheric particulate pollutants. Furthermore, inhalation of fine and ultrafine particles contributes to their rapid transfer to the lung. Their size allows them to reach the deep lung, and the smallest of them can be transferred to the entire human body. Recent studies have demonstrated that exposure to inorganic particles was associated with an increased risk of morbidity and mortality related to respiratory and cardiovascular diseases. Indeed, with the complexity of the bronchial tree, inhaled ultrafine particles are in regular contact with lung epithelial cells and may favor the emergence of chronic inflammatory diseases (asthma, COPD, etc.). With the development of nanotechnology and the increasing use of nanoparticles, a question emerges: “what are the effects of this nanoparticles on health?” The specific properties of nanoparticles such as their smaller size, larger surface area and higher reactivity are major points of concern. Consequently, an essential step in nanoparticle study is their physicochemical characterization in their pristine state, or suspended in an aqueous solution. The present study, aim to investigate the lung effects of iron-engineered nanoparticles (representative of industrial smoke emitted by metallurgical industries). After characterizing these particles at the physico-chemical level and verifying their capabilities to penetrate lung cells using transmission electron microscopy, we assessed their cytotoxicity on primary lung cells and their capacity to modulate gene expression and oxidative stress. After 6-hour exposure of primary cells to low dose of iron-engineered nanoparticles, we highlighted expression modifications of genes involved in inflammation but we failed to detect a significant induction of oxidative stress.

Soil and water contaminants: evaluation, biomonitoring and bioindicators for effective management (I)

492 Which pollutant should we be measuring? - Broad spectrum screening provides multiple answers in a day

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There is still little information on which chemical pollutants are present in the South African environment. To prioritise economic investment in expensive analytical techniques, valuable information should first be gained from broad spectrum screening. A broad spectrum analytical screening technique was developed for the analysis of non-polar lipophilic compounds from abiotic and biotic matrices using comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS). Once a specific compound class is identified, the method can then be used for quantification. This provides a powerful tool in optimising the amount of data that can be generated in a single analysis. A LECO Pegasus IV, comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS) system was used with a non-polar Rxi®-5Sil MS primary column and a mid-polar Rxi®-17SilMS secondary column. The developed method was tested on sediment samples extracted using selective accelerated solvent extraction. Non-target compounds in complex samples can be provisionally identified due to the predictable compound grouping within the chromatogram (based on physicochemical properties) and full mass spectra collected. The analysed sediment samples from industrial areas included the presence of various PCBs, PAHs, PAH derivatives, as well as chlorinated PAHs, storanes, hopanes, and triphenols associated with petrochemical contamination. In agricultural areas terpenoids, terphenyls and PAHs were prevalent with various pesticides. PAH, PCB and OCP concentrations were quantified successfully using the data collected in the same analytical run. This analytical technique allowed petrochemical contamination to be identified even though the initial focus of the study was PAHs, OCPs and PCBs. Petrochemical contaminants can be of concern to human and environmental health, especially PAHs and related compounds that are known human carcinogens. This data can then be used to justify a project focused on the presence of petrochemical contaminants in the South African environment.

493 Raman SERS monitoring of Acetylcholinesterase activity for the detection of pesticides

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Pesticides are well-known chemical compounds highly toxic to health and environment with important adverse effects on the environment. Usually, chemical methods are implemented to detect pesticides such as gas chromatography and high performance liquid chromatography, although these can’t provide rapid, sensitive, simple and low-cost on-field detection. Over the last decade more sensitive and specific pesticides detection techniques have been explored, such as fluorescent and amperometric, even if they are not appropriate for routine application, they give a sum parameter of the presence of pesticides without any qualitative or...
quantitative information on the individual analyte. In this work, we present a new Raman SERS biosensing system for the qualitative and quantitative detection of pesticides by measuring the Acetylcholinesterase (ACHE) activity. The Raman SERS is not only used for measuring the ACHE activity, but also the direct detection of pesticides individually. Gold nanoparticles (AuNPs) were used as dyeing surface-enhanced Raman spectroscopy (SERS) substrate for measuring ACHE activity in the presence of very low levels of organophosphate and carbamate pesticide. The limit detection of paraoxon and carbaryl were May 2013.

It is not reported as more unpleasant. The results obtained permitted the odour untrained panel were presented. Now, additional data with a joint panel including chlorine dioxide in drinking water. Part II. R. Devesa et al. Chemical and sensory analysis of chlorine dioxide in drinking water. Water and sediment samples. Acute Daphnia toxicity testing ws carried out to assess potential harm of ozone to acute effects on organisms. Results of method development, environmental water monitoring and effective concentrations (ECs) are presented.

Bioavailability and Toxic Response of Bound PAHs in Natural Organic Matters for Oryzias latipes

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The research aims to carry out investigation on bioavailability of PAHs in the present of different NOMs for model animal pelagic Japanese medaka (Oryzias latipes). Our previous work has shown that the uptake rate of HOCs in triolein-embedded cellulose acetate membrane (TECAM), a kind of passive sampler, is independent on HA concentrations. Therefore, using TECAMs as a biomimetic tool, combined with biomarkers, the bioavailability of bound PAHs in NOMs to Oryzias latipes, as well as the aging effect on PAHs-NOM association and its mechanism was studied. Dihumic acid (HA), and four kinds of humins from different sources were examined in this study as different types of NOMs. The freely dissolved concentration (CDF) of PAHs were estimated by using TECAMs. A bioavailability model as followed was used to describe the bioavailability extent of bound PAHs: \[ F_{\text{BA}} = C_{\text{Solute}} + (C_{\text{HA}} + C_{\text{Cof}}) \times \left(1 + \alpha K_{\text{Bio}} \right) \text{mg/L} \text{mg/L} \]

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (I)

Knowledge, Assessment, and Management for AQUAtic Biodiversity and Ecosystem Services aCROSS EU policies.

A.J. Nogueira, University of Aveiro / Biology; A. Rossi, ACTEON; G. Delacamarra, Instituto IMDEA Agua; T. Hein, BOKU; A. Iglesias-Campos, IUC-UNESCO; S. Jähnig, IGB; G. Piet, IMARES; M. Lago, Ecologic Institute aquatic ecosystems are rich in biodiversity and home to a diverse array of species.
and habitats, providing numerous economic and societal benefits to Europe. Many of these valuable ecosystems are at risk of being irreversibly damaged by human activities and pressures, including pollution, contamination, invasive species, overfishing and climate change. These pressures threaten the sustainability of these ecosystems, their provision of ecosystem services and ultimately human well-being. AQUACROSS strives to advance the implementation of ecosystems management for aquatic ecosystems in an effort to support the timely achievement of the EU 2020 Biodiversity Strategy and other international conservation targets.

In this regard, AQUACROSS aims to develop and test an assessment framework which considers the full array of interactions, including human activities, within aquatic ecosystems, to identify policies that can increase the resilience of aquatic ecosystems against the trend of declining biodiversity of aquatic ecosystems. The current broad policy landscape such as the Water Framework Directive and Marine Strategy Framework Directive means that sustainable management solutions require coordination and cooperation between different policy areas spanning freshwater, coastal and marine ecosystems, in addition to innovative business solutions and public-private engagement. The AQUACROSS project will support the achievement of EU and international biodiversity targets by delivering a consolidated and coherent outlook on EU policy for aquatic ecosystems; increasing knowledge on biodiversity and drivers of aquatic ecosystem change; supporting the management of Natura 2000 sites and invasive alien species; and testing environmental and business models for the provision of ecosystem services that will contribute to ecosystem protection.

498 Assessing dynamics and stability of River Ecosystems under multiple stressors conditions: Iberian rivers as case study

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River ecosystems are complex systems that naturally fluctuate on space and time around a regular pattern or dynamic equilibrium, governed by external environmental conditions. If these are varied up to a threshold, the system may shift towards a new contrasting steady-state or regime. Characterizing the ecosystem stability and predicting the conditions under which such shifts are produced is a challenging task that has become an area of growing interest in the last years [1, 2]. The present work aims at progressing on the understanding of the relationships between the dynamics and stability of Mediterranean River ecosystems and the environmental conditions, taking Iberian Rivers as case study. We also aim at bringing closer modelling and field ecology under a common framework that enables the exploitation and interpretation of field measurements. To do so, we applied a mathematical dynamic model structurally equivalent to the well-known Lotka-Volterra that takes explicitly into account spatial relationships [3]. The model considers both the spatial connection and similarity of biological and other environmental descriptors associated to each river site. Its application provides relevant information regarding questions like the system’s stability, transition between states, as well as the interpretation of the longitudinal patterns of the rivers and their evolution according to shifting environmental conditions. By doing so, we will approach modeling and experimental environmental practices under a common framework that facilitates the understanding of ecosystems’ behaviors and links to multiple stressors effects. References [1] Rohr RP, Saavedra S, Bascompte J. 2014. On the structural stability of mutualistic systems. Science 345: 416-425 [2] Scheffer M, Carpenter SR, Lenton TM, Bascompte J, Brock W, Dakos V, van de Koppel J, De Ruiter PA, Levin SA, van Nes EH, Pascual M, Vandermeer J. 2012. Anticipating critical transitions. Science 338: 344-348 [3] Dakos V, van Nes EH, Donangelo R, Fort H, Scheffer M. 2010. Spatial correlation as leading indicator of catastrophic shifts. Theor. Ecol. 3: 163-174 Acknowledgement - This work is supported by the European Communities 7th Framework Programme funding under Grant Agreement No. 603629-ENV-2013-6.2.1-Gloebaqua

500 Natural variability of biochemical biomarkers in the macro-zoothenths: dependence on life stage and environmental factors

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Biomarkers have been widely implied in ecotoxicology as indicators of exposure to toxicants. However, the usefulness of biomarkers in ecotoxicology and, in particular, their ability to provide unambiguous and ecologically relevant information on the exposure, composition and impacts of toxic contaminants. One of the major problems for the use of biomarkers in ecotoxicology is understanding if the measured responses are determined by the effects of stress factors or lie within the natural variability range produced by the effect of environmental parameters. In a previous work, the natural variability of enzymatic levels, measured in some macro-zoothenths taxa of Milanese rivers, was proved to be relevant across both space and time. In this work, the experimental design was improved by considering different life stages of the selected taxa and by including the measure of additional environmental parameters. The method aimed at evaluating the variability of some enzymatic biomarkers measured in freshwater benthic invertebrates collected in pristine alpine streams in order to limit any potential anthropic influence. The experimental design considered: sampling sites in two different areas; eight sampling dates covering the whole annual cycle; four taxonomic groups (Perlidae, Baetidae, Heptageniidae, Hydropsychidae); present in almost all samples; three to four different life stages considered for each taxonomic group; four enzymes: Acetylcholinesterase, Glutathione-S-transferase, Alkaline phosphatase, and Catalase. Biomarkers levels were related to several environmental parameters (temperature, pH, conductivity, oxygen level, nitrite and phosphate concentrations, metals and metalloids concentrations), to verify any kind of dependence. Data were elaborated using multivariate statistical methods. Natural variability of enzymatic levels was found to be relevant across both space and time. The results of this work proved that great care should be paid when interpreting experimental results on biological parameters among sites or dates; further research is needed to understand how the natural variability of biomarkers could be accounted for and managed in ecotoxicological studies.

501 How do anthropogenic pollutants affect the genetic structure of a macroinvertebrate freshwater population?

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Organisms are rarely exposed to only one single anthropogenic stressor in the environment, but rather to multiple human-derived threats acting simultaneously. Environmental pollution can modify genetic population structure via ecological bottlenecks, adaptation of species to contaminant levels, altered mutation rate. Organic micropollutants such as pesticides, biocides, pharmaceuticals, personal-care products, or industrial chemicals are ubiquitous in the aquatic environment and their effects are considered a relatively new and emerging anthropogenic pressure over evolutionary processes. Especially potential effects of pollutants on genetic population structure may be more disruptive regarding ecosystem functioning than individual-level effects. Despite the bunch investigations on genetic variation in wildlife, our understanding about the individual stressor effects on genetic variation is still limited. Recently, there has been an increased interest in integrating environmental chemistry and evolutionary ecotoxicology approaches into the assessment of direct and indirect effects of anthropogenic pressures on populations. To address these challenges the genetic structure of a shredder invertebrate, Gammarus pulex, was examined using evolutionary ecotoxicology and body burden approaches. Significant genetic depression was detected and remarkable presence of private/rare alleles occurred after first WWTP effluents. These effects occurred together with a drastic increase in pollutants together indicating their mutagenic activity. Thus, it is highly probable that mutagenic chemicals emitted by the WWTP have a significant impact on genetic variation of gammarids. Conversely, a slight though significant increase in genetic diversity was detected in a short stretch of the river (~four kilometers) marked by rainwater drainage, the second wastewater treatment plant effluents and a small backflow level upstream the weir. These sampling sites showed a strong significant correlation in RDA analysis performed in order to assess the influence of man-made stressors on genetic diversity and structure. This study provides evidence of direct and indirect effects of anthropogenic pollutants on genetic variation in an invertebrate freshwater population. Clear signal of pollutants-induced genetic changes were determined in the Holtemme River.

502 Influence of chemical and non-chemical stressors on the macroinvertebrate community composition of the Danube river

A. Focks, Wageningen University / Environmental Risk Assessment Team; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team

A key challenge for the evaluation of the impacts of chemical pollution in aquatic ecosystems has been to disentangle their isolated toxic effects caused by water contaminants from those originated by other sources of natural or anthropogenic stress. In this study we developed a method for the evaluation of single and combined effects of chemical pollution on aquatic communities of large rivers. The method was based on the combination of univariate and multivariate statistical techniques including GLMs, PCA, RDA and Variation Partitioning. The method was applied to the database obtained as part of the environmental monitoring campaign performed during 2013 in the Danube river and used in the SOLUTIONS project. The database contained information on the macroinvertebrate community from 55 sampling sites and measurements of more than 300 environmental parameters including hydromorphological conditions, physical river alterations, and biological parameters like water quality parameters (i.e., pharmaceuticals, pesticides, metals, industrial pollutants, etc). The results of
Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (I)

A conceptual framework for a solutions-focused management of chemicals J. Munthe, IVL Swedish Environmental Research Institute Ltd; M. Rahmberg, E. Brorström-Lundén, T. Rydberg, IVL Swedish Environmental Research Institute; L. Posthuma, RIVM / Centre for Sustainability Environment and Health; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health; G. Engelen, Flemish Institute for Technological Research VITO; D. Bunke, Öko-Institut e.V. / Sustainable Products - Material Flows Division; J. Slobodnik, Environmental Institute; B. Gawlik, European Commissions Joint Research Centre JRC; J. van Gils, DELTAres; W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis; R. Altenburger, UFZ Centre for Environmental Chemistry / Biota and Sediments; D. López Herráez, Helmholtz Centre for Environmental Research UFZ

Risk assessment and prioritisation procedures have in common that they are based on extensive research and evaluation and focus on one chemical substance at a time, in contrast to real world contamination comprising thousands of chemicals. Challenges remain to link the occurrence of chemicals to the status of waters, to identify major chemical stressors including mixtures, and to find solutions for abatement. The project SOLUTIONS will address these challenges. To manage the SOLUTIONS approach, a conceptual framework was developed with four main entry points: Chemicals, Environment, Abatement and Society based on needexpressed by end-users. The entry points are linked by four topics which describe the scientific challenges that will be addressed: Under the activity Identify and prioritize hazardous chemicals on different scales, the aim is to provide a hypothesis on causes and candidate chemicals starting with existing monitoring data and applying innovative approaches for improved monitoring such as target and non-target screening, bioanalytical tools and effect-directed analysis – EDA. Modelling using an integrated ‘model train’ to link emissions to impacts provides a useful tool to fill data gaps and to extrapolate. The activity Selection of abatement options is focused on developing guidance on selection of and placement of abatement options. The topic Decision support for management of chemical pollution is aimed at providing guidance on existing and future policy frameworks. Potentials for synergies will be assessed and gaps in current policies identified e.g. for groups of chemicals or specific sectors in society not or poorly covered by existing legislation. Under the activity Predict and prioritize future risks the approach is to assess future emissions based on an evaluation of relevant societal scenarios (economy, demography, industry, consumption, energy, agriculture) and how these trends and scenarios may affect fate and occurrence of chemicals. SOLUTIONS will deliver a number of important products which can be used for the implementation of a sustainable use of chemicals in Europe. The information and experiences will provide the basis for introducing an innovative solutions-focused approach in risk assessment for emerging substances. Acknowledgements The SOLUTIONS is a EU FP7 project, grant agreement 603437.

A 20-years record of emerging and priority pollutants in salmon tissues from southwestern France M. Monpertuis, Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; J. Barre, IPREM UMR CNRS Université de Pau et des Pays de l’Adour / Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; V. Boulet, UMR ECOBIO INRA - Université de Pau; J. Cavelheiro, IPREM UMR CNRS UPPA / IPREM UMR CNRS; E. Tesser, Université de Pau et des Pays de l’Adour / Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; L. Iancu, IPREM UMR CNRS; D. Amouroux, Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; J. Barre, IPREM UMR CNRS; E. Tesser, Université de Pau et des Pays de l’Adour / Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; L. Iancu; IPREM UMR CNRS; D. Amouroux, Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; J. Barre, IPREM UMR CNRS; E. Tesser, Université de Pau et des Pays de l’Adour / Laboratoire de Chimie Analytique Bioinorganique et Environnement LCABIE; L. Iancu.

Coastal rivers are subject to human pressures often combined with the emission of potentially hazardous organic and inorganic contaminants. In addition, some pollutants in previous decades have accumulated in sediment and can be leached into the water column under certain conditions such as floods. Without ending up at alarming levels in terms of toxicity, many contaminants can impact aquatic wildlife. Traditionally, the most studied pollutants correspond to the priority substances listed in the Framework Directive (WFD) such as pesticides, metals, organometals, PAHs and PCBs. There is however a growing awareness of the occurrence of emerging pollutants for which the elimination processes in wastewater treatment plants are not yet adapted. Among them, the musk compounds for which there is currently no environmental quality standards set by the WFD. Synthetic musks are primarily components of personal care products. Because of their ubiquity and lipophilic properties, some of them are found in aquatic organisms, but few data still exist. The monitoring of priority substances and other pollutants, including new emerging substances, requires the implementation of temporal and spatial trend monitoring programs. The use of integrative matrices (biota and sediments) is strongly recommended to achieve such objectives. Fish are ideal for contaminant monitoring as they accumulate pollutants both from the surrounding water and their prey through processes bioaccumulation and biomagnification. This study aims to investigate the occurrence and concentration of musks in various aquatic organisms (liver, muscles and gonads) in Atlantic salmon (Salmo salar).

We investigate the temporal variations of several chemicals (including organochlorine pesticides, HAPs, PCBs, metals and organometals, synthetic musks) and their metabolisation in salmon caught in the Nivelle river (Southwestern France).

Several biophysical parameters are also monitor for each individuals (weight, length, sex, age, percent lipid) as well as ecological parameters (C and N isotopic signatures, otolith chemical signatures, scales fingerprinting) over a 20 years period. This monitoring permits to define status and trends for these contaminants, but this multi-contaminant approach gives also inputs to raise important questions for both science and policy (where does the contamination come from and how can the situation be resolved?).

Impact of untreated wastewater on a major European river evaluated with a combination of in vitro and chemical analysis M. Koenig, Helmholtz centre for environmental research - UFZ; W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis; P.A. Neale, Griffith University / Smart Water Research Centre; K. Hirschova, J. Novak, Masaryk University Faculty of Science RECETOX / RECETOX; M.A. Hashmi, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis; S. Seidensticker, Bioanalytical- Applied Gesellschaft; M. J. Hauska, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; B.I. Escher, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology

The pollution of river ecosystems by complex mixtures of micropollutants, such as pesticides, pharmaceuticals and industrial chemicals is of concern for ecosystem health and for use as drinking water source. Untreated wastewater is discharged into the River Danube in Novi Sad, Serbia, which triggered the
monitoring data and applying innovative approaches for improved monitoring of aquatic environment - the role of effect-directed analysis, and the capacity of single species to realise their niches within the pollution scenario. Does this differ with species: implications for adaptation?

Analysis was performed that indicates correlations between the presence of pesticides, HAPs, PCBs, metals and organometals, synthetic musks) and their biotransformation of microcystin-LR and the herbicide Roundup Flash® (which lead to enhanced accumulation of the cyanobacterial toxin. Detoxification by Roundup Flash® and could not increase in the mixed exposure. Thus the approach is to assess future emissions based on an evaluation of relevant pathways revealed prominent effects on the activation of metabolism (arylhydrocarbon receptor (AhR) and the constitutive androstane receptor (CAR)), endocrine disruption (estrogen receptor ER, androgen receptor AR) and adaptive stress responses (oxidative stress, inflammation). Of the analyzed 261 compounds 112 were detected at least in one sample. Both chemical and biological analysis revealed the same pattern. The site upstream and 7 km downstream of the discharge had a similar burden of chemicals and effects whereas directly downstream of the wastewater discharge effects and micropollutants’ concentrations were increased, particularly those typically associated with wastewater. The detected chemicals could explain 28% of AhR activation, 62% of AR inhibition and 24% of glucocorticoid inhibition in the water. The direct effect of pesticides on the aquatic organisms was therefore significant. The direct and indirect wastewater discharge increased the micropollutant load, but the large volume of the receiving stream Danube diluted them within a few kilometers to a status similar to upstream of the discharge. Keywords: bioassay; chemical analysis; Danube; Serbia

Detection of physiological activities of pharmaceuticals in river water: Suggestions to prioritization of pharmaceuticals in research involving environmental monitoring and toxicity testing

M. Ibara, S. Hanamoto, Kyoto University; H. ZHANG; H. Tanaka, Kyoto University

Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Although these are generally found at very low levels (e.g., ng/L to μg/L) in these waters, concern about their potential risks to aquatic species has been raised because they are designed to be biologically active. Whether pharmaceuticals in aquatic environments pose risks to aquatic organisms, we must know the extent to which such organisms may be exposed to those pharmaceuticals as determined by the pharmaceuticals’ respective modes of action. G protein-coupled receptors (GPCRs) are the largest group of these cell surface receptors in eukaryotes, and participate in various physiological and pathophysiological processes. It is estimated that nearly half of all marketed pharmaceuticals act by binding to GPCRs: for example, antihypertensives, antipsychotics, antidepressants, antiallergics, and antiasthmatics. In 2012, Inoue et al. developed an in vitro transforming growth factor-α (TGFα) shedding assay, in which GPCR activation is measured as ectodomain shedding of a membrane-bound preform of alkaline phosphatase-tagged TGFα and its release into conditioned medium. The TGFα shedding assay can detect not only activation but also inhibition of GPCRs, and is a very simple and rapid tool. In this study, we measured the agonistic activities of GPCR-acting pharmaceuticals in WWTP effluents, and upstream and downstream of the WWTP outfall in Japan using the TGFα shedding assay. We selected receptors for angiotensin (AT1), dopamine (D2), acetylcholine (M1), histamine (H1), and adrenergic family member (β1). Activities detected in waters were quantified as antagonist equivalent quantities (EQs). Antagonistic activity against AT1, D2, M1, H1, and β1 receptor were detected in the final effluent of the WWTP A through two years. Particularly, antihypertensive activity was observed at the AT1 and H1 receptor were stronger than 0.5 μM as olmesartan-EQ (μg/mL) or dihydroehydramine-EQ (μg/DIP-L), respectively. Antagonistic activities against AT1, H1, and M1 receptor in downstream of river were higher than those in upstream due to the effluent from the WWTP A. Based on these results, we propose that pharmaceuticals antagonistic to AT1, D2, H1, M1 and β1 receptors should be prioritized in future environmental monitoring and toxicity testing.

Enhanced Environmental Non-target Screening: Connecting Mass Spectrometry, Big Data, Knowledge Sources and Predictions

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Non-target screening using high resolution mass spectrometry (HR-MS) is essential to help prioritize and identify the tens of thousands of unknown chemicals detected in complex environmental mixtures, but is also time-consuming and requires compilation of information from a plethora of sources. A high-throughput, consolidated non-target identification workflow for HR-MS was developed within the European project SOLUTIONS. The in silico framework lever MetFrag, a search engine to increase the discovery and patent information, spectral similarity, suspect list screening, element and/or substructure selection and exclusion as well as user-defined scores [1, 2]. References and patents provide vital clues for high-use substances, while suspect screening allows candidate retrieval from large compound databases (several million entries) combined with tagging entries in various suspect lists (e.g., STOFF-IDENT [3], NORMAN Suspects[4]). User-defined scores allow e.g. the inclusion of per-substance toxicity predictions relevant for effect-directed analysis (EDA). MetFrag2.2 was evaluated on 1308 merged spectra of 975 environmental reference standards from MassBank, with 71% ranked correctly in first place using fragmentation, RT and reference/patent information. The workflow in R covers peak-picking to identification with enviPick, enviMass, enviPat, non-target, RMassBank and ReSOLUTION. A graphical user interface, enviVsyp, is also available. The workflow was tested on upstream, downstream and effluent samples from three locations in the SOLUTIONS Rhine case study. Masses (159 positive, 137 negative mode) were prioritized for identification using peak picking, componentization (isotope, adduct information) and pattern analysis. Transformation products to U1 and effect-directing surfactants were clearly formed during direct treatment and present downstream. Downstream of Muri WWTP, 110 high intensity non-targets were investigated, with 83 targets from STOFF-IDENT (7602 substances, including REACH) among 72,331 total candidates retrieved from ChemSpider. The top matches for 54 masses are highly promising; confirmation efforts are underway. The workflow greatly exploits high-throughput non-target screening and has huge potential to assist prioritization and EDA. [1] Ruttkies et al 2015 J. Cheminformatics, under review [2] http://c-ruttkies.github.io/MetFrag/ [3] http://b-b-stoffident.hswt.de/ [4] http://www.norman-network.com?3=mode/236

Revisiting the Mutagenicity in River Rhine: Identification of Mutagenic Aromatic Amines

m. musz, UFZ - Helmholtz Centre for Environmental Research / Effect Directed Analysis; D. Cassier, A. Bahlinm, M. Krauss, W. Brack, Helmholtz Centre for Environmental Research – UFZ / Cell Toxicology; B.I. Escher, Helmholtz Centre for Environmental Research-UFZ / Effect Directed Analysis; M. Koenig, Helmholtz centre for environmental research - UFZ / Environmental Institute; B. Gawlik, European Commissions Joint Research Institute of Aquatic Science and Technology / Environmental Chemistry; M. Kirby, Cefas / Ecotoxicology; B. Bowles, Cefas / OCNS; C. Phillips, Cefas acts as the UK Government regulators or advisors to the regulators in a number of Marine Science fields and operates separate regulatory capacity which enables us to view the use of standardised testing from more than one perspective. We discuss how the availability of standardised tests affects the regulator and those involved in submitting reports and testing. A case study is examined where the use of standardised tests allowed the regulator to re-assess data from previous submissions. Aspects of standardised tests that allow regulations to operate effectively are discussed. Is it critical for the standardised test to be chosen?
511 Standards in Environmental Toxicology - Strengths and Weaknesses - Practicability versus Complexity

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The strength of Standards in Ecotoxicology is that the users not only know exactly - but also have to abide by - all the detailed descriptions. The Standards prescribed by national and international organizations for toxicity testing unfortunately often differ - sometimes even to a substantial extent - on many aspects. These differences cloud the use of the tests and the comparison of the results. Numerous examples of the differences of prescribed standard toxicity test methods will be highlighted with regard to e.g. selection of test species, effect criteria, exposure time, number of test organisms and replicates, validity criteria and data analysis. A major drawback of many Standards is that only little attention has been paid to their practicability/complexity aspects. It is often argued that for the evaluation of the hazards of chemicals - as is e.g. the case for REACH dossiers on chemicals - these "inherently" practicability/complexity aspects have to be coped with. Standard toxicity tests are, however, also needed for application in a regulatory framework for routine toxicity assessment of polluted environments, and practicability and cost problems are in this regard a major point. Independently of Standards, scientists have presented the former drawbacks by developing "sand-scale" toxicity test methods, and the name "microbiotests" was coined in this regard in 1991 by Dr. C. Blaise. Microbiotests must be inexpensive or cost-efficient, generally not labor intensive, have high sample throughput potential, be based on cultures which are easily maintained or maintenance-free, require modest laboratory and incubation space, can sample site-specific bioavailability assessment or passive sampling approach is best to use and the bioavailability assessment or passive sampling approach is best to use.

514 Advancing the use of passive sampling in risk assessment and management of contaminated sediments: Results of an international passive sampling ring test


Acknowledgement - The authors thank the Swiss Federal Office for the Environment (FOEN) for financial support and industry partners for providing their products for testing.

512 Implementation of the TRIAD approach for the ecological risk assessment of an old lead mine.

N. Pho Oue, INERIS; S. ANDRES, INERIS / Toxicological Ecotoxicological Assessment of chemical Substances; P. Pandur, INERIS; C. GRAND, ADEME/ Site specific ecological risk assessment of soil contamination goes with intrinsic difficulties. Observed differences are heterogeneity of soil properties, landscape, local communities, diversity and ecosystem structure, and contamination nature and pressure. In this context, the International Standard ISO/DIS 19204 “Soil quality – Pollution management – Ecological risk assessment (Soil quality TRIAD approach)” describes in a general way the application of a combined three lines of evidence (chemistry, ecotoxicology and ecology). The TRIAD includes different tiers in which each consecutive tier is increasingly fine-tuned according to the site-specific situation. Data collected on an open pit mine operated during 60 years and which the activities stopped about 100 years ago as been used to evaluate the ease and efficiency in using the different tiers of TRIAD. The pollution pressure still present in the mine is of metallic origin (mainly Pb, Cd, Zn) and 5 areas with different levels of contamination, vegetation and quantities of organic matter in soil were chosen. It was possible to apply the tier approach on three selected locations. The combined use of chemical concentration in soil (Chemistry), plant screening test (Toxicology) and simple vegetation survey (Ecology) were sufficient to assess the risk on some of the areas. In consequence, some of the expensive and time consuming data collected in these locations were not needed to assess the risk. On other locations, uncertainties were still too great and the implementation of higher tiers was needed. The focus during this applied case was on the outcome of the TRIAD method in order to obtain practicable results according to the complexity of the study. It reveals that for some locations, the use of TRIAD method can save financial and time resources. The use of the highest tier on particularly complex zones will need additional data, future investigations are planned for the year 2016.

513 Ecotoxicological assessment of corrosion protection products used on hydraulic steel structures

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PCBs). The results demonstrated that standardizing methods significantly decreased the overall inter-lab variability. The resulting variability however still largely exceeded the intra-lab/intra-method variability. By performing all analyses in one laboratory, this difference in part could be explained by the substantial variability introduced through analytical chemistry (i.e., identification, integration, calibration of target chemicals). Overall, the findings of the ring test suggest that passive sampling is fit for implementation in risk assessment and the management of contaminated sediments when following standard protocols.

A focus on research and education tools in environmental toxicology and chemistry

512 A coordinated set of ecosystem research platforms open to international research in toxicology, AnaEE-France

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Human activities have altered continental ecosystems worldwide and generated a major environmental crisis. To truly develop integrative ecosystem biology and to assess the consequences of various toxic factors, such as pollutants, we therefore need new approaches and tools that bridge the traditional gap between life and environmental sciences. To deal with that challenge, the infrastructure for Analysis and Experimentation on Ecosystems (AnaEE-France) is an integrated network of the major French experimental, analytical and modeling platforms dedicated to the biological study of continental ecosystems, both aquatic and terrestrial. This infrastructure aims at understanding and predicting ecosystem dynamics under global change. It comprises five complementary nodes offering access to the best experimental facilities and associated biological resources and data: Ecotrons, semi-natural experimental platforms to manipulate terrestrial and aquatic ecosystems, in natura sites equipped for large scale and long-term experiments. AnaEE-France also provides shared instruments and analytical platforms dedicated to environmental (micro)-biology. Finally, AnaEE-France provides users with data bases and modeling tools designed to represent ecosystem dynamics and to go further in coupling ecological, agronomical and evolutionary approaches. AnaEE-France offers adequate services to tackle the new challenges of research in ecotoxicology, positioning its various types of platforms in an ecologically advanced ecotoxicology approach. AnaEE-France is a leading international infrastructure and it is pioneering the construction of a European AnaEE infrastructure in the field of ecosystem research. This infrastructure is open to the international community of scientists in the field of continental ecotoxicology.

516 MOSAIC: a web interface with modelling and statistical tools for ecotoxicology

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MOSAIC stands for “Modelling and Statistical tools for ecotoxicology”. In ecotoxicology, bioassays are generally conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC is a user-friendly web interface dedicated to the mathematical and statistical modelling of such standard bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists and regulators. Without wasting time on extensive mathematical and statistical technicalities, users are given advanced and innovative methods for a valuable quantitative risk assessment. MOSAIC is available at http://phil.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers three operational tools: (i) MOSAIC_CSV, a tool dedicated to the species sensitivity distribution (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the calculation of the so-called hazardous concentration for p% of the species (HCp), even when the toxicity values are censored; (ii) MOSAIC_repro, which provides users with a complete statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. Concentration-response models are fitted within a Bayesian framework to provide ECx values; (iii) MOSAIC_surv, which provides users with a "new" survival data. A log-logistic model is fitted within a Bayesian framework to provide ECx estimates. This presentation is an overview of MOSAIC features and a demonstration of illustrative examples as provided within the web interface.

517 ImpactE: an emerging innovation center in environmental performance of processes, products and ecosystems

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The innovation center ImpactE, created in 2015, aims at transferring scientific knowledge and technical skills in ecology, ecotoxicology, microbiology, biochemistry and physico-chemistry, from the laboratory to the industry. The targeted partners are manufacturing industries concerned with the environmental impact of their activities, environmental engineering companies, and companies involved in sustainability development. The activity is presently focused on assisting small and medium size companies to improve their eco-efficiency, and thus to increase their economic health. Therefore we raised co-funding from National and Regional Authorities, the French State and Europe. ImpactE offers: Studies to improve environmental performance of manufactured products, taking into account ecotoxicity risk assessment, according to the physical and chemical properties of the products, Studies to reduce the environmental impact of manufacturing, according to sustainability development concepts: waste and secure management, monitoring of contaminated sites, remediation technologies. Training courses for companies personal in ecology, ecotoxicology, risk assessment, … The ImpactE team provides high level scientific skills acquired by a long time experience in the multi-disciplinary research areas. We also can help our partners to perform funding arrangement of collaborative projects. Keywords: environmental risk assessment; environmental performance; research and education tools; innovation.
It is widely agreed that the entire Life Cycle of nano-enabled products is the appropriate unit of analysis to consider its sustainability aspects, including its social impacts. However, there is limited information on which social impacts are relevant for nano-enabled products, and a methodology to monitor them is lacking. In the EU SUN (Sustainable Nanotechnology) project a quantitative methodology based on Social Life Cycle Assessment (s-LCA) also new Multi-Criteria Decision Analysis (MCDA) is proposed to assess the social impacts of nano-enabled products through their Life Cycle. The s-LCA conceptual scheme (i.e., impacts and indicators for different stakeholders) is developed through an appraisal of literature on social impacts of products and Ethical Legal and Social Impacts of nanotechnology. It comprises of nine impacts each for workers, consumers and community and six impacts for value chain actors. Out of them, five indicators associated with impacts of nano-enabled products, with two impacts in Worker category (Professional training and Non-fatals accidents) and three impacts in Community category (Education, Employment, Research and Development expenditure and Non-fatals accidents). A quantitative MCDA methodology is proposed and applied to a case study that compares the social impacts of two nanotechnology applications. One advantage of using MCDA with s-LCA is that qualitative stakeholder preferences on indicators can be combined with quantitative indicator scores. The gaps to be addressed to expand the future development of methodologies to assess social impacts of nano-enabled products are also considered in the work.

523 Exploring correlation between spatial distribution of fertilizers emissions and social disfavour indicators: a case study in Luxembourg
S. BORRELLY, Université Lyon III Jean Moulin, A. ARAGULAS, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRTE/Resource Centre for Environmental Technologies CRTE
This work is the first step of a research aiming at a coupling of health geography and LCA to i) calculate and map the impacts of agricultural practices on ecosystems and 2) find their correlation with human factors that we call social disfavour indicators (such as low incomes, high distance from hospitals, low level of education, high rate of unemployment, etc.). The aim of the analysis is exploring the existence of a statistically relevant correlation between ecosystems’ damages and human health, and providing a representation of this correlation at a fine spatial scale (at the level of the territory of single communes). In this respect, LCA can nicely complement health geography by providing spatial information (to calculate emissions and translate them into potential impacts) and health geography can provide to LCA powerful computational techniques to evaluate spatial correlations. In this presentation we show the application of a clustering methodology very commonly used in computational geography in order to groups communes by classes of criticality with respect to risk of environmental contamination and their correlation with variables of social disfavour. The presentation also introduces the limits of this method and proposes a possible enhancement of the methodology.

524 Life cycle sustainability assessment for agricultural holdings: integrating environmental, economic and social aspects
A. Roese, Agroscope Reckenholz-Tänikon Research Station ART / Agroscope; T. Nemecek, G. Guillard, Agroscope / Institute for Sustainability Sciences ISS
Despite the widespread acceptance of the ‘three-pillar’ model for sustainability – environmental, social and economic – there is still a serious lack of technologically and practically feasible data-collection solutions for conducting a social life-cycle assessment (SLCA) and life-cycle costing (LCC) for a large sample of agricultural holdings. Despite extensive research there is still a considerable lack of methods for a comprehensive assessment of the sustainability at the farm level as many of these reference from gaps in the present frameworks. Therefore the analysis is focused on how EcoBIM researchers have approached the problem in the field involving the design of educational tools in aquatic ecotoxicology. The PROFECIA project, its objectives and its implementation will be presented.

Advances in Social Life Cycle Assessment and Life Cycle-based Sustainability Assessment

520 PROFETIA: Open and Online Training Program in Aquatic Ecotoxicology
L. Parent, Télé-université / UER Science et Technologie; S. Betoulle, URCA / UMR SEBIO; M. Fournier, INRS-Institut Armand Frappier / ISMERUQAR; M. AUUFFRET, IUEM / LEMAR UMR
ECOBIM, the international research network in aquatic ecotoxicology was initiated in 2001 by researchers from Quebec (Canada) and France, who shared projects research in aquatic ecotoxicology. While several researchers from countries in Europe, Africa and the Americas participated in the annual meetings, it seemed important to share their teachings to promote transfer of knowledge, harmonizing approaches and methods, and to supplement courses with notions sometimes absent. It was proposed to create a training program that understand different credited modules consisting of digital educational resources available online, as well as methodological harmonization workshops offered in presence at the annual meetings of EcoBim. This training program that would be offered to the Master and doctoral level, and initial training or continuous would be the scientific knowledge how EcoBIM researchers. This strategic partnership in the field involves the design of educational tools in aquatic ecotoxicology. The PROFECIA project, its objectives and its implementation will be presented.

521 Data quality management in Social LCA studies
A. Cirioth, F. Eisfeldt, GreenDelta
Typically, many different information sources are consulted for a social LCA case study, from surveys to company information to public sources of various origins. As social data can change rather quickly, and since several sources can contribute, following a triangulation approach, to a final data point used in a study, an assessment and effective handling of the data quality of social information is important for social LCA. This holds both for social LCA case studies and for generic databases used for them. Understanding the data quality is important especially if the study is used for decision support. An approach for addressing data quality for social LCA studies is presented. The approach uses a specific pedigree matrix to evaluate the quality by 5 different indicators, following evaluation scores from 1 to 5. This matrix is implemented in an LCA software (openLCA), and it is also supported by the social LCA database PSILCA. In a case study on latex products, it is shown how both together support the management of data quality for social LCA in decision support, to detect e.g., whether hot spots appear in the case study to some extent because of weak data quality, or whether hot spots call for immediate action because they are based on reliable information. As of course, vice versa, there may be cases where hot spots are not visible because the available information is weak, hiding hot spots.

522 A methodology for social impact assessment of nano-enabled products
A. Kherajmanian, Ca Foscari University of Venice / Dept of Environmental Sciences Informatics and Statistics; E. Semenzin, Ca Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics; A. Zabot; D. Hristozov, Ca Foscari University of Venice; I. Malsch, Malsch TechnoEvaluation; P. Saling, BASF SE / Sustainability Strategy; T. van Harmelen, T. Ligthart, TNO; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics
A methodology for social impact assessment of nano-enabled products was developed and in the following years, it was tested and validated in the scope of several European projects. It has been successfully used in the SUN project in which MCDA was developed to couple environmental and social aspects. The results indicate that the pipeline is likely to reduce the total workload, and the aesthetic value of the agricultural landscape, and (iii) a set of quality, (ii) social sustainability, including animal welfare, physical and mental workload, and the aesthetic value of the agricultural landscape. In this respect, LCA can nicely complement health geography by providing spatial information (to calculate emissions and translate them into potential impacts) and health geography can provide to LCA powerful computational techniques to evaluate spatial correlations. In this presentation we show the application of a clustering methodology very commonly used in computational geography in order to groups communes by classes of criticality with respect to risk of environmental contamination and their correlation with variables of social disfavour. The presentation also introduces the limits of this method and proposes a possible enhancement of the methodology.
indicator for the workload of both the farming family and the employees was developed. The landscape quality will be assessed by the diversity Shannon index based on preference rating scores for typical agricultural landscape elements. Animal welfare will be included by a pragmatic approach based on a credit point system. The implementation of an efficient data collection process will be tested in a follow-up project based on a small sample of farms, with the mid-term objective to extend the sample on a substantial part of the Swiss farming sector.

525 Life cycle sustainability analysis - a procedure and a case study
T. Ekvall, H. Ljungkvist, IVL Swedish Environmental Research Institute; A.F. Sandvall, E.O. Ahlgren, Chalmers University of Technology
This presentation aims to contribute to the development and demonstration of an operational approach to life cycle sustainability analysis (LCSA). This approach originates from the framework developed within the EU project CALCAS. The framework is a life cycle sustainability assessment tool outlined by Klöpffer in that it not only broadens the scope of life cycle assessment (LCA) to include economic and social aspects, but also allows for deepening of the analysis. It is also different in that it does not predefine the LCSA to be the sum of LCA, life cycle costing (LCC) and social LCA. Instead, the sustainability indicators, the systems investigated and the methods used for the analysis are all decided case by case. Our LCSA approach has two distinct features: 1. the case-specific research questions are defined in a participatory procedure that involves an Open Space workshop; 2. the analyses are carried through by a network of researchers and experts. A network is necessary because the research questions are not known in advance. We applied the approach in a sustainability assessment of a 50 km pipeline for transport of residual heat from industries to a large district-heating system. The LCSA included 14 research questions on economic, environmental and social aspects. The results indicate that the pipeline is likely to reduce the total costs of the system, but the expected profit is rather small and uncertain, and it is difficult to find a market model that ensures everyone a share of this profit. The environmental benefit of the pipeline is dependent on what electricity production increases when the residual heat in the DH systems reduces the combined heat and power production in these systems. The pipeline is likely to have no significant impact on the employment and a somewhat negative impact on the land owners. In conclusion, our LCSA approach proved to be operational. The Open Space format for workshops can generate a good basis for the research questions; however, care must be taken to ensure a balanced participation at the workshop, and complementary research questions might have to be added after the workshop. We found that an LCSA that is the sum of LCA, LCC and social LCA does not cover all sustainability aspects that stakeholders can consider important. We also found that the sustainability of a pipeline for residual heat is uncertain in this specific system and in the time frame investigated.

Exploring links between the biodegradation of chemical contaminants, the metabolic capability of microbial communities and environmental variables

526 Biodegradation of volatile hydrocarbons in five surface waters tested as composed mixtures in the µg/L range
H. Birch, DTU Environment / Department of Environmental Engineering; R. Hammershøj, P. Mayer, Technical University of Denmark / Department of Environmental Engineering
Biodegradation is an important removal process for many chemicals that are released to the environment. High quality experimental data are thus needed for the development and verification of biodegradation models. In biodegradation tests as well as in the environment, the microbial population is not controlled, and therefore a source of variability. In this study the variability of biodegradation in five different surface waters in Denmark with different characteristics regarding pre-exposure to petroleum hydrocarbons was investigated. The study was conducted with a composed mixture of 9 hydrocarbons that were all within a narrow carbon number range (9-12 C) but diverse in molecular structure. The 9 test compounds were biodegraded by low soil microbial populations at lower environmental concentrations, and all experiments were thus conducted well below solubility in and the µg/L range. The test method was based on OECD 309 but adapted for hydrophobic and volatile test chemicals. Stock solution of ~1/100 of the solubility for each chemical was prepared by partitioning based dosing from a loaded silica polymer. 20 mL test systems were then prepared using 135 mL of the surface water inoculum and 1.5 mL stock solution. Test systems were incubated at 20 °C for a maximum of 28 days on a roller and at fixed time intervals three replicate test systems and abiotic controls were analyzed on GC-MS using fully automated Head Space Solid Phase Micro Extraction (HS-SPME). Primary biodegradation (substrate depletion) was determined based on the HS-SPME chromatograms and the abiotic controls. The general order of biodegradation in the five surface waters was n-Decane > Bicyclohexyl > 1,2,4-trimethylbenzene > Biphenyl > Naphthalene > Tetratin > 2,3-Dimethylpentane > Decalin > 1,3,5-Trimethylcyclohexane. Lag phases were between 0 and 8 days. The half-lives were similar in four of the surface water samples and lower than predicted using the BioHCwin model. However, 1,3,5-Trimethylcyclohexane had a predicted half-life of 3.5 days but was only degraded in one sample after all other test chemicals were degraded. In the fifth sample from the clear lake half of the half-lives were higher and half lower than predicted in BioHCwin.

527 Exploring biotransformation of micropollutants in phytoplankton species: overview and influencing factors
M.A. Strazy, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; F. Porrati, Swiss Federal Institute of Aquatic Science and Technology / Aquatic Ecology; J. Hollender, Eawag / Environmental Chemistry
The role of phytoplankton in the biotransformation of micropollutants in surface water is largely unstudied; however recent studies indicate a potential role of phytoplankton organisms in the degradation of crop protection products. With three model species (Microcystis aeruginosa and Synechococcus sp., cyanobacteria, and Chlamydomonas reinhardtii, a green alga), we investigated biotransformation reactions of a set of 24 micropollutants, composed from strobilurin and azole fungicides representing diffuse source pollutants and pharmaceuticals representing point source pollutants. Among the reactions observed were hydrolysis of esters, dealkylations, methylations, deaminations and dehydryations, as well as some amino acid conjugations. Biotransformation activity was apparently not sensitive to the presence of low-concentration chemical stressors, however factors like pH and cell density effected some transformation rates. Selected reaction pathways, in particular associated with Chlamydomonas in single-strain experiments could be observed also in experiments with phytoplankton communities sampled from a lake. The study provides a general overview over biotransformation reactions present in phytoplankton species and influence of experimental or environmental conditions.

528 Glyphosate is biodegraded via two pathways in soil and water-sediment systems - a stable isotope co-labelling approach
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Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in soil and freshwater sedimentary environments, but there are no comprehensive studies on glyphosate behaviour in these systems. Microorganisms can use C and N from a pesticide to synthesise their biomass compounds, e.g. amino acids (AA) and fatty acids (FA). The extraction of known microbial biomarkers from soil or sediment after addition of C and N isotope tracer allows an estimation of microbial activity in the transformation of pesticide. We investigated the degradation pathways of glyphosate with the particular focus on the metabolic incorporation of the isotope label into AA, FA and their fate over time. An agricultural soil and water-sediment were incubated with co-labelled glyphosate (13C, 15N-glyphosate) in the dark and at constant temperature (20°C). 13C, 15N-glyphosate was used to its turnover to biomass balance over a period of 80 days. Soil and sediment samples at the respective sampling date were analysed for the amount and the isotopic composition of AA, FA, CO2, solvent-extractable parent compound and metabolites and total NER. In the water-sediment system, 55.7 % of 13C of glyphosate was ultimately mineralised, whereas the mineralisation in the water system (without sediment) was low, reaching only 2.4 % of 13C of glyphosate equivalents. Glyphosate was mineralised in the soil more rapidly and at the end labelled CO2 constituted about 73% of 13C-glyphosate equivalents. A rapid increase in 15N-AMPA after 10 days was noted in water-sediment system and these transformation products ultimately constituted 26.2 % of the 13C-glyphosate and 78.5 % of the 15N-glyphosate equivalents. In contrast, in the soil, 15N-AMPA increased initially but after 20 days decreased slowly reaching ultimately 12.3 % of the 13C-glyphosate and 39.6 % of the 15N-glyphosate equivalents. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labelled 15N-sarcosine-glycine. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of 15N-glycine. The presented data provide the first evidence of the utilization of glyphosate as a C and N source and highlight the relevance of both the sarcosine and the AMPA pathways in the water-sediment and soil system.

529 NfA-FRP nitroreductase enzyme family: a new tool to link biotransformation pathways and environmental contamination by mesotrione, a beta-triketone herbicide
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ionization as a mass spectrometry imaging technique. The sample is scanned in three dimensions at a preselected spatial resolution while the mass spectrum is recorded. Advantages, like measuring the distribution of a large amount of analytes at one time without destroying the sample, make it a useful method in solid-surface study. The present study aims at developing an analytical quantitative method for the study of biodegradation of a commercial homopolymer to be further used as a probe for the evaluation of the so-called “river metabolism”. Polycaprolactone (average MW=1250) was selected as suitable candidate polymer. MALDI-ToF imaging experimental conditions were optimized. The method was tested in the lab using different environmental conditions. Progress of biodgradation after several days of exposure was reflected on the changes in the mass spectra corresponding to variations in the chain length distribution pattern. Results were investigated using different image processing tools

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (II)

532 Toxicity exerted by AgNPs in OECD and LUFA 2.3 soils: From molecular to organism level responses in Eisenia fetida earthworms


Over 400 consumer products contain AgNPs (24% of the total products containing nanomaterials; e.g., detergents, paints, printer inks and textiles), which are also used in biomedical devices and water treatments due to their antimicrobial properties. Since the number of applications is widely increasing the release of AgNPs into different environmental compartments is already occurring, thus, the concern about the still scarcely known hazards of nanosized materials to environmental health has considerably grown. To date, the potential risk of AgNPs has been mainly studied in aquatic environments, while their effects on soils are less investigated despite the great complexity of soil matrix and the potential interactions of soil components with pollutants. These components (O.M, cations, soil colloids and water) together with the soil type varying pH and ionic strength factors, may affect the behavior of NPs, with particular effect on the aggregation and subsequent effect on their toxicity in organisms inhabiting soils. Eisenia fetida, the earthworm, has been widely used as model organism for soil health assessment due to their capacity to accumulate pollutants and their quick and measurable responses at different levels of biological complexity. Hence, the aim of this work was to compare the toxicity exerted by AgNPs in two widely used standard soils through molecular, biochemical and cellular responses measured in this earthworm. With such a purpose, E. fetida earthworms were maintained in OECD artificial soil (10% O.M) and in LUFA 2.3 natural standard soil (1.62% O.M) spiked with environmentally relevant and high but sublethal concentrations of AgNPs (0, 0.05 and 50 mg AgNPs/Kg). After 3 and 14 d of exposure, Ag accumulation, weight loss, coelomocyte number and viability, metallothionein (MT) levels and catalase (CAT) activity, damage in DNA and MT transcription levels were recorded. Results indicated that earthworms exposed to 50 mg AgNPs/Kg presented the highest weight loss, affection on coelomocyte parameters, an increase in MT levels and CAT activity and DNA damage in both soil types, although these effects were enhanced in earthworms maintained in LUFA 2.3, where mortality (7.5-10%) was also observed. It can be concluded that apart from the intrinsic properties of NPs (shape, size, surface chemical and charge) the characteristics of the exposure scenario (i.e. soil type, pH, O.M) are to be considered when assessing toxicity of AgNPs in soil.

533 Toxicity of Pristine and Aged Coated Copper Oxide Engineered Nanomaterials (CuO ENMs) to the Earthworm E. fetida

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Many engineered nanomaterials (ENMs) are coated to enhance their technical properties, but these coatings may also change their environmental fate and potential toxicity. There is currently little knowledge on the biological effects of aged nanomaterials and therefore two experiments were conducted to assess the differences in the effects of different coatings on ENMs to adult earthworms (E. fetida) as pristine ENMs and after 1 year of aging based on OECD TG 207. In both studies, worms were exposed for 14 d to negatively/positively charged, organic coated or uncoated (core) CuO ENMs (indiscriminate size range of primary particle 20-50 nm) at maximum concentrations of 1000 and 10,000 CuO/Kg with a comparison to iconic copper (200 mg Cu/kg as CuSO₄) in standard Lufa 2.2 soil. Survival, weight of earthworms and tissue metal concentrations (after a 24 h depuration period) were assessed at 7 and 14 d in each experiment. Biochemical endpoints were also analysed in worms from both experiments at 14 d to enable the determination of oxidative and osmoregulatory stress. Total Cu concentrations and pH (in 2.1 soil:water mixture) were measured in soils at 0, 14 d and after 1

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Multiple transformation product formation was investigated for compounds belonging to a relatively high mobility and bioavailability. Two major metabolites resulting from biotransformation are commonly found in soil: 4-methylsulfonfyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonfyl benzoic acid (AMBA). Several mesotrine-transforming bacterial strains have been isolated from various environments such as farmland and cloud water. Although these strains belong to different genera, the mesotrine transformation pathways are well conserved and characterized. They belong to the NfsA-FRP family of Nitro FMN reductases, of a NR in the transformation of mesotrine into AMBA. A screening of Mes11 NR activities strengthened this hypothesis. Preliminary biodegradation studies in water of commercial homo-polymer being linked to nitroreductase (NR) enzymes [2]. These results were consistent with the chemical structures of metabolites, strongly suggesting the involvement of a NR in the transformation of mesotrine into AMBA. A screening of Mes11 NR revealed two enzymes (NfrA1 and NfrA2) capable of reducing the nitro group of mesotrine. Both purified Mes11 NR were structurally and functionally characterized. They belong to the NfsA-FRP family of Nitro FMN reductases, previously shown as having activity on different nitro-aromatic compounds. Interestingly, members of the NfsA-FRP family have also been identified in other mesotrine-transforming strains, suggesting a key role of these enzymes for mesotrine transformation in various environments. The broad range of temperature and pH determined for Mes11 NR activities strengthened this hypothesis. This study constitutes the first identification of enzymes involved in mesotrine biotransformation. These enzymes (or the corresponding genes) may be used as biomarkers to predict natural attenuation of mesotrine in the environment and to assess potential contamination by the parent molecule and/or metabolites. NfsA-FRP NR could also be used in bioremediation processes or in the development of in situ biosensors for detection of nitro-aromatic compounds.

530 Effects of Solids Retention Time on Micropl pollutant Biotransformation Rates and Pathways in Activated Sludge

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An important parameter in wastewater treatment is the solids retention time (SRT), defined as the time during which the suspended biomass, on average, is maintained in each reactor and SRT was observed. However, we found that individual compounds showed strongly deviating behavior from this average trend. A clustering of compounds according to the trends of the transformation rate constants with SRT was therefore sought. When doing so, we observed different trends amongst the compounds, three of which correlated well with nitrification rate, oxygen uptake rate and SRT, respectively. For three of the clusters we identified groups of chemicals similar compounds that (mostly) showed the same trend. The group of p-aminobenzenesulfonamides, for instance, showed a slight decrease of transformation rate constants with SRT, highlighting the importance of a biological oxidizing system at the level of compound classes. In a follow-up analysis, we will then attempt to link the observed trends for types of biotransformation reactions with microbial community features, including taxonomic richness, composition and gene expression of relevant functional genes. References: [1] Clara M., et al. 2005. Water Research 39(1), 97-106.
year of age. There were no statistically significant differences between soil pH in all treatments (ANOVA, P > 0.05) so the data were pooled and the pH was 5.3 ± 0.2 and 5.2 ± 0.3 (mean ± SD, n = 40) at the end of the pristine and aged soil experiment, respectively. After 14 d, control group survival was 100% in both pristine and aged soil experiments. In worms exposed to 1000 mg Cu g⁻¹ CuO ENMs, survival was only 50 and 40% in the negatively and positively charged CuO ENM treatments, respectively, while in aged soil experiment only exposure to positively charged CuO ENMs resulted in a 30% survival. In both pristine and aged soil experiments, body weights were significantly reduced (ANOVA, P < 0.05) between 10 and 30% and 30% and 50% in all low and high Cu treatments, respectively, compared to controls. Total Cu in earthworms exposed to 200 mg Cu kg⁻¹ and 1000 mg Cu kg⁻² ranged from 0.02 – 0.14 and 0.05 to 0.3 mg Cu kg⁻¹ dw, respectively, at the end of the pristine soil experiment, analysis of the worms from the aged soil experiment is in progress. Early findings indicate aged ENMs are less toxic to earthworms than the pristine ENMs. The research is funded by EU FP-7 NANOSOLUTIONS Project, Grant Agreement No. 309329.

534 Toxicity of silver nanoparticles to soil organisms: an integrated in vitro-in vivo approach
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As engineered nanoparticles (ENPs) continue to find increasing applications in soil science, elucidation of their potential to pose environmental risks is urgent. We investigated the effects of size (20, 35 and 50 nm) as well as surface coating/charges (chitosan/positive CHIT; bovine serum albumin/negative BSA, and polyvinylpyrrolidone-neutral PVP) on the toxicity of silver nanoparticles (AgNPs) in vitro (Lambriacus rubellus) and in vitro (RAW 264.7 cell line) models. A 28-day sub-chronic exposure in soil spiked with varying concentrations (0 – 250 mg/kg soil) revealed highest uptake of AgNP_BSA by earthworms (body burdens 50 – 100 mg/kg BW), with already high uptake after only 72h. The number of cocoons produced was most affected by negatively charged AgNP_BSA particles. Similarly, results from cellular in vitro tests indicate the highest induction of TNFα with AgNP_BSA (70x higher than control). Significant in vitro ROS induction was only observed for the 20nm positively chitosan coated particles. Cytotoxicity tests showed no significant differences between the different types of AgNPs. Gene expression profiling using whole tissues of earthworms exposed to the different AgNPs was conducted and analyses are on-going. Early indications of gene alterations suggest the AgNP_BSA to be more effective. The present study provides further evidence of the influence of ENP properties in driving their bioaccumulation and toxicity.

535 Trophic transfer of engineered nanoparticles in terrestrial food chains
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The trophic transfer of engineered nanoparticles from soil through terrestrial food chains is being evaluated. Cerium oxide and lanthanum oxide bulk and nanoparticles (NP) were added to soil (0-1000 mg/kg) with zucchini or lettuce plants, respectively. Additionally, studies have been initiated in which lettuce was actively amended (0.025 mg/cm² d) after amendment with copper oxide NP, bulk and ions (0-400 mg/kg). The plant Cc, La and Cu accumulation was determined by ICP-MS and leaves were fed to crickets or darkling beetles for 14 days. Crickets were then fed to secondary consumers; wolf spiders, mantids or lizards for 7 or 14 days. The Ce, La and Cu accumulation in the primary/secondary consumers and crickets/lizard feces were also measured. Results from the Ce experiment showed that zucchini Cc content was significantly greater with the NP exposure. The flowers, leaves, stems, and roots of bulk exposed plants contained 93.3, 707, 331, and 110,000 mg/g, respectively; nanoceria-exposed plants contained 153, 1510, 479 and 567,000 ng/g, respectively. Crickets fed bulk and NP-exposed leaves containing Ce and La showed significant differences in Ce retention. Both crickets had non-detectable Ce but NP-fed spiders contained Ce at 4.9 ng/g. Upon lanthanum exposure, lettuce mass was reduced across all treatments but there was no difference in lettuce La content based on particle size. Although the CuO experiment is ongoing, preliminary results from pigment content/production showed no significant effects in lettuce from the two soils (0 or 70-d weathered CuO upon Cu treatments exposure. Interestingly, expression levels of several target genes involved in Cu transport suggest that the mechanisms involved in CuO NPs accumulation are differentially regulated as compared to ionic Cu. Additional results focused on accumulation of Cu in plant, crickets and lizard tissues will also be presented.

536 Approaches towards predicting the teotoxicity of nanomaterials
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Nowadays, nanomaterials are highly integrated into our daily life. However, recent studies have shown obvious toxicity of some nanoparticles to living organisms, and their potentially negative influence on environmental ecosystems. The goal of the present study was to develop predictive models that allow to efficiently predict the ecotoxicological properties and effects of inorganic nanomaterials (metals and oxides). The numerical data on toxicity of nanoparticles to different organisms have been taken from the literature and were updated in a data base. This study characterizes of nanoparticles such as chemical composition of nanoparticles, average particle size (APS), shape and information about the biological test species were used as obligatory condition for all properties in the database. 20 QSAR-type of models were compared by following the same procedure with different combinations of descriptors and machine learning methods. The QSAR methodologies applied, used Random Forests (WEKA-RF), k-Nearest Neighbors and Associative Neural Networks. The predictive ability of the models was tested through leave-one-out cross-validation, giving a 0.69-0.78 for regression models and total accuracies AUC=73-99% for classification models. Predictions for external evaluation sets obtained accuracies in the range of 69-85% (for low/high toxicity classifications) and q2=0.70-0.78 for regressions. The method showed itself to be a potential tool for estimation of toxicity of new nanoparticles at early stages of nanomaterial development.

537 QSAR Models for heterogenous Nanoparticles: Results from the Cost Action TD1204 MODENA - WG3 Exercise
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This study shows partial results generated by the author within the context of the COST Action TD1204 ‘Modelling Nanomaterial Toxicity’ (MODENA). This project was launched in 2012, and generated a network of scientists currently active to promote trans-disciplinary cooperation on the topic of computational nanotoxicology. A main output of this cooperation has been the creation of a large dataset with cytotoxicity data measured in different cell lines for heterogeneous nanoparticles (NPs) by 9 laboratories operating within MODENA. This dataset was shared among members of the Working Group 3 (WG3 - modelling) in order to investigate the possibility to develop and validate QSAR models that could include 192 heterogeneous NPs representative for 11 different metals and materials (i.e. Ag, Au, Fe, Co, Ni, Si, Ce, C, Ti, Zn, PLGA-PEO), structurally characterized by experimentally measured properties, such as shape, size, surface area etc.. Approaches based on Quantitative Structure Activity Relationships were applied to predict different responses of biological activity represented by EC₅₀ and EC₉₀ values measured in human cell cultures, using four cytotoxicity assays (i.e. ATP assay, LDH assay, MTT assay, and WST-1 assay). The models were developed by using the software QSARINS by Multiple Linear Regression (MLR - Ordinary Least Squares (OLS) method), performing exhaustive search i.e. by exploring all the possible combinations of variables. The best models were chosen as those with the best statistical performance according to various validation parameters. This study confirmed the scientific relevance of the dataset created within MODENA starting from data generated independently by different laboratories, and without a priori supervision. The coherency of the information included in the data set was captured by the modelling approach which identified robust structure-activity relationships on the basis of interpretable descriptors. A main issue emerged regarding the dependency of the quality of the models on the metric used to express toxicity. Results show that the metric “number of NPs/ml” gives the best modelling performances, and thus may be suggested as preferable metric to generate QSAR models for heterogeneous datasets.

Polar ecotoxicology: hot issues in cold climates!

538 Influence of environmental and ecological factors on plasma perfluoroalkyl substances (PFASs) in female polar bears (Ursus maritimus) from the Barents Sea
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Perfluoroalkylated substances (PFASs) are synthetic chemicals used for various industrial purposes since the 1950s. The most commonly detected groups of PFASs in biota are perfluoroalkylsulfonic acids (PFASs) and perfluorobcarylic acids (PFCAs). Many PFASs are highly persistent and have similar biomagnification capacity as lipophilic persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and chlorinated pesticides in Arctic marine food webs. In contrast to PCBs and chlorinated pesticides, PFASs have strong affinity to protein-rich tissues such as liver and blood. The
concentrations of these emerging compounds in Arctic marine predators such as polar bears (Ursus maritimus) are comparable or higher than PCBs and chlorinated pesticides. The high concentrations of PFASs in Arctic apex predators are of great concern due to their potential health effects such as metabolic disruption, developmental toxicity, thyroid disruption, immunological effects and neurotoxicity. This can impair their life cycle, deteriorate immune system and cause obesity in marine mammals by accumulating these emerging compounds that can bioaccumulate and bioconcentrate.

Humpback Whales

suggest that several pollutants can disrupt lipid storage and possibly lipid stores' physiological and reproductive success and population recruitment. Peroxisome proliferation of ice-free summer seasons will lead to prolonged fasting periods of Norwegian Polar Institute

RBC) implying an influence of diet on PFASs exposure. Moreover, δ15N RBC and higher plasma concentrations of PFASs compared to local females. Our results suggest that the influence of dietary exposure on plasma concentrations of PFASs is driven by the space use strategy of females. This further suggests a possible indirect role of climate change in PFASs exposure through alterations in migration patterns of polar bears (i.e., the ecology of species).

539 How do pollutants and fat get along? Insights in a “fast and feast” specialist, the polar bear.

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Changing climate will challenge arctic animals energetically. For example, prolongation of ice-free summer seasons will lead to prolonged fasting periods of polar bears, which use sea ice as a platform for hunting. Fasting is physiologically demanding and requires optimal control of energy homeostasis. Recent research indicates that several currently used chemicals, may disturb the function of PPARs. We hypothesize that contaminant exposure may affect the energy homeostasis of polar bears and thus decrease their ability to respond to climate change. To explore our hypothesis, we measured PPARα mRNA expression in adipose tissue and related it to legacy chlorinated and brominated pollutants. PPARα mRNA expression was positively related to most pollutants. We also analyzed metabolomics, which is a comprehensive study of low molecular weight water-soluble metabolites. This methodology can give mechanistic insight into changes in metabolism due to a stressor such as fasting. We tested the relationships between metabolome and fasting, as depicted by the urea/creatinine ratio in plasma. We observed a negative relationship between metabolome (characterized by lipid fingerprints) PCA score and urea/creatinine. Additionally, we observed a positive relationship between PCA scores and δ13C and δ15N in adipose tissue. Overall, our results suggest that several pollutants can disrupt lipid storage and possibly lipid stores' functionality, which could impair the ability of polar bears to adapt to climate changes.

540 Lipophilic Persistent Organic Pollutants as Indicators of Adiposity in Humpback Whales

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Southern hemisphere humpback whales are naturally obese mammals, highly adapted to the productivity extremes of Polar latitudes. Multiple research disciplines are alerting us to progressive weight loss and changing foraging habits in contemporary humpback whales. Despite the potential gravity of this trend, unravelling the nutritional signals of humpback whales to provide an unambiguous evaluation of species energy reserves, and therefore vulnerability to ecological change, is hindered by the inadequate power of existing non-lethal, cetacean research approaches. Humpback whale external blubber adipocyte area analysis has recently found, by the investigative team, to be adequately powerful to distinguish between “fed” and “fasted” migrating cohorts. Similarly, the investigative team has previously shown the dramatic concentration effect that occurs in outer blubber Persistent Organic Pollutant (POP) levels between the two migrating cohorts, lending support for both parameters as indirect measures of energetic reserves. Here we demonstrate individual metabolic fluxes of energetic reserves to be measured in combination with stable isotope analysis. We advocate the scrutiny of POP burdens in long term monitoring programs, as indirect indicators of energetic health.

541 From Antarctica to the sub-tropics: latitudinal differences in trace element and organic pollutant contamination in Southern Ocean skuas (Catharacta spp.)

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Polar seabirds integrate bioaccumulative contaminants via food intake and have revealed geographical and temporal trends of contamination, especially in the Arctic. By contrast, spatio-temporal patterns of contamination of Antarctic and subantarctic food webs are still poorly known. Pre-fledging seabird chicks are particularly interesting in bioaccumulation studies. Here, we measured POPs, including organochlorine pesticides and polychlorinated biphenyls in blood of skua chicks from four breeding locations that encompass a large latitudinal range in the southern Indian Ocean: from Antarctica (Terre Adélie, Catharacta macrorrntic), through subantarctic areas (Crozet and Kerguelen Islands, C. lonnbergi), to the sub-tropicals (Amsterdam Island, C. lonnbergi). Concentrations of the highly-toxic Hg increased from chicks in Antarctica to chicks in the subantarctic and subtropical islands, with a factor of eight between the populations with the lowest and highest burdens (0.5 ± 0.1 vs. 4.0 ± 0.8 µg g-1 dry weight, mean ± SD, at Terre Adélie and Amsterdam Island, respectively). Selenium (Se) concentrations were extremely high in some skua populations, reaching levels never shown before in any bird species. Se was particularly associated to Hg, suggesting its involvement in protective mechanisms against Hg toxicity. Chicks' POP pattern was largely dominated by pesticides, in particular DDT derivatives and hexachlorobenzene (HCB). Overall, POP concentrations were low, although HCB levels were comparable to related species from the Northern Hemisphere. Skua chicks from the sub-tropical site had lower levels and diversity of POPs than those of the subantarctic and Antarctic populations. The strong inter-site differences in Hg and POP levels highlighted in the present study are consistent with previous results showing a latitudinal trend in the incorporation of these contaminants in Southern Ocean food webs. The skua populations studied here could serve as useful models to investigate the potential effects of these contaminants on seabird physiology and demography.

542 Contaminant loads in relation to wintering area and breeding habitat in the Arctic Skua

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Seabirds are often used as model species in ecotoxicological studies as they are near the apex of most marine food chains, and therefore bioaccumulate high concentrations of certain pollutants. Most seabirds that breed in temperate and Arctic areas are migratory, with a significant proportion of the year away from their breeding areas thereby functioning as biovectors. Several studies have implied that migration may have considerable effects on seabird ecotoxicology. In this study, we have studied migration strategies in the arctic skua Stercorarius parasiticus. It breeds in arctic and subarctic areas, and in Svalbard and Northern Norway, it spends the nonbreeding part of the year (September through May) away from the breeding grounds. We have used Global Location Sensor (GLS) loggers to track the migration strategies of the same individuals over several years. This revealed different individual wintering areas spanning large parts of the Atlantic Ocean. We report that the individual birds repeatedly targeted the same wintering area year after year. We have analyzed various pollutants such as heavy metals and PCBs to look for individual differences in contaminant load related to different wintering areas and breeding habitat in the Arctic.

543 Do reproductive strategies affect pollutant levels in pinnipeds? A meta-analysis

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In highly seasonal environments, marine food availability is limited, such as the Arctic, animals depend on the use of stored energy reserves. Species store energy as lipids which they can allocate towards survival, growth and reproduction. Animals that store energy prior to reproduction are described as ‘capital breeders’, and those that feed during reproduction as ‘income breeders’. In marine mammals such as seals, breeding substrates (fast ice and land vs. drift ice) and access to food resources during the breeding period can determine which
strategy a species may adopt. In polar habitats such as the marginal ice zone where ice is unstable and food is temporally limited, capital breeding is favoured. However, the use of lipids means that persistent organic pollutants (POPs) are also stored, remodelised and maternally transferred during reproduction, especially during lactation. Most studies on maternal pollutant transfer have investigated only a few species, and no study synthesising the overall pattern of lipids and POPs as a consequence of the overall life history strategy on the bioaccumulation pollutants is lacking. A meta-analysis was conducted to test the effects of reproductive strategy (income and capital breeding) on pollutant levels in mother-pup seal pairs. Reproductive strategy was measured as pup daily mass gain during lactation, with capital breeding pups gaining more weight than income breeding. The meta-analysis showed that capital breeding mothers transferred more lipid soluble POPs such as polychlorinated biphenyls (PCBs) to their pups than income breeders. On the other hand, protein associated contaminants such as mercury (Hg) was not transferred as efficiently by either reproductive strategy, compared to lipid soluble POPs. Studies with data on nine species of juvenile and adult female seals in the same population were also compared and pup daily mass gain was found to be a poor descriptor of pollutant levels across seals, most likely because using direct mother-pup seal pairs are best suited to studying the effects of reproductive strategies. In summary, offspring of capital breeders are at greater risk to negative health effects as they are exposed to higher levels of pollutants than income breeders, given similar POP levels across female seal species. These findings are of particular importance in polar regions, where species have adapted to utilise stored energy reserves, and are especially relevant when considering how these areas are affected by climate change.

Soil and water contaminants: evaluation, biomonitoring and bioindicators for effective management (II)

544

Nutrients in urban surface waters: urban aquatic ecosystem threat and water re-use
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Introduction Domestic and industrial discharges into urban surface waters including streams and rivers can result in nutrient enrichment, de-oxygenation and reduction of freshwater quality. This could lead to increased hazard in waste water re-use, and threat to urban aquatic ecosystem diversity and human health. This study investigates the levels of dissolved oxygen (DO), oxygen demand (BOD, COD), inorganic nutrients (N-NH4, N-NO3, N-NO2, N-NH4+ and P-PO4) and organic carbon (OC) in Ogungu River (an urban surface water) in Ibadan metropolis, Oyo State, Nigeria.

Materials and methods
Water samples were pooled at the Ogungu River source and at different sampling stations in different area along the Ogungu River. The water samples were prepared and analysed using standard methods. Results and discussion pH Organic carbon % DO mg/L BOD mg/L COD mg/L N-NH4+ mg/L N-NO2 mg/L N-NO3 mg/L P-PO4 mg/L Minimum 6.8 2.1 8.59 7.39 2.11 7.13 6.4 19.0 0.85 2.83 Maximum 7.4 3.6 5.73 10.5 2.67 9.58 7.85 0.51 9.68 The DO, BOD, COD ranged, 2.85 – 5.37 mg/L, 7.39 – 10.52 mg/L, 2.11 – 8.67 mg/L respectively. Organic carbon and pH ranged 2.1 – 3.6 and 6.8 – 7.4 respectively, while N-NO3, N-NH4+ and P-PO4 were, 7.34 – 9.58 mg/L, 6.19 – 7.85 mg/L, 0.085 – 0.51 mg/L, and 2.83 – 9.65 mg/L respectively. The high nutrient concentrations of the water, may be attributed to unsustainable release of high nutrient streams and disposed solid wastes. This results in the undesirable effects of corrosion and incrustation occurred in most sampling stations, leading to the drop in DO and thus the high COD and BOD. Conclusion In effect, the self-purification and regeneration capacity of the water is low due to consistent exposure. This suggests high cost of water treatment for re-use. An appropriate monitoring and management procedure is therefore required to improve the water quality, and sustain the ecosystem.

545

New tool of multi component three-phase exposure: Bioavailability and mobility of organic contaminants in solid phase samples
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The bioavailability plays crucial role in ecological risk assessment of soil contaminants. There are two approaches of environmental risk assessment, the biological and chemical, which complement each other. We developed a novel approach for rapid and reliable estimation of environmental and organic pollutants. The response of the same test organism to the organic extract, water extract and solid phase of soil was recorded and compared. This approach was designed to give an initial estimate of the total organic toxicity (response to organic extractable fraction), as well as the mobility (response to water extract) and bioavailability fraction (response to solid phase) of soil samples. The selected soil samples with different level of contamination (PAHs, PCBs, HCH and DDT) and different amount of organic carbon was used to demonstrate the efficiency of proposed method for analysis of contaminants fractionation in solid samples. The results showed a low potential mobility of organic toxicants in tested soils. The bioavailable fraction was significantly greater than the mobile fraction. Still, the large fraction of the total contamination was strongly bound to soil particles and this shows significant dependence on the amount of organic material. The fraction bioavailable to the test microorganism was likely elevated by the effect of driven desorption. The presented new tool of “multi component three-phase exposure” utilise the exposure of the same test organism and it is useful for evaluation of total toxicity, water soluble and biologically available fraction of the solid samples. It is a promising tool for understanding the presence of potential risk and provides significant information about behaviour of contaminants in soils in the context of mobility and bioavailability. The knowledge of mobility and bioavailability of chemical compound in a soil is useful for the hazard identification (provides useful toxicity and exposure information) and environmental risk assessment for organic contaminants in soils.

546

Novel β-FeOOH/NiO composite material as potential catalyst for ozonation of organics
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This study reports the on novel synthesis of an organic linker mediated nanocomposite comprising of β-FeOOH and NiO and its possible application to heterogeneous ozone catalysis. The synthesized composite material was characterized using XRD, FTIR, TEM. Elemental mapping of single composite material under dark field shows the colloid formation and confirmed the presence of Ni and Fe in the composite. 4-chlorophenol (4-CP) was used as target molecule to evaluate the catalytic activity. Above 5 % β-FeEOOH loading on NiO, catalytic efficiency reduced. The composite material was stable under acidic (pH 2.5) and alkaline (pH 10), with the resulting solutions having no traces of Ni or Fe as evident from AAS measurements. Catalytic ozonation removed 85 % of 4CP in 20 minutes compared to 47 % removed by ozonation alone. The catalyst showed good recyclability as the catalytic activity of the material could be restored after calcination. The catalytic activity of the composite was due to the higher generation of OH· as shown by photoluminescence experiments. This novel composite material therefore shows as a future catalyst in water purification processes.

547

The sorption of active pharmaceutical ingredients onto soils under different pH and microbial conditions
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Population growth, increasing affluence, and greater access to medicines have led to an increase in active pharmaceutical ingredients (APIs) entering sewage networks. In the low and lower-middle income countries (LLMIC) of Asia, Africa and Central and South America, the use of human pharmaceuticals increased by 23-29 % between 2000 and 2011. As a consequence, the loadings of residual APIs and other down the drain chemicals (including personal care products (PCPs) and cleaning agents to soils, surface and ground waters of these countries are increasing. In areas with high wastewater reuse residual quantities of APIs may enter agricultural soils via irrigation with treated, partially treated, or even untreated wastewater and sludge. Wastewater used for irrigation is currently not included in chemical environmental risk assessments (ERAs) and requires further consideration in areas with high water reuse. The aims of this research were to measure the influence pH and the soil microbial community has on the sorption of three APIs to soil. Propanolol, naproxen and ofloxacin were chosen as the APIs to focus on in these experiments as they cover a wide range of API characteristics and are widely used throughout LLMICs. The OECD 106 technical guideline was used to generate data on the adsorption and desorption kinetics for the three APIs using two soil types under natural, pH altered and stabilised soils. Future work as part of this project will include applying minimally treated wastewater to the soils and measuring how API sorption and degradation is affected and column experiments to assess leaching potential to groundwater sources. The data gathered through these experiments will aid the development of robust ERAs for APIs in soil for wastewater irrigation.

548

Fate of metamitron in soil - a detailed insight into its biotransformation pathways and biogenic residues formation
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behaviour in the soil systems are available, the detailed insight into its microbial turnover is still missing. We investigated biotransformation of MTR with the particular focus on the metabolic incorporation of the isotope label into FA and AA and their fate in soil over time. In biotic system, the mineralization of MTR started immediately without any lag reaching finally 60% of the initial. 13C-metamitron coexists. In contrast to the abiotic system, where a lower mineralization, amounting 7.4% of the initially added 13C-metamitron MTR was degraded initially to desamino-metamitron in soil and water-sediment might be assigned to microbial growth. Desamino-metamitron is ultimately converted to pyruvate and acetalddehyde. Pyruvate is used for direct synthesis of alanine as proven by the dominant presence of 14C-alanine throughout the experiment, including both growth and starvation conditions. In addition, pyruvate and acetalddehyde entering the tricarboxylic acid cycle contributed indirectly to glutamate synthesis via 2-oxoglutarate and may explain high contents of

13C-labelled glutamate. The complete dissipation of desamino-metamitron from soil after 16 days together with a rapid mineralisation of MTR leaving only traces of MTR in soil on day 16 suggests the relevance of this pathway in active metabolism. Based on the measured content of total AA (14.85% of 13C-labelled metamitron equivalents) we could estimate that 29.7% of carbon-derived MTR was assimilated by microorganisms. At the end, 13C-label derived from MTR was distributed mainly between 13C- and microbial biomass indicating a complete degradation of this herbicide in soil. Using stable isotope label tracer (13C), we established a detailed MTR turnover mass balance and metabolic fate analysis including biogenic residues formation. The presented data showed that nearly all NER were biogenic and were based on non-toxic biomass compounds stabilized in the SOM. In addition, we proved for the first time that MTR was utilized as a C source and was biodegraded via two pathways the “desamino-metamitron" and via the "Rhodococcus pathway".

549

Threatened southern African soils: Status and challenges from an (eco)toxicological risk assessment perspective

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Soils in Southern Africa (SA); comprising South Africa, Namibia, Botswana, Zambia and Zimbabwe are, in terms of area, not a limited resource but are in terms of arability. This is due to limited rainfall in the area and inherent poor physical condition of these soils. As in the rest of the world soils in SA are threatened by a range of anthropological activities e.g. mining, agriculture and industry. Ecosystem risk is an assessment to protect ecosystems and their biodiversity. Although some risk assessment of soils has been done in SA it has not been extensive. Soil screening values exist in South Africa, which enables soil quality assessment, but lack a clear risk-based scientific foundation and site-specific risk assessment. This is especially important because of the vast mining activities and the proximity of mine tailings disposal facilities to residential areas; exposing people living close by to a wide range of possible contaminants. We ask the question; to what extent the developed general RA systems used in other parts of the world are valid for the specific conditions in SA? We also investigated the status of the (eco)toxicological research in SA. This presentation intends to give an analysis and overview of the present situation, highlight the improvements needed, and assess current research as well as identifying knowledge needs and gaps.

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (II)

550

Cross effects of salinity and ceria nanoparticles on two endobenthic bivalves

Corbicula fluminea and Scorbocularia plana: biomarker assessment

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Cerium nanoparticles (CeNMs) are integrated in numerous consumer products (e.g. wood stain, painting, risk additive) because of their high power, high resistance and UV-shielding properties. Given the production volumes of CeNMs and their potential release into the aquatic environment, assessment of the potential environmental risks of CeNMs is a priority. While the impacts of Engineered Nanomaterials (ENMs) in freshwater media have been largely studied this last decade, data covering a salinity gradient are scarce. The aim of the present study is to assess the fate, behaviour and toxicity effects of both CeNMs included in a commercial fuel additive (EnviroxTM) at two stages of its life cycle and of NM-212, a standardized CeNMs provided from Joint Research Centre, across a salinity gradient (1.5, 15.0 and 30.0, practical salinity unit) during 28 days. Two euryhaline bivalves were selected: Corbicula fluminea and Saccorhiza polyspila to represent the diversity of the stress response, respectively. Both species are well recognized as good models for biomonitoring purposes. These filter-feeding species may be particularly at risk of ENM exposure since they live at the water-sediment interface. C. fluminea were exposed to a constant water volume at 1.5 and 15 psu while S. plana were exposed to an artificial rhythm of tide at the laboratory (6 h high tide/ 6 h low tide) during the whole duration of experiment. The size, shape and crystallography of the uncombusted and combusted EnviroxTM were performed by Transmission Electron Microscopy (TEM). Total Ce in the water column, labile forms of Ce accumulated in Diffusive Gradient Thin film (DGT) tools and Ce concentrations in digestive glands of bivalves were estimated by ICP-MS. After 7, 14, 21 and 28 days of exposure, individuals were submitted to burrowing tests. Toxicity responses of both bivalve species were evaluated using a multi-marker approach at different levels (sub-cellular, individual) of biological organization using an adjustable packed-size marine and freshwater mesocosm platform. Results showed in both species a significant difference of biomarker responses according to salinity, exposure condition and exposure time. Moreover, the differences between the conditions of exposure were discriminated with a reduced set of biomarkers: cellular damage, antioxidant defences, cellular respiration condition.

551

Environmental health assessment of the Seine estuary using a battery of indicators at different levels of biological organization

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It is estimated that the global economic scale of environmental damage due to anthropogenic activities in coastal zones and estuaries is approximately $12.6 trillion per year. The Seine estuary is of particular concern as it is influenced by twenty-five percent of France’s population and forty percent of the countries’ industrial and agricultural runoff. The aim of this study, which is a part of the ECOTONES project, led by the GIP Seine-Aval, is to utilize a multi-disciplinary approach to assess the health status of a key invertebrate of estuary functioning: the annelid polychaete Hediste diversicolor. The approach is based on endpoints at multiple levels of biological organization (cellular, individual, population) so as to estimate the overall health of this species in the Seine estuary. For each biomarker studied (acetylcarnitine: AChE, catalase: CAT, glutathione S-transferase: GST, superoxide dismutase: SOD, thiobarbituric acid reactive substances: TBARS, energy reserves), results of the current campaign (March 2015) in Seine were compared to historical data previously acquired from samples collected in reference sites (Authie and Bourgneuf). H. diversicolor’s population trends depicted lower biomass and density, smaller individuals with delayed reproductive potential compared to samples from reference sites. Biochemical biomarker results also show that acidification was being severely impacted at the cellular level exhibiting low levels of energy reserves (glycogen and lipids), of AChE activity (neurotoxicity), and of GST values suggesting defense mechanisms being overwhelmed. The high levels of Catalase and TBARS compared to values of reference sites suggest an oxidative stress endured by organisms. In addition, a predictive model for BAC and EAC determination using a linearized regression model (with “K-means clustering” method) has been developed. Keywords: Levels of biological organization, Hediste diversicolor, Biomarkers, Background and Environmental Assessment Criteria.

552

Adaptive responses of a marine filter-feeder, the common blue mussel, to chronic contamination in a harbour located within urbanized environment.

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 Conjuncted effects of chronic contamination and temperature increase affecting coastal waters are to be addressed by marine biologists when considering global change in the ocean. Adaptive responses of a marine filter-feeder, the common blue mussel Mytilus spp., have been investigated in a pseudo-estuarine area, the Rance estuary (Brittany, France), to chronic contaminants in the port of Brest with both trade and navy port have generated for decades a chronic contamination by POPs. Actual contamination levels have been established in mussel tissues. To assess biological responses to environmental pressure, the activity of several enzymes involved in major physiological functions, including metabolism and biotransformation, metabolic enzymes, and stress proteins, were examined to determine robustness and consistency within a grouping afterwards. They colonized a wide range of environments from estuary to open ocean. They have a high adaptability to environmental stress response and its consequences in organisms (II)

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variability, several cohorts were recognized based on shell length and further sampled. Considering that two species, M. edulis and M. galloprovincialis, and their hybrids are sympatric in this geographical area, the genetic status of each individual has been determined by using molecular methods. As a complement to these observations in native individuals, mussels collected at a distant and less contaminated site in the Bay of Brest were kept in the harbor. After one month, contrasted, biochemical responses among groups suggested an adaptive process in native mussel in order to overcome deleterious effects of environmental stress. This set of observations could contribute to a better understanding of how biological responses are induced in the blue mussel which is becoming a major sentinel species in the observed and budgeted global changes in coastal ecosystems.

Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (II)

555


The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites. The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites. The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites. The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites. The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites. The approach is based on endpoints for the assessment of environmental exposure to pharmaceuticals and their metabolites.

554

Influence of environment on the response capacity to thermal stress in the blue mussel using experimental approach I. BOUTET, Université de Bretagne Occidentale / IUEM; C. LACROIX, Université de Bretagne Occidentale / IUEM; A. Tanguy, UPMC; D. Moraga, Université de Bretagne Occidentale / IUEM.

In the intertidal zone, organisms are permanently exposed to strong environmental gradients and long periods of stressful conditions including hypoxia, temperatures and eutrophication. Temperature variations impact organisms at both biochemical and physiological processes directly, by restricting enzyme activity efficiency or promoting protein catalysis. Mussels of the genus Mytilus are one of the most important marine invertebrates in the French coastal ecosystems. This study focused on two major questions from an ecological point of view. They colonized a wide range of environments from estuary to open coastal ecosystems and consequently challenged a large variety of fluctuating parameters such as salinity, temperature, tide, anthropic effluent. They are largely used as model species in biomonitoring programs and laboratory exposure studies. The present study aimed to investigate the cellular and transcriptomic response of mussels having been exposed to various levels of chronic chemical contamination (having distinctive environmental history) may respond to an experimental increase of temperature. In these experiments, blue mussels collected at two sites of the Bay of Brest were exposed in the laboratory to graduated warming and cellular and molecular responses relevant for physiological stress assessment were measured. In addition, considering that three mussel species (M. galloprovincialis, M. edulis) and their hybrids are present in the study area, we investigated possible differential responses in order to identify genetic basis of metabolic responses and further, differential sensitivity of species to environmental, thermic stress. Results indicated various pattern of response when considering the different parameters analysed (population origin, temperature effect, tissue, hybrid status). Metabolic depression is observed at highest temperatures in both populations studied but some differences remains at lowest temperature according to the site origin. Hybrid mussels show some difference in their response compared to “pure” species M. galloprovincialis highlighting the importance of hybridism in the species adaptation.

553

Multiple stressors influencing seagrass dynamics in a shallow Mediterranean lagoon N. Diepens, Wageningen University; Y. Cherain, E. Coulet, National nature reserve of Camargue; A. Proebst, E. Buffan-Dubau, ECOLAB UMR CNRS UPS INPT; J. Kallerhoff, ECOLAB UMR CNRS UPS INPT / Ecolabo Laboratoire déologie fonctionnelle et environnement; G. Merlina, ECOLAB UMR CNRS UPS INPT; J. Silvestre, ECOLAB UMR CNRS UPS INPT / ECOLAB UMR CNRS UPS INPT; O. Bouton, P. Grillas, Tour du Valat, Research centre for the conservation of Mediterranean wetlands; A. Elger, University of Toulouse / Ecolab.

Seagrasses have a wide spatial distribution, form an important component of many coastal ecosystem and provide essential ecosystem services. Seagrass declines have been observed worldwide due to a variety of (multiple) stressors. The Mediterranean seagrass, Zostera noltei is the dominant macrophyte species and suffered two major declines in 1997 and 2008. The first decline was well explained by salinity and turbidity, whereas the second decline could not be explained by these parameters. Other stressors such as chemicals, nutrients or temperature could be explanatory variables for the second declines. The main aim was to understand the influence of multiple stressors on seagrass physiology and population dynamics in a typical Mediterranean shallow lagoon (Vaccarès lagoon) by analysing the biological, environmental and chemical data. Contamination by pesticides and metals was monitored in water and sediments both in the lagoon and in its main tributaries from 2011 to 2014. The recent dynamics of seagrass was quantified across the lagoon using a 55-points sampling design. Three stress biomarkers were measured in situ: photosynthetic pigment composition, chlorophyll fluorescence and thymol sugar content. Biological, environmental and chemical parameters were analysed by co-inertia analysis. GIS was used to interpoleate between measured data points. Co-inertia analysis showed a significant relationship between environmental data and seagrass status matrices. Seagrass dynamics were negatively correlated to the concentrations of some herbicides in water (2,4-MCPA and bentazon) and with metals in sediments (arsenic). Thymol starch content in winter was negatively correlated to those herbicides and several metals in water and/or sediments. These results suggest that environmental contamination may be responsible for the decline of seagrass in some parts of the Vaccarès Lagoon. However, complementary investigations, such as longer monitoring and additional toxicity tests, are required to address the causal link between contamination and seagrass decline. Understanding the influence of multiple stressors on seagrass dynamics and thus possible declines is crucial for environmental risk assessment and to preserve these valuable systems with integrated management strategies.

544


Environmental management strategies are required to protect coastal ecosystems and provide essential ecosystem services. Seagrass declines have been observed worldwide due to a variety of (multiple) stressors. The Mediterranean seagrass, Zostera noltei is the dominant macrophyte species and suffered two major declines in 1997 and 2008. The first decline was well explained by salinity and turbidity, whereas the second decline could not be explained by these parameters. Other stressors such as chemicals, nutrients or temperature could be explanatory variables for the second declines. The main aim was to understand the influence of multiple stressors on seagrass physiology and population dynamics in a typical Mediterranean shallow lagoon (Vaccarès lagoon) by analysing the biological, environmental and chemical data. Contamination by pesticides and metals was monitored in water and sediments both in the lagoon and in its main tributaries from 2011 to 2014. The recent dynamics of seagrass was quantified across the lagoon using a 55-points sampling design. Three stress biomarkers were measured in situ: photosynthetic pigment composition, chlorophyll fluorescence and thymol sugar content. Biological, environmental and chemical parameters were analysed by co-inertia analysis. GIS was used to interpoleate between measured data points. Co-inertia analysis showed a significant relationship between environmental data and seagrass status matrices. Seagrass dynamics were negatively correlated to the concentrations of some herbicides in water (2,4-MCPA and bentazon) and with metals in sediments (arsenic). Thymol starch content in winter was negatively correlated to those herbicides and several metals in water and/or sediments. These results suggest that environmental contamination may be responsible for the decline of seagrass in some parts of the Vaccarès Lagoon. However, complementary investigations, such as longer monitoring and additional toxicity tests, are required to address the causal link between contamination and seagrass decline. Understanding the influence of multiple stressors on seagrass dynamics and thus possible declines is crucial for environmental risk assessment and to preserve these valuable systems with integrated management strategies.
Identifying the single most dangerous chemical present in UK surface waters following a risk ranking exercise

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The aim of this project was to identify which of the many chemicals we contaminate our environment with are likely to be causing the greatest harm. Whilst this may at first seem obvious, the reality is that environmental science can be pushed in many directions with actual risk not being the sole criterion for the research. In this exercise the focus was on chemicals subject to chronic discharge from the human population, rather than those more associated with spill accidents, or chemicals discharged from a particular industry in a discrete location. The 73 chemicals studied in detail included metal-, pharmaceuticals, persistent organic pollutants (POPs), pesticides, biocides, surfactants, plasticisers and nanoparticles. For each class, selected representatives were studied in detail to hopefully act as a guide to the risk of that group as a whole. The principle of the data collection was to be representative of the spread of species and effects reported. Typically around 50-100 effect data points and 50-100 measured data points were used. What stands out from the results is the magnitude of the differences in risks between the chemicals. So for example Zinc based on its toxicity and dissolved river concentration would appear to represent a 10,000-fold greater risk to UK rivers than say nano-silver or propanolol and 1000-fold greater risk to freshwater fish than can be assessed as only using recent UK water measurements, dissolved concentrations, lethal or sub-lethal effects and only those compounds with BCFs higher than a 1000 still led to metals such as zinc and copper dominating the top 10 risk.

Estimating emissions, concentrations and combined effects of REACH substances in EU waters


In this contribution, we present the analysis of the emissions, exposure and net expected mixture impacts from 4000+ monomeric organic chemicals, registered under REACH up till February 2015. A train of models was used to describe, predict and analyze potential impacts of REACH chemicals in aquatic ecosystems. Starting from registered EU tonnages, emission rates to an imaginary downstream local EU-environment consisting of air, water and soil were estimated by means of a newly developed, REACH-based release estimation model which accounts for all major use stages and known usages during the substance Life Cycle. In a subsequent fate modeling step, a simple unit-world fate model, accounts for all major use stages and known usages during the substance Life Survey (JDS3). Total market volume of REACH results of water quality monitoring programs, such as the third Joint Danube calculation. Derived emission rates, expected environmental concentrations and Prediction of environmental concentrations of multiple industrial compounds by means of a newly developed, REACH-based release estimation model which downstream local EU-environment consisting of air, water and soil were estimated describe, predict and analyze potential impacts of REACH chemicals in aquatic ecosystems. These findings will be used in the evaluation of monitored and simulated bulk concentrations using field data from the Joint Danube Survey 3 (JDS3) with a reasonable agreement found between model estimates and measurements. Our newly developed model constitutes a large step towards wider understanding and quantification of industrial chemicals’ exposure. Its applicability to any type of organic material, any field of application and both in EU and non-EU countries such as only using recent UK water measurements, dissolved concentrations, lethal or sub-lethal effects and only those compounds with BCFs higher than a 1000 still led to metals such as zinc and copper dominating the top 10 risk.

Pollutants 2030: Predictions based on developments in society

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Patterns of pollution in river basins change with time. Management strategies should address such developments. Many scenarios are published which describe future changes in society; climate change, demographic change, urbanization and others. Specific trends can be predicted (with uncertainties) already now. Does this help to get a picture on future pollutants? In most of the existing scenarios, impacts on (aquatic) ecosystems are not directly addressed. However, scenarios often describe trends which can be linked to the use of chemicals. Therefore, they can be the starting point to analyse implications on future use and emissions of chemicals and consider how to anticipate in terms of risk prevention. Sector-specific trends have been analysed with a wide range of experts from the respective sectors with focus on health care, food production and supply, urbanization and changes in land use and new technologies. It could be shown that these developments have many and often complex implications on future pollutants. Patterns of use will change e.g. for pharmaceuticals, biocides, household chemicals as well as for specific groups of industrial chemicals. In many areas legacy chemicals will contribute significantly to the future impact on the environment – besides really unexpected new substances. This refers not only to substances which have been used in society decades ago and are still present in the environment or in “stocks”, e.g. buildings, so called legacy chemicals. It refers also to a large number of “new materials”, which are often based on the use of already existing chemicals in processes or technologies for which they were not originally conceived. Furthermore, completely new molecules and materials have to be expected with not only new and changing exposure processes and transformation products. Some of the future trends can be integrated in exposure and risk modelling. Examples are predictions on demographic change and changes in the consumption pattern of pharmaceuticals during life time. Other trends can have implications for effect monitoring. The expected increase of emissions of pharmaceuticals could support the development of new exposure endpoints, e.g. behavioural changes in fish. These findings will be used in the project SOLUTIONS to develop prioritization and risk reduction approaches for individual chemicals and mixtures and for assessment of abatement and policy options.
561 Comparison of P, B and T properties of parasiticides and harmonisation of the basis for an environmental assessment at the EU level

J. Roembke, K. Dais, P. Egele, D. Gilberg, C. Schuh, ECT Oekotoxikologie GmbH; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological Chemistry; M. Herrchen, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; L.E. Holzle, University of Hohenheim; B. Heilmann-Thudium, University Hohenheim; R. Dürring, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation; M. Woehde, J. Behn, University of Giessen; N. Adler, UBA FG IV 2.2 Arzneimittel Persistant, bioaccumulative and toxic (PBT) substances are of high concern and their identification is part of various regulations. The environmental risk assessment of veterinary pharmaceuticals includes a PBT screening and, if required, a PBT assessment for the active substances. However, so far it is not clear how PBT properties should be considered in the risk/benefit evaluation of veterinary pharmaceuticals, and which consequences the classification of an active substance as PBT might have. Furthermore, there is no complete and consistent database for a comparative assessment of P, B and T properties of different parasiticides. The present project contributes to filling these data gaps by deriving octanol/water partition coefficients and fish bioconcentration factors for selected parasiticides, and by compiling available data on the excretion of parasiticides by pasture animals and on their effects on dung organisms. Octanol/water partition coefficients ($P_{ow}$) for ivermectin (log $P_{ow}$: 5.6) and selamectin (log $P_{ow}$: 6.0) determined with the slow-stirring method (OECD test guideline 123) are higher than previously available values. Bioconcentration of ivermectin in zebrasfish (Danio rerio) is currently investigated according to OECD test guideline 305. Fish bioconcentration indicates that ivermectin is relatively fast. In a second test, bioconcentration of doramectin will be studied. Based on the evaluated literature data the toxicity of parasites to dung organisms can be classified as follows: doramectin $>$ ivermectin $\approx$ eprinomectin $>$ moxidectin. The obtained results will be used to identify effective and feasible risk management measures for parasiticides and PBT properties that will be discussed with competent authorities, veterinarians and farmers.

562 The Origin and Evolution of Persistence Criteria for PBT Chemicals and Persistent Organic Pollutants

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Public concern over the effects of persistent chemicals began in the early 1960s. Since then, significant scientific advances have increased our understanding of persistent, bioaccumulative, and toxic (PBT) chemicals and the properties and processes that influence their fate in, and adverse effects on, humans and the environment. In addition to the scientific advances, a number of legal instruments and agreements for global, international, and national identification and control of persistent organic pollutants (POP) and PBT chemicals have been adopted. However, some of the rationales and thoughts that were relied upon when the first criteria were developed to identify and categorize PBT chemicals and POPs have not been carried forward. Criteria for identification of persistent chemicals are simplification-based criteria which, based on available data for neutral hydrophobic “reference chemicals”, derived under laboratory conditions, and consensus-based policy decisions. Criteria have evolved over the last decades due to the diversification of the protection aims under various national regulatory frameworks and international agreements, advances in methods for estimation of physical/chemical properties, and the identification of chemicals which are non-traditional POPs. The numerical criteria serve as ‘yardsticks’ for assessing persistence properties of POPs and PBTs. From a scientific perspective, it is logical to use the same reference conditions as were defined for setting criteria values, when assessing a substance under review. If conditions, e.g. temperature or organic matter content, are changed for the determination of the properties of a substance undergoing assessment, the comparison becomes less meaningful, and longer apply. A better understanding of the robustness of persistence indicators, in particular for water-sediment systems, and a consensus on the interpretation of non-extracable residues in soil and sediment, would improve the persistence criteria for screening and classification. Setting appropriate numerical criteria for $P_{ow}$ would ensure that environmentally half-lives obtained from available data for neutral hydrophobic “reference chemicals”, derived under laboratory conditions, and consensus-based policy decisions.

563 Use of monitoring data to assess PBT, vPvB and POP properties of chemicals

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564 Screening and prioritization of chemicals for PBT behavior: state of the art on the consensus approach by PBT Index and PBT Profiler

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The limited availability of comprehensive data to describe Persistence, Bioaccumulation and Toxicity (PBT) properties pose a serious hindrance to the categorization of chemicals as PBTs or vPvBs, which use is regulated in REACH through authorization and additional plans for safer alternatives. A possible solution to this problem is the use of in silico approaches, such as those based on QSARs, which are valid alternatives to animal testing. These approaches generate predicted data and help in early identification of chemicals of high concern and in the definition of priority lists. In the context of screening and prioritization tools for PBT-assessment, we proposed a screening method founded on a consensus approach between two different QSAR-based models for the identification of PBTs. We developed this approach by combining predictions generated by the Insubria PBT Index model (based in the STREAM-EU model) and the PBT Profiler (developed at University of Insubria), with those calculated by the US EPA PBT Profiler, a freely available online tool for the preliminary PBT screening of organic chemicals. Predictions which were in agreement between the two models were more consistent, reliable and with a higher probability to correctly identify PBT chemicals, than those generated by one of the two models taken singularly. During the last years, we have applied this consensus approach to screen and prioritize big datasets of chemicals of environmental concern with heterogeneous molecular structure, and to screen specific groups of chemicals such as Flame Retardants, Personal Care Products and Pharmaceuticals. The good agreement between the two models and the utility of this approach highlight the need for urgent experimental tests on compounds prioritized in agreement as PBTs. The screening case studies, described in this presentation, are examples of how results generated by the Insubria PBT Index and by the US-EPA PBT profiler, which are both based on chemical structure, can be combined in order to identify the most suitable method for the screening of chemicals. These evidences suggest that the reliability of the predictions is higher for compounds that are predicted in agreement by different methods. The consensus approach combines the perspectives given by multiple modelling methods, which are based on different structural features and mathematical approaches, and is useful to integrate PBT/vPvB assessment procedures on thresholds criteria.

565 Regulatory PBT/vPvB assessment of UCVB-substances


Setac Europe 26th Annual Meeting Abstract Book 127
The experience in assessment of PBT/vPvB properties of substances of Unknown or Variable Composition or Biological origin (UCB- substances or UCBVs) has increased considerably in recent years. During the last four years alone over 50 UCB- substances were assessed for their PBT/vPvB properties by REACH competent authorities in Europe. Furthermore, a very high number of UCBVs have been registered so far. We present an overview of the tools and methods to assess the PBT properties of all relevant constituents of a substance. However, due to their complexity and variable nature, accurate assessment and testing of all individual UCB constituents is often not technically feasible or resource and cost-effective. Efficient assessment and testing strategies are therefore essential. The following pragmatic approaches have been applied to date: (1) Whole substance approach; (2) Fraction profiling approaches; (3) Known worst case constituent approach; (4) a combination of one or more of these three approaches. This presentation explains the approaches and gives an overview of their application with a few examples: MCCPs, styrened phenol, coal tar pitch, high temperature, polybrominated diphenyl ethers and tert-dodecylmercaptan.

566 How to apply socio-economic analysis to REACH-authorisations of PBT and vPvB chemicals? A critical synopsis of approaches and decision-criteria

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A core aim of the European chemicals legislation REACH is to ensure that the risks caused by substances of very high concern (SVHC) are properly controlled. Authorisation – i.e. the formal approval of certain uses of SVHC for a limited time - is a key regulatory instrument in order to achieve this goal. For persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) SVHC listed on Annex XIV of REACH, decision-making on the authorisation is conditional on a socio-economic analysis (SEA), in which companies must demonstrate that the gains from keeping a chemical in use outweigh expected damage costs for society. The current setup of the REACH authorisation process, however, raises a number of questions concerning the practical applicability of this tool. We suggest a tiered framework for which we discuss key analytic steps and informational requirements which we consider crucial in order to operationalise SEA of PBT and vPvB chemicals. Using the case of hexabromocyclododecane (HBCDD), an Annex XIV PBT substance which had a sunset (phase-out) date in August 2015, we investigate key challenges that need to be met in order to ensure that a SEA can be operationised in a meaningful way. We illustrate that, if monetary estimates for health and environmental impacts are available (e.g. from impact-specific valuation studies), a SEA will deliver the optimal period of use of a chemical. Thus, an authorisation should be granted with a review period that extends until marginal discounted benefits of use equal marginal discounted damage costs. A crucial aspect of this path is the selection of a concern-based discount rate. Another challenge is to examine the use of environmental monitoring data for determining media-specific impact functions. However, a monetary impact valuation is unlikely to be feasible for most PBT chemicals. Still, if sufficiently reliable information on a chemicals’ toxicity is available, non-monetary evaluation approaches, for example multi-criteria or cost-effectiveness analysis, can be applied in a SEA. For PBT chemicals, in particular, a SEA applying (explicitly or implicitly) efficiency decision-criteria turns out to be inappropriate because of deep uncertainty regarding PBT vPvB properties and risks. Therefore, an alternative authorisation route for PBT chemicals, which should be based on robustness criteria such as, for example, the ‘minimisation of maximum regret’.

How can we improve the link between academic research and policy-making in order to advance chemical risk assessment and management?

567 CRED - Criteria for Reporting and Evaluating ecotoxicity Data

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The derivation of safe concentrations of chemicals for ecosystems, such as Predicted No Effect Concentrations (PNECs) or environmental quality standards (EQSs), is an important aspect in a large number of (legal) frameworks worldwide. Different datasets are used in the various frameworks, and combined with the use of different assessment methodologies this often leads to the derivation of different PNECs/EQSSs for the same compound. However, even when the same set of study reports and/or references from the open literature is available and the same guidance documents are used, there is no guarantee that risk assessors will arrive at the same list of usable endpoints from these studies. The evaluation of scientific reliability and relevance of ecotoxicity studies is often subject to expert judgment, and results may differ between frameworks and between assessors within one framework. To improve the evaluation of aquatic ecotoxicity studies, the CRED project was started. CRED stands for Criteria for Reporting and Evaluating ecotoxicity Data. The aim of CRED was to improve the reproducibility, transparency and consistency of reliability and relevance evaluations amongst frameworks, countries, institutes and individual assessors. We also aim to improve the usability of peer-reviewed literature for substance evaluations, and in the end improve the extractability of assessment results between frameworks. We will present the CRED method, which includes a set of 20 reliability and 13 relevance criteria with an extensive guidance, as well as a guidance on how to report data from ecotoxicity studies. To test the applicability of the methodology, we performed a ring test with over 80 risk assessors from Europe, Asia and North America, representing academia, regulatory agencies, companies and industries. The data required for the CRED evaluation method to be more accurate, applicable, consistent and transparent than the often-used Klimisch method. The CRED evaluation method is accompanied by reporting recommendations for aquatic ecotoxicity studies, with 50 specific criteria divided into six categories: general information, test design, test substance, test system, exposure conditions, and statistical design and biological response. An ecotoxicity study in which all important information is reported is more likely to be considered for regulatory use, and proper reporting will support the peer-review process.

568 Lessons learned from a multi-country project under the SAICM Quick Start Programme

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Under the SAICM Quick Start Programme, a multi-country project on “Training on risk assessment for chemicals at national level in a global context” was implemented by the governments of Armenia, Chile, and Ghana in the period of 2010–2014. The International Panel on Chemical Pollution, IPCC, was the executing agency of the project. The overall aim of the project was to make available, in each of the three countries, a set of methods, tools and data for chemical risk assessment (“materials”) and to conduct workshops and national training activities that enable scientists, government representatives and other stakeholders to access and use these materials. In the initial phase of the project, a kickoff meeting with representatives of all countries, IPCP scientists, and experts from international organizations (WHO, OECD) as well as national workshops in each of the countries were conducted. This was followed by a phase of national case-study work (case-study topics were PCBs and organochlorine pesticides in Armenia, heavy metals in Chile, atrazine in Ghana) in collaboration with IPCP scientists and international experts. Finally, the results obtained were collected, discussed and compared at a synthesis workshop at the end of the project. The project required coordination of partners from different types of institutions: government institutions in each of the countries; a range of national stakeholders such as national research institutes, NGOs, universities, and industry associations; experts from international organizations, IPCP scientists, and the SAICM secretariat. Important conclusions from the project are: (i) Methods for first approximations or rough estimations are sufficient to define the chemical risk assessment, such as emission rates of a chemical, were found to be useful and of immediate benefit. (ii) Approaches that enable project participants to establish a “big-picture” understanding are important because often there is an extensive body of data from various projects available in a country. Offering a framework that can accommodate existing data helps reconcile existing data and supports long-term thinking and collaboration of experts from different fields. (iii) More time than anticipated may be needed because local stakeholders, interest groups, and societal actors need to be involved; it is not easy to estimate in advance how much time will be needed for this process and what the outcomes of the local stakeholders discussions will be.

569 Using the ecosystem services framework to link scientific research and policy-making: a case study of Lake Tai, China

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One way of linking scientific research and policy-making is to increase public participation into decision making processes, because public perceptions can play a significant role in identifying protection goals for use in chemical risk assessments. However, in the context of LCA in support to policy, weighting may help in the: i) Development of weights to integrate different ecosystem services and their relationships to chemical risk assessments; ii) Different sections of society may not have the same ecosystem values and this should be considered during the establishment of protection goals. Clear protection goals are important for many developing countries with rising economies, such as China, which suffer from unsustainable development and environmental degradation. The aim of this study was to investigate the perception of ecosystem services surrounding the lake were investigated by conducting interviews with 255 rural residents of a freshwater lake in China and is affected by severe anthropogenic threats, including eco-innovation policies and strategies towards the most effective solutions for changing this.
freshwater lake in China and is affected by severe anthropogenic threats, including chemical pollution that can negatively impact ecosystem services. Four cities surrounding the lake were investigated by conducting interviews with 255 rural and 252 urban respondents. There are similarities and differences between the value of services across cities, as well as across rural and urban communities. Each group of respondents ranked freshwater provision as the most important service but other services were valued differently between respondents situated at different locations around the lake. Rural communities ranked directly-used services (i.e. food provision and flood regulation) higher than urban communities, who ranked indirectly-used services (i.e. cultural services) higher. Prioritised services can be adversely impacted by chemical pollution in Lake Tai and 40% of the respondents were concerned about water pollution. By identifying services prioritised by the public, we can then identify drivers that support the services and specify protection goals. This study sets out a framework for investigating the ecosystem values of different communities. In addition, it highlights the question of whether or not biodiversity is a driver of ecosystem services of varying ecosystem values. By using the ecosystem services framework, this study has linked scientific research, social sciences and policy-making.

570 Use of the SEA and DSFA in risk assessment and management for chemicals of concern
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To alarm inapprrehensive upcoming concerns so as to avoid them beforehand is grounded as the ultimate goal of risk management and communication. This goal necessitates comprehensive, systematic appraisal of possible adverse impacts with a holistic consideration of social, economic and environmental impacts on a long-term temporal scale before decision making. Here we propose that a cost-effectiveness-oriented socio-economic assessment (SEA) framework, coupling with a dynamic substance flow analysis (DSFA), is competent for such appraisal in national management of hazardous chemicals to implement multilateral environmental agreements. We exemplified the application of the two approaches using the case of phasing out hexabromocyclododecane (HBCDD) in China. The SEA results indicate that the schedule of phasing out HBCDD production and new uses within two five-year exemption periods generates higher environmental and health benefits while that within a five-year exemption period leads to smaller economic and social costs. A decision can be biased if we ignore the environmental and health benefits and their related positive externalities. The DSFA results show that a whole century or longer time will be needed to eradicate HBCDD from the present in-use and waste stocks although production and new uses will stop soon. Therefore, future management efforts should be devoted to end-of-life disposal management. Our case highlights the implication and importance of the SEA and DSFA in national chemical management and risk communication.

571 Participative valuation of ecosystem services of aquatic ecosystems: Evrotas river basin Greece
P. Ker Rauli, J. Luttik, Alterra

Little is known beyond the described effects of single stressors on the chemical and ecological status of aquatic bodies and on their ecosystem functionality. This lack of knowledge limits our capacity to understand ecosystem responses to multiple stressors and to define a programme of measures that can improve the ecological status of a water body as sought by the European Water Framework Directive. The ecosystem services framework aims to support informed decision making by explicitly linking the goods and services produced by functioning ecosystems to human well-being illustrating the broad impacts of various land-use scenarios. The Economics of Ecosystems and Biodiversity (TEEB) approach provides a framework for assessing multiple stressor and multiple outputs of a river basin, facilitating management of a complex system. Economic valuation includes monetary and non-monetary methods. Monetary methods although under continuous development and construction are well documented [3, 4, 2]. Non-monetary valuation relies on perception and values of stakeholder at large. The main research question addressed is: How do stakeholders perceive Ecosystem services and functioning at river basing level? The practical framework to elicit stakeholder knowledge and to enable stakeholders around the Evrotas river basin to identify the relevant elements of ecosystem services is based on a participative workshop with representatives from the public and private sector involved in water management, nature management, cultural heritage and water related economic activities, municipal, and regional planning. A brief presentation of Ecosystem definition and types was given in order to provide a common ground for the following process. To utilise the basic knowledge of TEEB. Group-dynamic was rhythmised by group exercises, restitution of group findings in plenary session enabling expression of view-points and social learning. The core activity of the workshops is co-construction of ecosystem services, in three steps: (i) identification of ecosystem services in groups of four to six participants, (ii) plenary exchange of the findings, in order to identify major ecosystem services, multiple uses for the river basin, and (iii) individual ranking of ecosystem services by participants. The case study is the Evrotas river basin in Greece, 25 valid questionnaires were collected and analysed.

Challenges in data analysis, weighting, valuation and visualisation - How to enable decision makers to make trade-offs while being transparent for all stakeholders

572 Combining Fuzzy sets with LCA to weight global environmental impacts with local limits of ecosystem capacity
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Small hydropower plants (SHP) are discussed very controversially. They are essential for the realization of the Swiss Energy Strategy 2050 and looking at the impacts like climate change, energy demand or emissions SHP belong to the most eco-friendly power plants. However they can have relevant impacts on flora, fauna and the local ecosystems and these impacts are specific to each location. An overall weighting taking into account the relevant impacts and local aspects of ecosystems capacity is crucial for a decision support. The goal of this project is to build an evaluation tool for SHP, taking into account all the different environmental impacts, local tipping points and economic aspects. So the challenges are: To combine quantitative indicators e.g. from LCA with qualitative criteria on the local ecosystems. Taking into account the limits and capacities of the local ecosystems. Expressing the expert knowledge on the relation of different factors and ecosystem capacity even if they are controversial. To get a cost efficient method to be applied at SHP projects with limited budgets. After discussions with a variety of experts and studied different evaluation methods it become clear that for modelling expert knowledge given in quantitative as well as in linguistic terms the best method is fuzzy set theory (FST). This method gives the possibility to weight the different LCA results, to introduce qualitative expert judgment and furthermore different even contradictory judgments to be taken into account in the same model. A group of experts from different fields like hydrologist, biologists, from NGOs, administrations, technicians and LCA specialists has been formed to build the model. The first evaluation has shown promising results but further improvements are necessary. The experience from evaluation and modelling has shown that this method is very suitable to evaluate complex systems like ecosystems, taking also into account their capacity. The important advantages of this method are: representation of qualitative expert knowledge with linguistic terms bringing together in a mathematically exact way quantitative and qualitative data and knowledge nonlinear relations can be handled and FST makes the interpolation floating transitions which are typical for environment can be handled FST can give more than one ‘right’ result typical for human reasoning. Doing all this in a transparent way.

573 Reversibility assessment: how to improve the weighting of impacts in LCA
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Weighting impact categories in LCA is an optional step according to ISO14040. However, in the context of LCA in support to policy, weighting may help in the: i) identification of the most relevant impact categories, i.e. to define Product Category Rules (PCR) or for product labelling (as in the case of Product Environmental Footprint – PEF – of products (EC, 2013)); ii) to address eco-innovation policies and strategies towards the most effective solutions for decoupling impacts from production; iii) presenting results as a single score. Currently, the ILCD recommendations for life cycle assessment (EC-JRC, 2011) as used in the product environmental footprint (PEF) (EC, 2013) entail 15 impact categories at midpoint. The identification of the most important impact categories at the moment is done adopting a 1:1:1: weighting. Over the years, several weighting set has been proposed in literature (see a recent overview in Ishibashi, 2015) such as: proxy methods, midpoint or endpoint methods following different approaches such as e.g. distance to target, monetisation, panel- based sets etc. However, all those methods present strengths and limitations among which the capacity of assessing the direct and indirect relevance of the impacts from a sustainability point of view. The present work aim at discussing to which extent sustainability principles, such as the avoidance of irreversable impacts (see e.g. NRC, 1999), could be a criteria for determining the relative weight of an impact category. Some qualitative attempts have been made (e.g. Soares et al 2006), including the topic amongst criteria for weighting), proposing to a panel of expert to judge the degree of reversibility with a 5-levels score systems spanning from: a natural istantaneous reversibility, to a sole artificial up to irreversible. Moreover, Fanai and Burn 1997 listed specified characteristics of some impacts and direct measures of the severity of other impacts to be used in a “distance metric” formulation to evaluate a reversibility index. A case study on the reversibility of lake eutrophication is reported to discuss potential and limitation in using reversibility as a criteria for weighting. The results stem from the analysis of the evolution of the state of a lake over time, at decreasing environmental pressure in term of human induced nutrient’s loads.
574 LCA single score results about PCB-contaminated sediment disposal options lead to reassessment of authorities’ decision

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The Klingnau reservoirs main problem today is the silting of the lake. Dredging is being considered to extract the sediments. The dredged sediment can be disposed of by resuspension into the river or by disposal in a landfill. Article 40 states that resuspension can be considered as an option if the flora and fauna downstream is not negatively affected. As the sediment of the Klingnau reservoir is contaminated with pollutants such as heavy metals and PCBs, a qualitative environmental impact assessment commissioned by the authorities was carried out. The concentrations were below the maximum permissible values and indicated that no negative effects were expected by resuspensing the sediments. The assessment showed on the other hand that the emissions of the disposal to a landfill option would exceed the maximum permissible limits. Thus "Carrying capacity for Construction Transports". The environmental impact assessment concluded that the resuspension of the sediments to the river has a lower environmental impact than their disposal to a landfill. In order to verify the results of the environmental impact assessment, the two options were analysed by Life Cycle Assessment. The LCA shows very clearly that the disposal of the dredged sediment by resuspension into the river has a much higher environmental impact than the disposal in a landfill. This is mainly due to the PCB and heavy metal contents in the sediment which in case of the resuspension option affects the river ecosystems negatively. This example shows how important it is to perform a comprehensive assessment in order to evaluate the environmental consequences. The consequence of the LCA results for the Klingnau reservoir dredging project was that the planned resuspension of the dredged sediments to the river was discarded and the concept is currently being revised. As it has been shown in this paper it can prevent severe misjudgements compared to decisions that are only based on legal limits for single pollutant emissions. The presentation of single score LCA results plays an important role in the process of persuading decision makers to change their mind for an environmentally preferable option as such results usually are clear and easily understandable because they don’t need further interpretation.

575 How far can changes in consumer behaviour take us on the path to environmental sustainability?

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It has recently been proposed to normalise environmental impacts according to environmental carrying capacity in LCA, to arrive at normalised indicator scores expressed as occupations of carrying capacity. This can facilitate the evaluation of whether a studied anthropogenic system is environmentally sustainable. This paper presents the first application of carrying capacity based normalisation to a real-life comprehensive case study. The case study aims to cover the total annual consumption of goods and services of 1283 urban Danes, based on their response to a lifestyle survey. The goal of this study is to demonstrate the applicability of carrying capacity based normalisation by exploring the extent to which Danes can become the minimum sustainable by practical changes in behavior. The EcM of total personal consumption were compiled by combining process-based inventory modelling (e.g. for consumption of food and residential heat) with environmentally extended input-output analysis (e.g. for expenditures on electronics and clothing) and extrapolations for other impact categories than climate change. A “minimum impact”-profile was constructed by combining the environmentally soundest qualitative and quantitative consumption behaviour observed in the respondent pool across consumption categories, while disregarding potentially erroneous outliers having unrealistically low consumption values. The average respondent was found to exceed his or her fair share of carrying capacity for five impact categories, albeit less so than the average profile. The case study results indicate that (quite large) behavioural changes alone are insufficient for achieving the goal of personal environmental sustainability for all LCA impact categories in a Danish setting and must be supplemented by policies targeting, for example, eco-efficiency improvements of the energy system and public transport. The application of carrying capacity based normalisation in LCA could help the decision-maker design policies that are more likely to achieve the environmental goal of personal environmental sustainability for all Danes.

576 Incorporation of Lifecycle Thinking in Nanotechnology Risk Control and Sustainability Assessment: The Case of Sustainable Nanotechnology Decision Support System

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Early identification of risks and impacts of engineered nanomaterials over the lifecycle can enable stakeholders to minimize these concerns at an early stage of nanoproduct development. SUNDS conceptual decision framework integrates tools for evaluation of the environmental, economic and social impacts of nano-enabled products, and supporting their development toward safety and sustainability. SUNDS conceptual decision framework comprises of two tiers of varying analytical complexity and data requirements. Both tiers utilize Life Cycle Thinking (LCT) and optimise outputs “of individual modules and sub-modules in terms of hotspots to be addressed through remedial measures for risk management and sustainability assessment. The first tier of SUNDS comprises of LICARA NanoSCAN, provides a semi-quantitative evaluation of the environmental, social and economic benefits and the ecological, occupational and consumer health risks of nano-enabled products from lifecycle perspective in comparison to conventional products with similar uses and functionality. SUNDS Tier 2 implements an integrated Risk Control (RC) and Socioeconomic Assessment (SEA) module, in which the RC module comprises three risk sub-modules (Ecological Risk Assessment (ERA), Public Health Risk Assessment (pHRA) and Occupational and Consumer Human Health Risk Assessment (eHHRA and cHHRA respectively)), and SEA comprises all the sub-modules (ERA and HHRA, Life Cycle Impact Assessment (LCIA), Economic Assessment (EA), Social Impact Assessment (SIA)). Within both RC and SEA modules in SUNDS Tier 2, most of the sub-module outputs are organized within four lifecycle stages: Synthesis, Production, Use and End of Life. RC module pinpoints the hotspots for risks through the lifecycle of existing and new applications of nanomaterials and nano-enabled products and system technologies. By prioritizing the hotspots for risks, the decision-maker on where to focus remedial action. The proposed abstract discusses the benefits and methodological challenges in incorporating LCT to SUNDS conceptual decision framework, as well as insights gleaned from its application to case studies.

577 Harmonised decision-support and guidance in the European SPIRE programme to achieve overarching sustainability targets

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The SPIRE (Sustainable Process Industry through Resource and energy Efficiency) programme is a public-private partnership brought to life by the European Commission in the EU’s research and innovation framework programme Horizon 2020. A financial contribution of €44 million from the EU and €550 million from a consortium of 53 companies to the project. The EUR 900 million is foreseen over a seven year period in order to engage eight sectors of the European process industry; chemicals, cement, ceramics, minerals, steel, non-ferrous metals, industrial water and process engineering in the development of novel technologies for improved resource and energy efficiency. By means of this measure, the ambitious SPIRE initiative (being part of the Europe 2020 strategy) aims to reduce the energy consumption in the European process industry by 30% and the utilisation of primary (non-renewable) raw materials by 20%, compared with the period of 2008 - 2011. The European project MEASURE, a coordination and supporting action within the SPIRE framework, will provide a roadmap for sustainability in the process industry. MEASURE is building on the key messages and broader utilization of the stage-gating approach and established decision support and project management methodologies in European funded SPIRE projects. Within a given team, the stage-gating approach could be highly effectively used as a project planning and monitoring tool, to ensure successful delivery of the key objectives against which project success will be evaluated. Consequently, the stage-gating approach should be used pragmatically to stop research activities that are less likely to contribute to the goals of the project within its lifetime. However, this requires some degree of flexibility in funding within the project and access to other sources of funding by research groups. To aid the pragmatic use of stage-gating, existing multi-criteria decision methodologies should be integrated into the development process to aid consistent and transparent decision making.
Contaminants of Emerging Concern in the Environment and their Management (P)

MO001 Vertical distribution of legacy and current used pesticides in marine interstitial water from eight Iberian Mediterranean areas
Y. Perez, J. Moreno-González, Instituto de Oceanografía / Centro Oceanográfico de Murcia; C. Martínez-Gomez, Instituto Español de Oceanografía / Marine Contamination and Biological Effects; J.A. Campillo, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia Organochlorinated and current used pesticides (CUPs: organophosphorus, triazines and others) were determined in interstitial water of sediment cores from eight Iberian Mediterranean coastal areas (Barcelona, Tarragona, Ebro Delta, Valencia, Castellón, Cartagena, Almería and Málaga). Three sediment cores were taken at three different sampling sites per area by using a box corer at bottom depths higher than 50 m. Cores were cut into 1-5 cm-thick sections between 0 and 18-cm depth, interstitial water was obtained by centrifugation and samples from every site and depth were pooled. Pesticides were analyzed in interstitial water by stir bar sorptive extraction coupled to gas chromatography with mass spectrometry. Triazines, organochlorinated pesticides, organophosphorus and other pesticides were found in interstitial water of the study areas. The total number of pesticides found per each area varied from 6 to 17 compounds. Pesticides from the Linuron Alone and Linuron + DCPM cocktail were found at concentrations below 100 ng L\(^{-1}\). Other commonly detected compounds were a-endsulfan, m-parathion, alachlor, chlorothalonil and simetryn. Overall the concentrations of CUPs decreased with depth in the considered areas. However, the presence of some legacy pesticides, such as p,p′-DDE was mostly detected in deeper layers.

MO002 On-site large-volume solid-phase extraction device - A solution for sufficient sample volumes in comprehensive toxicological and chemical analysis of wastewater effluent
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Recently, more comprehensive approaches such as multi-target, non-target and bioanalytical techniques and combinations of these, have risen next to the more traditional way of monitoring hazardous compounds in wastewater effluents. However, one big issue related to extensive ecotoxicalogical profiling and chemical analysis is the amount of sample needed for such analysis. In addition to volume, sufficient concentration factors are needed because many of the compounds causing harmful effects are present at very low concentrations (ng/L). The main objective of this study was to apply and test the suitability of a recently developed onsite large-volume SPE device (LVSPE50) to achieve sufficient sample volumes for the comprehensive toxicological and chemical analysis of wastewater effluent. The performance of the device was evaluated by determining recoveries for selected compounds of perfluorinated alkyl acids (PFAS), hormones and bisphenol-A (BPA) from spiked artificial and real wastewater effluent by liquid chromatography mass spectrometry (LC-MS/MS). The LVSPE50 device is based on a system where the water is pumped through multiple steps through the machine (pre-filtration, sampling chamber, SPE cartridge). The device performed well with influent and effluent samples and with some WWTPs even more than 40L of sample was collected and extracted. The recoveries for PFAS were between 1-96% and 35-100% for hormones and BPA in artificial effluent depending on the compound. Large extraction volumes made it possible to analyze the samples with multiple bioassays (FET, ER-CALUX, AR-CALUX, p53-CALUX, NRR-assay, umu-test, EROD assay) and chemical analysis of more than 400 target compounds (LC-MS/MS).

MO003 Gene expression profiling of bacteria for environmental monitoring
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Major water pollution includes the collective discharge of domestic and industrial waste together with human bacterial pathogens into the environment. Wastewater treatment plants (WWTPs) do not completely remove all the pharmaceutical and organic contaminants, which subsequently end up in environmental waters. A major concern is that contaminants contribute to increased virulence and/or antibiotic resistance of human bacterial pathogens and thus pose a health risk to the community. We have designed and applied a gene expression system for Pseudomonas aeruginosa to predict the potential impact of soluble components released from sewage sludge on bacteria. P. aeruginosa was selected since it is both an environmental bacterium with bioremediation capacity and an opportunistic pathogen. Thirty-nine selected genes associated with stress response, virulence and antibiotic resistance were analysed by quantitative reverse transcriptase - PCR in bacteria treated with either antibiotics or heat shock for validation or sewage sludge extracts from WWTPs servicing 3 different cities (Eskilstuna, Västerås and Örebro) in Sweden. The molecular profiling of the slugge sludge extracts from WWTPs was assessed using the principle component analysis. The sludge extracts from Eskilstuna affected a greater number of P. aeruginosa genes, responsible for general stress response and virulence, while Västerås sludge leachate affected fewer genes and clustered more closely to the control. Örebro sludge leachate had intermediate influence on the gene expression in P. aeruginosa. This suggests that the presented strategy of P. aeruginosa toxigenicomics has potential applications in evaluating the effects of soluble contaminants on bacterial pathogens in environmental waters for considerations in risk assessment.

MO004 Pyrethroid occurrence and distribution in Brazilian fish tissues
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Pyrethroids are organic pollutants with high hydrophobicity used as insecticides. Current exists about aquatic organisms’ exposure to their toxicity. They produce a neurotoxic effect by altering the sodium channels of the nerve cells. Their LC50 values can be as low as 0.2 μg/l for aquatic macroarthropods considering a 4-day exposure, and 1 μg/l for fish. They were believed to be excreted or converted to non-toxic metabolites. But a recent work has detected them in wild river fish for the first time at significant levels in 100% of the samples [1]. Our study investigates the occurrence of pyrethroid compounds in fish from three locations in Rio de Janeiro (Brazil), as the first attempt to determine the occurrence and distribution of pyrethroids in marine fish tissues from Brazil. This is the first study to report pyrethroid levels in wild fish tissues from the sea. Samples were collected from Rodrigo de Freitas lagoon, Ipiranga bay and Itaipu in 2009-2010, including different tissues—muscle, liver and gills—from 20 fish including 10 males and 10 females. The analytical method monitored 10 different pyrethroids. For the sample preparation lyophilized sample was spiked with internal standards, extracted by sonication and underwent a clean-up with alumina and C18 SPE cartridges. Extracts were analysed by GC-NCI-MS/MS. Method recoveries ranged 53-116% and method LODs and LOQs were 0.02-0.46 ng/g lipid weight (w/w) and 0.08-1.54 ng/g lw, respectively. Pyrethroids were detected in all the fish and most tissues. Total concentrations were in the low limit of the range found in the wild fish from Spanish rivers (12-1,508 ng/g lw) and those found in dolphins from Brazil (7.0-69 ng/g lw) [1,2]. Half of the selected analytes were detected; cypermethrin and permethrin in 8 of the main contaminants to the pyrethroid profiles. Acknowledgements – This work has been funded by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2009-SGR-965). References [1] C. Corellas, E. Eljarrat, D. Barceló. 2015. First report of pyrethroid accumulation in river fish: A case study in Iberian river basins (Spain). Environ Sci Technol 75: 110-116. [2] Alonso MB, Feo ML, Corellas C, Vidal LG, Bertozzi CP, Marigo J, Secchi ER, Bassoli M, Azevedo AF, Dorneles PR, Torres JPM, Lailson-Brito J, Malm O, Eljarrat E, Barceló D. 2012. Pyrethroids: A new threat to marine mammals? Environ Int 47: 99-106.

MO005 Target and non-target screening analysis using gas chromatography-quadrupole-time-of-flight (GC-Q-TOF) to prioritize emerging pollutants for seafood monitoring
S. Lee, Hanyang University; M. Choi, National Institute of Fisheries Science; H. Min, Hanyang University / Analytical Sciences and Convergent Technology
Human is exposed to organic contaminants with several pathways, depending on target contaminants and their usage. Seafood consumption has known as a major exposure pathway to toxic organic contaminants including persistent organic pollutants (POPs). In order to prioritize the emerging pollutants in seafood, we employed non-target screening analysis using gas chromatography/quadrupole-time-of-flight (GC-Q-TOF). Surface seawater, sediment, and seafood samples collected from Ulsan Bay, Korea, to investigate the occurrence and migration efficiency for detected organic contaminants. To make our analysis effective, we made database with 200 target chemical standards based on nation-wide monitoring programs (e.g. POPs). Non-target screening analysis was also performed to present the feasibility of P. aeruginosa matrices. To remove matrix effect in each environmental sample, we developed
clean-up method based on solid phase extraction (SPE) with HLB cartridge. Using matching with NIST library and deconvolution techniques, we found approximately 4000 compounds in seawater, sediment and seafood samples. The predominantly identified compounds were siloxanes, phthalates, musk fragrances, and phosphate or chlorinated flame retardants, implying the strong candidates for seafood monitoring in industrial wastewaters. Our approach or framework for prioritization of emerging pollutants in seafood could be effectively utilized as occurrence-based prioritization in various environmental compartments.

MO006 Occurrence and assessment of Perfluorinated compounds in fisheries from Korea
M. Choi, I. Lee, R. Jung, National Institute of Fisheries Science
Residues of 15 perfluorinated compounds (PFCs) were investigated in 58 fisheries including 31 fish from Korea. The total concentrations of PFCs in muscles of fishes ranged from 0.04 to 32.2 ng/g wet weight for some fish samples.ควบคุมสุขภาพและสภาพแวดล้อมของมนุษย์. The muscles of crustaceans and cephalopods had greater concentrations of PFAAs than the oysters, and clams showed the concentrations higher than 10 ng/g wet weight. The main compounds among PFCs were perfluorooctane sulfonate (PFOS) for fish, perfluorooctanoic acid (PFOA) for crustaceans, perfluorooctanoic acid for bivalves, gastropods, and seaweeds, and perfluoroundecanoic acid for cephalopods. This suggests that bioaccumulation of PFCs in marine organisms is dependent on their taxonomic features (sources of food, feeding type, and metabolism). The concentrations of PFCs in liver and intestine were greater than in muscles. The muscles of crustaceans and cephalopods had greater concentration of perfluorotridecanic acid than intestine. We also found the difference in PFC profiles between wild fish and farmed fish. Farmed fish tended to accumulate relatively greater amounts of perfluorooctanoic acid in wild fish, while perfluorodecanoic acid was abundant in farmed fish. The calculated hazard ratio of PFCs for all fisheries muscle samples, was less than 1.0, and could be classified at safe levels for the general population. The main route of PFOAs to human exposure was fish consumption, and of PFOA was crustacean consumption.

MO007 Nitrate: an Environmental Endocrine Disruptor?
R. Poulsen, University of Copenhagen / Department of Pharmacy; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences; M. Hagman, University of California, Berkeley / Civil Environmental Engineering
Integrative Biology
This poster presents a review of the increasing experimental evidence that inorganic nitrate acts as an environmental endocrine disruptor. The double Nobel Prize awarded Haber-Bosch process, which fixes atmospheric nitrogen to ammonia as a feedstock for agricultural nitrate-fertilizers, kick-started the Agricultural Revolution. Subsequently, environmental nitrogen emissions have increased tremendously. Even though nitrogen levels in surface waters during pre-industrial times cannot be precisely estimated it is unarguable that present day nitrate-levels (1-9 mg L$^{-1}$ in larger European rivers) are severely above those concentrations where most aquatic life has evolved. During the past decades, deviations in wildlife reproductive hormone levels and sex ratios have been reported in organisms ranging from alligators, newts, fish and frogs to small crustaceans. Despite much research effort, no single major cause of the observed changes has been found. This poster presents a compilation of the growing experimental evidence in the aquatic environment of inorganic nitrate. It furthermore describes three hypotheses regarding the mechanisms by which nitrate may cause endocrine disruption: 1) Nitrate affects homeostasis by interfering with chloride and/or iodide ion transport, 2) Nitrate is converted to nitric oxide, which affects homeostasis by altered transduction of relevant genes and/or altered activity of relevant enzymes, and 3) Nitrate acts synergistic in combination with other environmental pollutants. It is likely that different species have very different sensitivities, as they have evolved in environments of different nutrient status and have inherently different hormonal systems. Future research must therefore include different ecologically relevant organisms and provide information on threshold concentrations, developmental “sensitivity windows” as well as addressing the hypothesized mechanisms.

MO008 Fate of antibiotics in pulsed corona discharge oxidation
M. Kahlström, Stockholm University / Department of Chemical Engineering; A. Sokolov, M. Louhi-Kultanen, Lappeenranta University of Technology / Chemical Technology; L. Kronberg, Abo Akademi University
Antibiotics in the environment are of concern due to the development of antibiotic resistant bacteria. Conventional wastewater treatment plants are not designed to remove micropollutants such as antibiotics. Thus, a significant amount of these compounds are released to the aquatic environment. Our approach is pulsed corona discharge process (PCD) is an effective, environmentally friendly method for removing recalcitrant organic compounds in waste water. In PCD organic compounds are transformed through reactions with hydroxyl radicals and ozone. The objective of this work is to optimize PCD oxidation for the antibiotics sulfamethizole, doxycycline and amoxicillin. A meta-analysis of the antibiotics and their oxidation products will be performed and the main oxidation products will be fully characterized. A preliminary structural characterization of the oxidation products was performed on the basis of molecular weight determination by a time of flight mass spectrometer, and fragmentation pattern recorded by an ion trap mass spectrometer. Final structural determination will be performed by NMR studies (1H and 13C NMR) on isolates obtained through semi-preparative LC fractionation of reaction mixtures. Subsequently, an exact quantification of the main products will be performed with a triple quadruple mass spectrometer using the isolated compounds as authentic standards. The preliminary results show that amoxicillin forms nine transformation products of which five have been tentatively identified. Sulfamethizole forms many minor products of which the structures of five products were preliminarily elucidated. Doxycycline forms two major products, both of which have been tentatively identified. The most common transformations are those where one or hydroxyl groups are added to the parent compound and ring opening of the β-lactam ring in amoxicillin.

MO009 WAST EFFECT: Life cycle management of emerging contaminants in waste with a focus on flame retardants
H. Arg, N. Morin, NGI / Environmental Engineering; G. Okkenhaug, NGI; A. López, University of Toronto / Department of Chemistry, University of Toronto; K. Breivik, Norwegian Inst. for Air Research; F. Wania, University of Toronto Scarborough / Physical and Environmental Sciences; G.D. Breiveld, NGI / Department of Environmental Engineering
Waste treatment has become a dynamic sector. In many countries, waste-handling infrastructure is changing from doing less landfilling and more incineration or recycling, in order to maximize energy and material re-capture. In addition to materials and energy, waste also contains a large variety of emerging contaminants (CEC), and yet how landfilling, incineration, and recycling can effect environmental emissions and exposures of such CECs remains largely overlooked. Two such emerging contaminants found in increasing levels in waste include brominated flame retardants (BFRs) and antimony. The Norwegian research project WAST EFFECT seeks to understand how these CECs and other contaminants behave in different waste streams, like landfilling, incineration and recycling. WAST EFFECT researchers sampled air and water emissions around several waste-handling facilities, and ultimately established a Norwegian waste emission inventory of BFRs and antimony in electronic waste, car waste, plastics, glass and combustible waste. Collectively these data show the contribution of the waste sector to emissions of CEC in the environment, as well as how effectively the eliminate CECs from the environment. The main output of this research will help guide the waste management sector on which of the treatment methods provide the lowest risk in regards to emerging chemicals, and which processes could be optimized. These results are also of relevance to a better understanding of potential future risks to areas around and down-stream of waste handling facilities.

MO010 The occurrence of antibiotics in wastewaters, recipient waters and sediments
E. M. Kortesmäki, Abo Akademi University / Department of Natural Sciences; A. Meierjohann, Abo Akademi University / Laboratory of Organic Chemistry; L. Kronberg, Abo Akademi University
In recent years, the occurrence of antibiotics in the environment has generated a major concern among the public and decision makers because of their possible impact on public health and the aquatic ecosystem and possible development of antibiotic resistant bacteria. Antibiotics have been shown to enter the environment through target organisms’ excretion mainly via discharges from wastewater treatment plants (WWTPs), through the use of animal manure in agricultural fields and through direct discharge from aquaculture. It has been shown that even the relatively low concentrations of antibiotics found in WWTPs and in the environment can give rise to the selection of antibiotic resistant bacteria. However, more data on the occurrence and fate of antibiotics is needed before an actual risk assessment of their impact can be done. The overall objective of the work was to determine the occurrence and fate of 17 most used antibiotics in Finland in WWTPs, in recipient waters and sediments. Twenty-four hour composite samples were collected from the influent and effluent waters in the WWTPs in three consecutive days. WWTPs were chosen to represent different profiles by their working efficiency, characteristics of their incoming wastewater and size. Additionally, surface and bottom water samples as well as sediment samples were taken from the discharge points and at various points along the wastewater conveyance line (WWTPs and catchment area to the aquatic environment). The analytical method developed for the analysis of 17 antibiotics and 3 metabolites (tetracyclines, β-lactams, macrolides, quinolones and sulfonamides) in this study combines on-line solid-phase extraction (SPE) extraction and LC-MS/MS identification and quantification (an existing pre-treatment technique of SPE with liquid chromatography mass spectrometry triple quadrupole through online connection). An important challenge was the drug ‘amoxicillin’ which is used as a tracer for wastewater contamination. Isotopically labeled isomers of the analyzed compounds were used for quantification. Obtained results show that nine antibiotics could be detected frequently at concentrations ranging from 500 ng L$^{-1}$ in the influent to low ng L$^{-1}$ in the effluent waters. Clarithromycin, erythromycin and carbamazepine were found close to the discharge point (at the nearest sampling location from the discharge point) at concentrations ranging from 5 to 52 ng L$^{-1}$.
Preliminary results also show that some antibiotics can be detected in sediment samples taken from the Archipelago Sea.

MO011
Application of Multimedia Urban Model to estimate the environmental fate of PAHs in Tarragona County, Spain
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Concern regarding polycyclic aromatic hydrocarbons (PAHs) continues because of their ubiquity in the environment and relatively high toxicity. PAHs are emitted from incomplete or low temperature combustion processes and as a result, environmental burdens are a function of proximity to emission sources. Numerous studies have demonstrated elevated concentrations of PAHs in urban areas. For this reason, predicting the environmental fate and transport of PAHs is an essential step in the process of assessing the impacts of these contaminants in the urban and surrounding environment. The goal of this research was to estimate the fate and dynamics of PAH in Tarragona County, Spain, that is home to the most important chemical/petrochemical industrial complex in Southern Europe. To achieve this goal we used the Multimedia Urban Model (MUM-Fate) of Diamond et al. (2001, Chemosphere 44, 1655-1667). This model is based on the Level III fugacity model of D. Mackay (Multimedia Environmental Models: The Fugacity Approach, Lewis Publishers, Boca Raton, 1991) and consists of seven bulk media compartments: lower and upper air, surface water, sediment, soil, vegetation and an organic film that coats impervious surfaces. This model is characterized by considering impervious surfaces, which are a unique feature of urban environments. Here, MUM-Fate was parameterized according to conditions in Tarragona County and then run with an illustrative emission rate of 1 mol/h for six PAHs, Naphthalene, Anthracene, Phenanthrene, Fluoranthene, Pyrene and Benzo(a)pyrene. Results from the MUM-Fate model showed that the film compartment achieves highest concentrations of PAHs studied, followed by soil, sediment and vegetation compartments in descending order. In contrast, soil and sediment were the greatest sinks for PAHs in Tarragona County, because they receive high inputs from air, water and vegetation relative to low losses. By far the greatest loss of PAH was due to advection from air, followed by photodegradative reactive losses from air. Although the general pattern was similar to that seen in Toronto, Canada, more PAH were lost via air advection and photodegradation whereas less PAH accumulated in vegetation, water, soil and sediment because of lower precipitation in Tarragona with its semi-arid climate. These results provide a first approximation of the fate of PAH in Tarragona County, an area highly impacted by the petrochemical industry.

MO012
Assessing the potential effects of reclaimed waters on aquatic organisms using a test battery with standardised and novel bioassays
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Climatic change is causing more persistent drought periods in the Mediterranean basin as they reduce availability of water sources and increase undesirable impacts on aquatic ecosystems. The use of reclaimed waters for irrigation and aquaculture purposes is generally considered a reliable alternative for sustainable water management, particularly in those regions with high water demand vs. water availability. Recently, a large number of organic compounds, which are generally categorised as emerging contaminants, has been detected in wastewater effluent. To date, no guidelines have been proposed for this group of compounds, so they cannot be controlled according to existing Spanish Legislation on reclaimed waters use (RD 1620/2007). Reclaimed water samples, supplied by four Spanish WWTPs that collect effluent of different origins (municipal only or municipal and industrial), which undergo distinct tertiary treatments, were used herein. Chemical characterization, performed and 44 emerging contaminants classified as pharmaceuticals, parabens, UV filters and endocrine disruptors, were analysed. A complete test battery was conducted to detect sublethal or chronic effects, which covered all toxicological taxonomic representative of the aquatic compartment. This bioassays battery included a modified standardised growth inhibition test on algae (OECD, 201), the standardised reproduction test on Daphnia magna (OECD, 211), the standardised reproduction test on Thamnoctena spec. and a reproduction test on Phya acuta, an embryo life cycle test on O. latipes, and a modified embryotoxicity test on X. laevis (ASTM, 1998). Reclaimed waters, dilutions of these waters (10%, 50%) and reclaimed waters spiked with high concentrations of a mixture of methylparaben, PFOs and fluoxetine were tested in this test battery. The results showed differences in the toxicity of samples, with good correlation between assays in some cases. This variability of endpoints observed reflected the complete biological characterization of these samples, which confirmed the utility of the test battery to assess the potential effects of complex mixtures as reclaimed waters. This work was possible thanks to Spanish Ministry of Economy and Competitiveness projects CTM2013-44986-R and CTM2014-52588-R.

MO013
Understanding the fate of active pharmaceutical compounds in surface waters receiving poorly or untreated sewage effluent and the development of appropriate environmental risk assessment approaches
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Active pharmaceutical compounds (APCs) have been classified as emerging contaminants, and their introduction in the environment might pose risks to ecosystems. The main source of pharmaceuticals in the environment is human use and excretion or the improper of unused medication to the sewerage system. In wastewater treatment works the lack of efficient removal of some pharmaceuticals, combined with pseudo-persistence, results in their presence in surface waters. As patient access to medicines increases in developing countries the environmental concentration of pharmaceuticals has the potential to be higher than in developed countries if the level of wastewater and drinking water treatment does not also increase. Untreated wastewater enters the environment via discharge into surface waters resulting in a downstream area characterized by high pollution, named “impact zone”. Characterization of the environmental risk posed by pharmaceuticals in such an area is challenging since the formal protocol for environmental risk assessment was developed for environmental conditions largely different from the ones encountered in the impact zone; High levels of BOD, ammonia, and other potential toxicants exist in combination with low concentrations of dissolved oxygen, meaning that there is an absence of traditional species used for toxicological endpoints. Therefore, the calculation of predicted environmental concentrations (PEC) in such conditions is not relevant. The aim of the research is to obtain a comprehensive overview of the gaps regarding the fate of pharmaceuticals in aquatic environment initially starting with an extensive literature review of available data for (1) occurrence, (2) degradation rates, (3) partitioning to dissolved organic matter, colloids and suspended solid matter, and (4) relevant endpoints. This will inform research aimed at gathering data about partitioning and degradation of selected pharmaceuticals in the impact zone at varying dilutions, and determination of proper endpoints. The information obtained will be used to develop an environmental risk assessment approach for impact zones, as only with more accurate exposure concentrations and impact data can the risks to the aquatic environment of APIs be quantified.

MO014
The Chemical Investigations Programme Phase 2: an assessment of the presence of pharmaceuticals in wastewater treatment works influent and effluent
V. Veere, M.J. Gardner, Atkins Ltd.; D. Leverett, WCA-Environment Ltd.

There is increasing interest in the presence of Active Pharmaceutical Ingredients (APIs) in the aquatic environment due to their potential environmental impact. The Water Framework Directive (WFD) “Watch List” currently includes three APIs (E2, EE2 and diclofenac) with the potential for inclusion of further such compounds in future revisions of the Directive. Wastewater treatment works (WwTWs) are the primary source of APIs into the environment. The Chemical Investigations Programme (CIP) Phase 1 took place between 2007 and 2013 and examined the presence of a range of trace substances in WwTW influent and effluent, including selected APIs, across mainland UK. The results of this large-scale study indicated the need for further study to examine issues at a more localised scale and consider solutions. The CIP Phase 2 started in 2015 and includes monitoring at 600 WwTWs over a period of 5 years across England and Wales. The study encompasses monitoring of 23 key APIs, such as pranoproanol, carbamazepine, ibuprofen, tamoxifen and four antibiotics. This poster will present an outline analysis from the first “tranche” of results from the CIP2 study focusing on APIs in influent and effluent, including interactions with Biologically Effective Concentrations (PNECs) which have been developed for each API will also be presented, taking into account the dilution of each WwTWs effluent, as well as an assessment of removal during the treatment process at different sites.

MO015
Presence of virulence genes in Enterococcus spp. isolated from South African environmental water systems
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Enterococcus spp. from environmental sources harbouring virulence factors may not always express functional gene products. However, their presence may be
indicative of potential pathogenicity and could also contribute to the spread of these genes in the environment. The aim of this study was to determine the relationship between the presence of selected virulence genetic determinants and the expression of these factors from environmental water from the North West province of South Africa. A total of 93 enterococci isolates, consisting of six species and two genera, were screened for the presence of virulence determinants, specifically cya, esp, gelE and hly by using PCR (polymerase chain reaction). Furthermore, the expression of cya, esp, gelE and hly were determined by phenotypic assessments illustrating β-haemolysin, hyaluronidase and gelatinase activity. Seventy percent of the screened isolates harboured at least 1 virulence gene whilst 15% harboured 2 or more. The most frequently detected virulence genes were cya and gelE. However, asa1 and hlyA were also detected. Enterococcal surface protein (esp) was the only virulence determinant absent in all screened Enterococcos sp. The presence of virulence genes was correlated with their extracellular enzyme production. Only 40%, 22% and 22% of the cya, gelE and hly gene carrying isolates exhibited β-haemolysin, gelatinase and hyaluronidase, respectively. The results of this study show that a large percentage of these environmental enterococci (including E. faecalis, E. faecium, E. munditii, E. casseliflavus, E. gallinarum and E. hirae) possess virulence factors and that these could be expressed in vitro. This could have implications for individuals using this water directly for recreational and other purposes. Therefore, measures to minimize their presence in such water sources are vital. Keywords: Enterococcus spp.; environmental water systems; virulence genes; extracellular enzyme production.

MO016 Occurrence of 16 illicid drugs in Ceyhan River, Turkey

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Illicit drug concentrations in surface waters which under the influence of waste water treatment plants can be an indirect tool to estimate the community level of consumption of illicit drugs. These drugs enter the surface waters unaltered or slightly transformed via waste water effluents by human excretion after illegal consumption, by intentional extermination from undercover drug laboratories or by accidental. The illicit drugs and their metabolites are very recalcitrant to elimination at conventional waste water treatment plants. In the current study, which is one of the first studies in Turkey, presence and seasonal variability of 16 illicid drugs and metabolites in Ceyhan River, which places in Southern Turkey was investigated. Water samples were collected from 9 stations at seasonal intervals for one year to characterize the seasonal variability of drugs. One of these stations was near the Osmaniye waste water treatment plant. As a part of this study, a sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method targeting 16 illicid drugs has been developed and employed to investigate the occurrence of these drugs in water samples. Solid phase extraction techniques were used for all samples. 16 different illicid drugs and metabolites (6-MAM, cocaine, codeine, fentanyl, hydrocodeine, hydroorphomone, JWH-018, JWH-073, ketamine, MDA, MDMA, amphetamine, methamphetamine, methyleconine, morphine, tramadol) were examined with LC-MS/MS. Fourteen of these drugs couldn’t be found in the river water samples. Cocaine and tramadol were detected in 77.8% of the river water samples. The concentrations found in the water samples in the low ng/L range. Median cocaine concentration was 0.404 ng/L and tramadol concentration was 0.420 ng/L. Highest illicit drug concentrations were detected in the station close to the wastewater treatment plant and summer season. Environmental concentrations of these illicit drugs are low, but they may be toxic to the aquatic organisms. Risks to the environment and human health are under consideration and further studies about the occurrence and toxicology should be done.

MO017 Mixture toxicity effect of bisphenol AF and sulfamethoxazole on thyroid endocrine system in zebras

K. Ji, B. Kwon, S. Jang, Yongin University; B. Kim, Department of Environmental Medicine

Bisphenol A (BPA) has been produced for the past decades with wide variety of paper products (mean: 1.5 µg/g). In particular, seventeen thermal receipts (14% of currency bills, and business cards. As the concerns for toxic effects of BPA grows, Bisphenol A alternatives cause reproductive endocrine disruption in zebrafish (Danio rerio) by affecting sex steroid hormones and thyroid function. Therefore, bisphenol A (BPA) and bisphenol F (BPF). However, limited information is available concerning the occurrence of these environmental pollutants in water sources are vital. Keywords: Enterococcus spp.; environmental water systems; virulence genes; extracellular enzyme production.

values was observed. The present study indicates that combined exposure to SMX could significantly increase an endocrine disrupting effect of BPA.

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MO018 Toxic and genotoxic potencies of rainwater samples from Greece on bacteria and human cells

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Rainwater is considered as the main route of pollution from the atmosphere to the aquatic ecosystems. Despite the significant effect of rainwater quality on the different levels of trophic chain, a relatively small number of studies has been conducted regarding the impact of rainwater to living organisms and microorganisms. The main focus of the present work is to provide novel knowledge in relation to the potential adverse effects of rainwater on aquatic ecosystem and human health. In this context, the toxic and genotoxic effects of various rain water samples collected from three urban cities of Western Greece were estimated. Acute toxicity of the rainwater samples was evaluated by monitoring the inhibition of the luminemence emitted by the marine bacterium Vibrio fischeri, using a Microtox system (Azur Environmental). The cytotoxic and genotoxic potential activities of samples was assesed employing the cytokinesis block micronucleus (CBMN) assay. The physicochemical composition of the samples was also determined. The pH values of rain samples ranged between 5.8 and 7.2, indicating a reduction of the pH index during the summer months. In contrast, conductivity increased, in the same sampling periods. Chemical analysis revealed that SO_4^- followed by Cl were the dominant anions in the majority of the samples. The increased concentration of chloride ions in the rainwater samples from cities situated in coastal zones is probably correlated with the transport of seawater aerosol. Concerning the % inhibition of luminescence of the bacterium Vibrio fischeri, the highest percent of inhibition was observed to the samples collected during the summer months. The results of the CBMN assay showed an induction of genotoxicity in the rainwater samples on the month of December in all urban areas, as well as a cytotoxic activity in the majority of the samples with a most pronounced increase during the summer months. Correlation between obtained toxicity data and chemical composition of the samples proved significant contribution of ions that originate from anthropogenic sources (nitrogen ion species) in the observed toxic effects.

MO019 Occurrence of seven bisphenol analogues in paper products from Korea: Implications for regulation and human exposure

T. Lim, S. Lee, Hanyang University; H. Moon, Hanyang University / Marine Sciences and Convergent Technology

Bisphenol A (BPA) has been produced for the past decades with wide variety of commercial products, such as polycarbonate plastics and epoxy resins. Humans are mainly exposed to BPA through diet and dermal absorption from the use of paper products. Bisphenol A is also considered as a major exposure route to BPA for humans. Recently, BPA has been used in paper products including thermal receipt paper, currency bills, and business cards. As the concerns for toxic effects of BPA grows, BPA is gradually being replaced to related compounds such as bisphenol S (BPS) and bisphenol F (BFF). However, limited information is available concerning the occurrence of bisphenol analogues (BPs) in the paper products. In this study, the concentrations of seven BPs (BPA, BPS, BPF, bisphenol AF (BPAF), bisphenol AP (BAP), bisphenol Z (BPZ), bisphenol P (BPP)) were measured in six types of paper products (n = 153), including thermal receipts, paper currencies, business cards, airplane boarding passes, cafe coupons, and newspapers collected from Korea. All paper products showed the highest detection rate (88%), followed by BPZ (47%) and BPS (16%). Among paper products, thermal receipt contained the highest levels (mean: 2.19 mg/g) of BPA, compared with other paper products (mean: 1.5 µg/g). In particular, seventeen thermal receipts (14% of total) showed the higher levels of BPS (mean: 1.04 mg/g) than BPA (mean: 0.017 mg/g), implying the evidences that BPA is replacing into BPS. These 17 receipts have ‘BPA-free’ labeled in common on the back of receipts. Our findings indicate that BPA is still used as a predominant analogue in thermal receipt papers, but BPA is replaced into BPS. The daily intake (EDI) of BPA associated with dermal absorption of paper products was estimated to be 2.91 ng/day for general population and 9095 ng/day for general population exposed to floor of the control group. Considering paper products, thermal receipt papers contributed to most (>70%) of the BPA exposures for dermal consumption. Considering high occupational exposure groups, survey and risk assessment for the exposure of BPs in thermal receipt would be necessary.

MO020 QuECHERS as perspective extraction technique for isolation of steroid
estrogens from sediments in environmentally relevant concentrations

MO022 Bisphenol A alternatives cause reproductive endocrine disruption in zebrafish (Danio rerio)

J.-K. Li, B. Kwon, S. Jung, Yongin University; B. Kim, Department of Environmental Health; j.; Lee, Yongin University

Although the toxic effects of waterborne bisphenol A (BPA) exposure were frequently studied in aquatic organisms, limited information is available on the toxicity of BPA alternatives, such as bisphenol analogues or Tritan™ copolysters. In the present study, reproductive endocrine disruption of bisphenol A (BPA), bisphenol S (BPS), as well as the mixture of one of the major Tritan™ copolysters (1,4-cyclohexanedimethanol (CHDM)) in zebrafish were investigated. Adult zebrafish pairs were exposed to environmentally relevant concentrations of BPAF (0.5–50 μg/L), BPS (0.5–50 μg/L), and CHDM (0.1–10 μg/L) for 21 d, and the effects on reproduction, sex steroid hormones, and transcription of the genes belonging to the hypothalamic–pituitary–gonadal axis were examined. The adverse effects on performances of F1 generation were further examined. The average number of eggs spawned was significantly less upon the exposure to ≥0.5 μg/L BPAF and BPS, while no significant changes were observed in fish exposed to CHDM. Exposure to two bisphenol analogues resulted in estrogenicity in male fish, showing significant increase in plasma 17β-estradiol (E2) concentration. The increase of E2 is well supported by significant up-regulation of aromatase (Cyp19a) gene, which catalyzes the final step in conversion of androgen to estrogen. Parental exposure to BPAF and BPS resulted in delayed hatching and increased malformation rates. Given the importance of endocrine function, further investigations on underlying mechanisms of sex-dependent responses are required. Acknowledgments: This study was supported by National Research Foundation of Korea (Project no. NRF-2015R1D1A101056628).

MO023 Characterization of samples by endocrine activity profiles using the planar yeast estrogen screen (pYEs)

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Recently, a protocol for the direct combination of thin layer chromatography and the yeast estrogen screen (pYEs) was developed as a screening tool for effect directed analysis (EDA). The advantages of this approach lie in its rapidness compared to an EDA-approach with e.g. HPLC and a subsequent analysis of fractions for biological activity and in its robustness. The method allows for the analysis of effects in demanding matrices. The pYEs supports compound identification by the possibility to quickly falsify alternative compounds and by the direct accessibility of the compounds on the TLC-plate to a subsequent analysis by mass spectrometry. Qualitative and quantitative results about specific effects in mixtures can be obtained within one working day. Activity profiles of samples can be generated easily which allow a comparative assessment of alternative processes for e.g. wastewater treatment without a detailed chemical analysis. Furthermore, sources of environmental contamination can be identified and characterized based on biological effects rather than compounds, i.e. source-identification is independent from the knowledge about defined chemical compounds. Due to the high sensitivity of the method it is possible to detect less than 30 pg/l 17α-ethinylestradiol and 17b-estradiol after a 100-fold concentration. The pYEs might thus serve as a screening tool for the characterization of these compounds in surface waters according to the watch list of the EU water framework directive. Taken together, the pYEs-approach seems to have a high potential to be used as a fast and robust screening tool for various applications in effect directed analysis. The approach is complementary to the more common combination of HPLC with a subsequent bioassay.

MO024 Evaluation of the acute toxicity and genotoxicity of pyrolytic tire chars

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The disposal of waste tires constitutes a major problem with environmental and financial impact worldwide. The last decades, pyrolysiss has received considerable attention for the management of waste tire disposal resulting simultaneously in the recovery of valuable products and gas as fuel. Pyrolysis of tire chars is a pyrolyticCHAR. Pyrolytic char from tire rubber (PyrolyticTyre Char. PTC) is a carbon material similar to carbon black (CB), extensively used as adsorbent or support. As adsorbent, PTC has exhibited promising results for the removal of organic and inorganic pollutants from aqueous media. In recognition of its potential application in aquatic remediation, this work examines the genotoxicity and cytotoxicity potential of PTC (untreated and acid (HNO3-treated) was investigated
for the first time. The acute toxicity of PTCs was evaluated using the marine bacteria Vibrio fischeri, which emit bioluminescence as a byproduct of cell respiration and normal metabolic process, by a Microtox Model 500 Analyzer (AzurEnvironmental). In each experiment, the % inhibition of the luminescence and the EC50 values (mean sample concentration that causes a 50% reduction in bacteria bioluminescence) were recorded after 5, 15 and 30 min of incubation. The genotoxic and cytotoxic activity of the untreated and acutely treated PTC was studied applying the cytokinesis block micronucleus (CBMN) assay in human lymphocytes in vitro. Both the untreated and acutely treated PTC were tested at three different doses i.e. 2.5, 5 and 10 mg/L. The EC50 values for the untreated and acutely treated PTC after 30 min of exposure were determined as 485 and 163 mg L⁻¹, respectively. Both the untreated and acutely treated PTC were found to be genotoxic at all the tested concentrations in the CBMN assay. In the case of acutely treated PTC a slight decrease in MN frequency was observed. Regarding the cytotoxicity, both PTCs induced cytotoxic activity in all the tested concentrations.

MO025 A comparison of micropollutant removal in different biological wastewater treatment plants
O. Komolea, P. Meynet, Newcastle University / Civil and Environmental Engineering; J. Dolling, Newcastle University / School of Civil Engineering and Geosciences; W. Mróz, Newcastle University / School of Civil Engineering Geoscience; R. Davenport, Newcastle University / School of Civil Engineering and Geosciences
Concerns over the effects of chemicals in the aqueous environment has seen the introduction of more stringent water quality regulations and tertiary treatment using advanced treatment processes such as ozonation. These interventions make existing wastewater treatment more energy-intensive; aeration in activated sludge alone accounts for up to 1.5% of UK electricity use. The transition to carbon-neutral wastewater treatment will require the adoption of low-energy biological treatment systems that operate with little or no active aeration, such as those extensively used in Latin America (upflow anaerobic sludge blanket reactors; UASBs and waste stabilisation ponds; WSPs). Little is known about the true biological limits of micropollutant removal especially in low-energy systems. In this study we wanted to investigate a range of structurally diverse micropollutants with varying degrees of halogenation towards that goal. We started by validating analytical methods for the quantification of triclosan, PAHs and PBDEs using SPE-GC-MS and SPE-LC-MS and using them to assess triclosan and PAH removal in two UK WWTPs. Recoveries of the compounds in wastewater was between 70 – 130% with a relative standard deviation of ≤ 20.

MO026 Extended application of a human pharmaceuticals emission model to the assessment of metabolites
E. Han, D. Lee, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies
Accurate prediction of emission is critical to assessing and managing exposure to pharmaceuticals in freshwater, particularly because extensive monitoring data are often lacking. In our previous work (1), an emission prediction model was presented with the uncertainties associated with its prediction of human pharmaceuticals discharged into the typical Korean river environment. The model covers the stages of pharmaceutical life cycle posterior to domestic production and import, including distribution, consumption, disposal, and waste treatment. In the present work, the model was considerably extended, namely, SimpleTreat into a single spreadsheet-type model for calculating the predicted environmental concentrations of the human pharmaceuticals and their metabolites. For metabolites, the stages of life cycle were appropriately adjusted from after intake of the parent pharmaceutical through to the discharge. This model was then used to determine the combined total human pharmaceuticals but also their metabolites in surface water of Korea. From all the human pharmaceuticals sold above 1,000 kg in 2011 in Korea, 6 pharmaceuticals (acetaminophen, allopurinol, cefepime, furosemide, ofloxacin, and theophylline) were selected of which metabolite information (species, formation and demethylation rate) is available. As predicted no effect concentration data of the selected pharmaceuticals and their metabolites are scarce, ECOSAR was used for toxicity estimation. The metabolites of acetaminophen and theophylline were found to have the hazard quotients that are of comparable magnitude with those of their parent pharmaceuticals. As the finding strongly suggests a need to assess the environmental risk posed by metabolites, the model was extended in the present work to as a quantitative tool to efficiently screen the human pharmaceuticals concerning the potential risk from both the parents and their metabolites. Reference (1) Eun Jeong Han, Hee Seok Kim, Dong Soo Lee; Environ. Health. Prev. Med., 19(1), 46-55, 2014

MO027 Exploring the use of synthetic musk fragrances as tracers of wastewater discharges in surface waters
Traces of almost all the substances consumed by humans or released by anthropic and industrial activities end up in the environment and if measured in the different environmental compartments can give some information on their sources. The need for determining the contribution of the different sources of contamination in a particular urban or rural area in the past or in the present can be undertaken by finding specific sources in order to find a quantitative correlation with specific human activities. Antropogenic compounds previously suggested as potential wastewater indicators include artificial sweeteners, persistent drugs, and personal care products. Since each of these tracers has different uses, human metabolism and transformation pathways in natural systems or in treatment plants, the simultaneous analysis of different tracers with their metabolites and transformation products under different hydrological and precipitation regimes can be a powerful mean to assess the relative contribution of different sources and processes. In the framework of a wider project aimed to compare different urban tracers, we developed and tested an Isotope Dilution GC-MS method for the analysis of synthetic musk fragrances in waste and surface waters. Synthetic musks are widely used as fragrances additives in many consumer products, they are widely present in the environment and they tend to accumulate in sediment, sludge and biota. The method, validated taking carefully into account the problem of blank contamination, allows to determine 9 compounds, including nitro-, poly cyclic-, macrocyclic musks and a transformation product (galaxolide lactone), which can be a specific tracer of WWTP discharge. The occurrence of this class of substances has been evaluated in the river Po basin, which is an area with a very high pressure from population, industry and intensive agriculture, through specific campaigns that will be designed to evaluate their suitability as markers of anthropic pollution. Treated wastewater from wastewater treatment plants of Milano and Monza, surface waters (River Lambro and Lambro Meridionale) and irrigation waters has been included in the analytical campaign to assess the overall mass balance of the selected tracers.

MO028 National Environmental Specimen Bank of Norway
M.V. Dimmen, Norwegian Environment Agency; E. Farmen, The Norwegian Environment Agency; E. Lie, E. Fjeld, NIVA Norwegian Institute for Water Research
The modern society are probably using chemicals today that will be regarded as pollutants in the future. The samples stored in Environmental Specimen Bank (ESB) Norway can be used to identify the presence and/or biological effects of such substances in the environment alongside development of new analytical methods for detection. ESB Norway contains frozen samples of animals, plants, air and mud from across Norway and the Arctic. Sample collection is coordinated with the monitoring network. Storing samples during a long period of time gives the possibility to correlate currently monitored parameters to future results. During 2016, the bank will open for applications for sample acquisition for the first time, marking the starting point of new knowledge as the interest rate from stored samples.

MO029 Transfer of POPs from amendments to agricultural soils
L. Lundin, Department of Chemistry; P. Haglund, Umeå University / Department of Chemistry
Recycling organic waste as soil amendments is beneficial for organic matter, soil organisms and soil structure. The ECOSOM project study the effects on soil transformation pathways in natural systems or in treatment plants, the specific sources in order to find a quantitative correlation with specific human activities. Antropogenic compounds previously suggested as potential wastewater indicators include artificial sweeteners, persistent drugs, and personal care products. Since each of these tracers has different uses, human metabolism and transformation pathways in natural systems or in treatment plants, the simultaneous analysis of different tracers with their metabolites and transformation products under different hydrological and precipitation regimes can be a powerful mean to assess the relative contribution of different sources and processes. In the framework of a wider project aimed to compare different urban tracers, we developed and tested an Isotope Dilution GC-MS method for the analysis of synthetic musk fragrances in waste and surface waters. Synthetic musks are widely used as fragrances additives in many consumer products, they are widely present in the environment and they tend to accumulate in sediment, sludge and biota. The method, validated taking carefully into account the problem of blank contamination, allows to determine 9 compounds, including nitro-, poly cyclic-, macrocyclic musks and a transformation product (galaxolide lactone), which can be a specific tracer of WWTP discharge. The occurrence of this class of substances has been evaluated in the river Po basin, which is an area with a very high pressure from population, industry and intensive agriculture, through specific campaigns that will be designed to evaluate their suitability as markers of anthropic pollution. Treated wastewater from wastewater treatment plants of Milano and Monza, surface waters (River Lambro and Lambro Meridionale) and irrigation waters has been included in the analytical campaign to assess the overall mass balance of the selected tracers.

MO031 Determination of Rodenticides in Fish Samples of the German River Systems
K. Voigt, P. Krömer, W. Löhr, D. Prossen, and S. Comber, Plymouth University / Environmental Science; C. Rowett, Plymouth University / Environmental Science; M. Heldberg, University of Oslo Chemistry; A. Ruus, NIVA / NIVA; L. Hanssen, L. Christoffersen, NILU Geosciences
Marking the starting point of new knowledge as the interest rate from stored samples.
but the contents are mainly low and vary greatly between the different types of amendments and the POPs concerned. Sewage sludge had the highest combined toxic potential (of PCD/F, PBDD/F and WHO-PCBs) at 20 ng WHO-TEQ kg⁻¹ amendment. The concentrations of the targeted POPs increased in most amended soils at the four field sites. However, the observed increase were inferior to the levels of POPs provided by the amendments. This may be explained by the degradation of the compounds, their interaction with soil organic matter which render them unrecoverable or their transport into deeper soil layers. However, the results from one of the field sites in Sweden differ from the other field sites in the study. It was the only site where the combined toxic concentration increased. The increase were 12 times, 36a and 1.4 ng WHO-TEQ kg⁻¹ soil compared to 3.1±0.5 ng WHO-TEQ kg⁻¹ soil in the control soil. All the targeted compounds, PCD/F, PBDD/F, PCB and PBDE have increased in concentration with 14, 2, 85 and 68 times since the establishment in 1956 compared to the concentrations in the control soil today.

**MO030**

**Determination of Rodenticides in Fish Samples of the German Environmental Specimen Bank**

M. Kottthof, Fraunhofer IME / Environmental and Food Analysis; H. Jürling, M. Bücking, Fraunhofer IME Institute for Molecular Nutrition and Applied Ecology

For the determination of eight rodenticides in fish muscle and liver an appropriate method was adapted. Initial analyses revealed that higher concentrations were detected in fish liver as compared to fish filet. Thus the method was further optimized for liver samples and validated. The final protocol applied high-resolution mass spectrometric detection and yielded a limit of quantification (LOQ) of 0.06 ng/g liver weight for most compounds (validated by repeated measurements). Applying the final method to liver samples from the German Environmental Specimen Bank (ESB), Bromadiolone, Flocou-mafen, Brodifacoum, and Difethialone were found at levels > LOQ. In a further step, very liver samples from the ESB archive sampled at 17 sites across Germany were analysed retrospectively. In this spatial comparison for the year 2011, the highest levels were found at the site Saar / Rehlingen: 0.8 µg/kg Bromadiolone, 4.6 µg/kg Brodifacoum, 4.0 µg/kg Difethialone. These three compounds were the only identified rodenticides. Bromadiolone was the most frequently detected rodenticide at all test sites (found at 10 of 17 sites). The other rodenticides occurred rarer (Bromadiolone at 3, Difethialone at 7 locations). Difethialone reached a concentration of 4.0 µg/kg in liver from the site Saar / Rehlingen. Based on the results of the rodenticide spatial screening, samples from Saar / Rehlingen and Elbe / Prosse were chosen for a temporal comparison. From both sites, ten liver samples from the ESB archive were retrospectively analysed (years 1992 to 2013). Examining the results, no clear trend can be observed, but year-to-year changes in rodenticide loads. Again, Brodifacoum was the most frequently detected rodenticide (detected in almost every investigated year at Elbe / Prosse), and the most abundant one (levels of up to 4.6 µg/kg at the site Saar / Rehlingen 2011). Bromadiolone was found only in some years (up to 1.8 µg/kg, Elbe / Prosse 2003). Difethialone was detected only in some years (up to 4.0 µg/kg liver sample, Saar / Rehlingen 2011).

**MO031**

**The impact of natural and anthropogenic Dissolved Organic Carbon and pH on the toxicity of triclosan to Gammarus pulex**

S. Comber, Plymouth University / Environmental Science; C. Rowett, Plymouth University; T. Hutchinson, School of Biological Sciences, Plymouth University / School of Biological Sciences

Routine ecotoxicology testing rarely accounts for the effect of natural water chemistry on chemicals bioavailability. Therefore, this study identifies whether key omissions in relation to Dissolved Organic Carbon (DOC) and pH have an impact on calculated effect concentration (EC) values. Laboratory ecotoxicology tests were undertaken for the widely used antimicrobial compound triclosan, using wild Gammarus pulex. These tests were undertaken in synthetic fresh water, humic acid solutions and wastewater treatment works effluent at mean pH values of 7.3 and 8.4. G. pulex immobilisation and triclosan concentrations were measured and used to calculate 24 and 48 hour EC values. The results showed that toxicity tests undertaken at a pH above triclosan’s pKa and in the presence of humic acid and effluent, containing 11 and 16 mg/l mean DOC concentrations respectively, resulted in decreased triclosan toxicity. This was most likely a result of natural water chemistry complexing the chemical, giving it a higher hydrophobicity controlling its bioavailability. The mean 48 hour EC50 values calculated under different conditions varied between 0.75 and 1.93 mg/l. These results demonstrated that both pH and DOC can have a statistically significant effect on triclosan’s toxicity. This suggests that standard ecotoxicology tests can cause inaccurate estimations of triclosan’s bioavailability and subsequent toxicity in freshwater ecosystems. This calls for the implementation of concurrent testing in both conditions, causing overly stringent Environmental Quality Standards. These results highlight the need for further consideration regarding the role that water chemistry has on the toxicity of organic contaminants and how ambient environmental conditions are incorporated into the standard setting process in the future.

**MO032**

**Sun light enhances toxicity of methyl paraben and 1,2-hexanediol to Daphnia magna and Danio rerio embryo**

j. Lee, Yongin University; K. Lee, Seoul National University / Graduate School of Public Health; K. Ji, Yongin University

Methyl paraben (MP) has been widely used as an antimicrobial preservative in cosmetics, pharmaceutics, and food products, and 1,2-hexanediol (1,2-H) has been applied for cosmetics as an alternatives of parabens. However, very limited information has been reported on the phototoxicity of these chemicals among aquatic organisms. In the present study, acute toxicities of MP and 1,2-H were evaluated using Daphnia magna (48 h exposure) and Danio rerio (96 h exposure) embryos. We also investigated whether phototoxicity of MP and 1,2-H would be affected by environmental level of ultraviolet light, i.e., exposure to 4 h/4 sun light. Changes in expression of genes related to oxidative stress were determined in D. magna juveniles after being exposed to sublethal levels of the chemicals and environmental level of ultraviolet lights. Greater toxicities were observed in both MP and 1,2-H at lower UV intensity. In D. rerio embryos, exposure to MP resulted in lesser rates of hatching and increased malformation rates. Expressions of hemoglobin and a-esterase genes in D. magna were increased by co-exposure to MP and to ultraviolet lights. Our experiments demonstrated that exposure to MP led to oxidative damage in aquatic organisms and it was aggravated by natural ultraviolet lights. Further assessment for sublethal effects and specific mechanisms of MP under environmentally relevant conditions should be followed. Keyword: Phototoxicity, methyl paraben, 1,2-hexanediol, ultraviolet light, oxidative stress

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**MO033**

**Evaluation of decamethylcyclopentasiloxane (D5) accumulation behavior in Herring gull eggs from the Oslofjord urban environment**

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Since its initial detection within the Oslofjord in 2005, decamethylcyclopentasiloxane (D5) has been the focus of monitoring and regulatory activities over the past several years. Through the combination of high production/use and hydrophobicity/lipophilicity, accumulation of D5 in aquatic environments (e.g., sediment, biota) have not only been observed in the Oslofjord, but many other aquatic ecosystems impacted by human activities and waste. Aquatic species are considered to be at greatest risk to CVMs exposure due to exposure through sediment compartments and lower elimination capabilities. Monitoring and research focus has mostly been placed on aquatic environments as cVMS accumulation potential in air respiring organisms is considered low due to high elimination through metabolic degradation and respiration processes. However, recent findings under the “Environmental Contaminants in an Urban Fjord” project of the Norwegian Environmental Protection Agency showed elevated levels of cVMS within Herring gull eggs collected from the Oslofjord region. Concentrations of D5 detected in Herring gull eggs collected in 2013 (51 ± 71.8 ng/g wet weight (ww)) and 2014 (61.4 ± 51.7 ng/g ww) were comparable with maximum concentrations reaching 265 ng/g ww. Similar levels were observed for PCB 153 in 2013 (130 ± 96.2) and 2014 (75.8 ± 75.1 ng/g ww), indicating substantial uptake of D5 from the surrounding environment. High variances in 13C and 15N stable isotope signatures were observed among mother birds. In addition, both 13C and 15N stable isotope signatures were not reflective of the local aquatic food web, indicating feeding of the mother birds has occurred elsewhere. Low 13C and 15N stable isotope signatures in Herring gull eggs may indicate greater feeding from terrestrial based carbon sources for energy. As opportunistic feeders, Herring gulls are known to scavenge on human waste, which are highly contaminated with volatile siloxanes and may represent an important source of D5 exposure to seabirds. Although air breathing organisms are considered to be of low risk for the bioaccumulation of D5, results presented here indicate D5 accumulation occurs within herring gull eggs. This is may indicate a potential risk to un-hatched birds and should be further monitored.

**MO034**

**Emerging pollutants in reclaimed water. Is there some concern about crop irrigation?**

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Reclaimed water is a strategic effective way to help solve the problem of water scarcity and droughts in the EU. In 2009 in Spain, the current level of water re-use was estimated to be about 500 Mm³ (Spanish Ministry of the Environment). It is projected that wastewater treatment in Europe will be 3.201 Mm³/year in 2020, with Spain in first position (over 1.200 Mm³/year). Agricultural irrigation is the main
samples from Environmental Monitoring Programs

J.A. Durham, Dow Corning Corp. / Health and Environmental Sciences; N.A. Warner, NILU - Norwegian Institute for Air Research / Environmental Chemistry; S. Knoer, R. Seston, D. Powell, Dow Corning Corp

When embarking on an environmental monitoring study, it is critical that the proper quality control (QC) parameters are evaluated to ensure integrity of the results obtained from monitoring activities. This is especially critical when analytes under investigation can be present in personal care products, equipment and analytical instrumentation is as the case for cyclic siloxanes. Selecting an appropriate QC matrix is imperative to accurately account for loss or contamination during collection, transport, and laboratory processing and analysis. In addition, it is important to evaluate analytical variation introduced by the sample matrix itself to the overall signal measured to avoid reporting false positives. It is therefore important that QC matrices be determined prior to the initial design of the monitoring program. Key aspects when designing a QC program include applying an appropriate QC matrix to the overall signal measured to the sample matrix being collected; determining background contribution from sample collection and laboratory equipment; evaluating initial concentrations present within QC matrices prior to application and how they will evaluate analyte loss or contamination. This presentation will provide an overview of quality control evaluations from several different programs and the best practice for creating a proper QC program. The Loss and contamination of the analyte in the field has been assessed using a variety of materials including fish muscle/liver, sediments and sorbents such as low density polyethylene and XAD® resin. Additionally, processing and analysis QC are assessed to evaluate contamination from equipment such as collection and analysis jars, use of nitrogen as well as analysis equipment.

MO038 Determination of toxic effects of environmental contaminants on gap junctional intercellular communication in T3M Leydig cell line

A. Yarar, RECETOX, Masaryk University / Biology of Toxicology; P. Macíková, Masaryk University Faculty of Science RECETOX, P. Babica, Masaryk University, RECETOX / RECETOX, I. Sovadínová, Masaryk University, Awar A., Macíková P., Babica P. Sovadínová I. RECETOX, Masaryk University, Kamence 753/5, 625 00, Brno, Czech Republic

Environmental contaminants are substances that, when accidentally or deliberately introduced into the environment, might induce adverse ecological or health effects. Male reproductive system is uniquely vulnerable to such toxic chemicals in the environment. It has been hypothesized that exposures to environmental toxicants is responsible for increasing incidence of congenital malformations of the reproductive organs and testicular cancer, deteriorating semen quality, and decreasing sperm counts, which has been reported in the past decades. Disruptions of hormonal regulation by so-called endocrine disrupting chemicals (EDCs) have been suggested as one of the principal mechanism underlying adverse effects of toxicants on male reproductive system. However, homeostasis balance, morphogenesis, cell differentiation and growth in testicular tissues are physiological processes strictly regulated by gap junctional intercellular communication (GJIC), a process known to be altered by various environmental contaminants, including well-recognized EDCs. We are currently investigating (i) the effects of selected EDCs as well as personal care products (PCPs) on GJIC inhibition in mouse somatic testicular cells, T3M Leydig cell line, and (ii) which mechanisms are involved in dysregulation of GJIC. The results showed that organochlorine pesticides (methoxychlor, lindane and DDT), and PCPs (triclosan and triclocarban) induced rapid (30 min) dose-dependent inhibition of GJIC in T3M. The effects became more pronounced after 6 or 24 h exposure, where significant reduction of GJIC was induced also by bisphenol A, vinclozolin and PCB153. As determined by Western blotting analyses, the inhibition of GJIC was accompanied by activation of mitogen activated protein kinase ERK1/2, which is the mechanism implicated in GJIC dysregulation induced by known tumor promoters, e.g. 12-O-tetradecanoylphorbol-13-acetate (TPA). Inhibition of GJIC and activation of MAPKs were observed at the concentrations of chemicals not associated with cytotoxic effects as evaluated by Neutral Red uptake assay and the reverse transcriptase-PCR of CFDA-AM assays. Our results indicate that EDCs and PCPs might disrupt testicular homeostasis and functions via alterations of GJIC and MAPKs.

Acknowledgment: This research is supported by Czech Science Foundation Project No.16-10773Y.

MO039 Performing a large scale xenobiotic removal efficiencies of activated sludge treatment plants with a cost-efficient sampling scheme in Luxembourg

T. Gallé, Luxembourg Institute of Science and Technology (LIST) / ERIN; C. Kochler, M. Plattee, M. Bayerle, D. Pittois, Luxembourg Institute of Science and Technology (LIST)

Xenobiotic removal by activated sludge treatment plants can be very variable depending on the design and the operational parameters of the sites. Accurate full scale balances are cumbersome to realize and labolatory sludge experiments have not been related to overall plant performance with enough cases for statistical relevance. Therefore the laboratory has been launched in Luxembourg to evaluate the performance of activated sludge treatment plants with an array of
techniques. Full scale plant balances will be performed with passive samplers covering an entire week of inflow vs. outflow. This approach circumvents the problem of mixing regimes and matching volumes in classical autosampler campaigns through the extended time-period of observation. Although for reasons of different suspended matter and DOC loads at the in- and outlet the sampling rates of the passive sampler are different, this can be normalized by constant or detergents like f.e. carabamazine or lidocaine. The ease of operation allows a multitude of plants to be monitored in parallel with much less workload than autosampler campaigns. The contribution will shed a critical view on the clogging of membranes of passive samplers and the representativeness of sampling in both in- and outlet with this approach. In addition the campaign features laboratory analyses including respirometric tests of the activated sludge, enzyme assays and degradation kinetics of selected substances. The goal is to compare the performance of a large amount of WWTPs (24 sites) and relate the elimination efficiencies with plant design and operational settings. Prioritisation of advanced treatment processes for operation or treatment improvements can be explored as part of this project. This contribution presents the results of a first part of the campaign.

MO040 Development of a counter-current chromatography-based extraction method for emerging contaminants
B. Huerta Bluinrago, Catalan Institute for Water Research (ICRA) / Institute of Environment Health and Societies; A. Pathmanathan, University Pierre et Marie Curie; M.D. Scrimshaw, Brunel University London / Institute for the Environment; S. Ignatova, Brunel University London
Complex mixtures of emerging pollutants which include pesticides, biocides, personal care products and pharmaceuticals (PPCPs) are becoming a major issue in the environment. They may pose a risk to aquatic ecosystems and human health. The vast and ever-increasing number of chemicals, often present at very low (sub ng/L) concentrations, complicates their monitoring and subsequent regulation. In this context, we propose the application of high performance counter-current chromatography (CCCC) as a novel tool for the extraction of a broad variety of pollutants from river waters. CCC is a chromatographic separation technique that has become a novel, worldwide separation and purification technique and is based on the partition of compounds between two immiscible liquid phases as they interact in a thin tube under a fluctuating centrifugal force field. The research explores the use of CCC, not as a separation technique, but as an extraction method for large volumes of water. The extraction is based on partitioning between the two immiscible liquid phases: the mobile phase and the stationary phase. The mobile phase is the water sample potentially containing the pollutants, while the stationary phase is an appropriate organic solvent(s), retained in the column by centrifugal rotation. The advantages of this technique range from little to no sample preparation, and highly versatile solvent systems, to high recovery efficiencies and potentially unlimited extraction capacity. We have applied this powerful tool for the extraction from water samples of a number of compounds, including pesticides, pharmaceuticals, and corrosion inhibitors, covering a wide variety of polarities (log K_w, between 1 and 5). Preliminary results demonstrating a range of recovery efficiencies for the majority of these compounds and method development will be presented and discussed.

MO041 The consortia of microorganisms for use in bioremediation
A. Wysoczka, M. Popowska, University of Warsaw, Faculty of Biology / Institute of Microbiology Department of Applied Microbiology
The continuous development of the industry results in the appearance of chemical compounds in the environment, which do not occur in nature. The different types of them and their metabolites are highly toxic and constitute a threat to humans – some of them are potent poisons, often exhibiting strong mutagenic and carcinogenic properties. Most of the aromatic compounds are characterised by stability and durability in biological systems, and what is more, considerable resistance to degradation. An additional concern is that the contamination of the soil by xenobiotics, is also a direct threat to groundwater, and consequently may reduce their potential as a source of drinking water. The problem of soil and groundwater contamination by xenobiotics from various branches of industry, concerns not only Poland and other European Union countries, but practically all industrialized world. The main objectives of our study were characterisation of microorganisations (bacteria and fungi) isolated from several environments polluted with petroleum compounds, heavy metals, pesticides for wood and the other xenobiotics. All soil samples were analyzed for the presence of pollutants: heavy metals, PAHs, BTEX, gasoline and mineral oils. The bacterial strains and fungi were identified by PCR amplification and characterised for their ability to degrade certain hazardous substances, for instance mazout, other petroleum products and polychlorinated compounds. The determination of degradation rate of each pollutant may help to choose those strains, which play the critical role in this process. Selected ones will be part of consortium, which in future might be commercially available as bioivacure. Currently, preliminary studies on this subject has been launched in laboratory systems - microcosms. This approach enable us to test different variants of our study and define optimal relative amount of each culture, as well as abiotic conditions. This method in easy and eco-friendly way of removing hazardous pollutants from the environment without introducing any synthetic products into it. The in situ bioremediation is based on natural processes occurring in the environment, which is associated with considerably lower costs than conventional physicochemical methods. The developed and deposited composition of strain (bioivacure) provides a possibility to quickly obtain the appropriate amount of the formulation and conduction of bioremediation in a short time.

MO042 Ecotoxicological endpoints to assess the impact of nanomaterials on soil and water biota
S. Sanchez-Fortun, UCM; G. Costa, Universidad Complutense de Madrid; G. Mengs, Natural Biotec; C. Fajardó; M. Nande, UCM; M. Martin, Universidad Complutense Madrid
Heavy metals are ubiquitous pollutants worldwide. The accumulation of metals in the environment from anthropogenic sources creates the potential for significant human health problems and soil contamination. For example, the use of metal-based health effects have been reported in all body systems, with exposure stemming from multiple sources, including water, air, or soil. Nanotechnology is a rapidly growing area of study with applications in numerous fields because engineered nanoparticles (NPs) unique physical and chemical properties allow them to have higher reactivity than larger particles with the same chemical composition. For the last decade, nanosized zero-valent iron (nZVI) has been used for the remediation of polluted soil, groundwater and wastewater, targeting a wide variety of common environmental contaminants, such as chlorinated organic solvents, organochlorine pesticides, polychlorinated biphenyls, inorganic anions and heavy metals. To this respect, the ecotoxicological assessment of the potential effects of the interaction nZVI-heavy metal on different organisms is an important approach to be considered. Thus, the choice of optimal species as well as sensitive ecotoxicological endpoints may allow the use of shorter testing intervals in the analysis of the interaction NP-heavy metal effects on water and terrestrial organisms. In the present study, we analyze the effects of the interaction NPs (2.5 and 5 mg/L) with novel tool concentrations of Cd, Pb and Zn (heavy metals with persistent presence in soil and water) on several species including the green alga Scenedesmus intermedium and the cianobacteria Microcystis aeruginosa, as representative aquatic biota from fresh ecosystems, and the nematode Caenorhabditis elegans as representative terrestrial organism from soil ecosystems. The results in both aquatic and terrestrial organisms showed that selected toxicity endpoints (growth, reproduction, survival and photosynthetic activity) were significantly affected by the heavy metal concentration used. However, these effects were reverted by the presence of nZVI in all cases. Together, these results suggest that: (1) the organisms as well as the endpoints considered have shown to be suitable to analyze the ecotoxicological effects of metal and nZVI interactions, and (2) nZVI have shown to be an excellent method to heavy metal immobilization thus rendering their deleterious effects and making nZVI as an excellent tool in the remediation of contaminated ecosystems.

MO043 THE UKWIR Chemicals Investigation programme Phase 2, 2015-2020
M.J. Gardner, V. Jones, Atkins Ltd; D. Leverett, WCA-Environment Ltd
The UKWIR Chemicals Investigation Programme is the response of the UK water industry to current and emerging legislation on trace substances in surface waters. The first phase of the Programme (CIP - 2010-2013) was aimed at the prioritisation of complementary monitoring by different treatment options for emerging contaminants. This programme did provide a generic overview of trace contaminant concentrations in wastewater treatment works effluents and the effectiveness of current treatment processes in reducing contaminant concentrations. The second phase of the CIP is now under way. This £140M programme of monitoring and investigations is focussed on: site-specific compliance status at over 600 wastewater treatment works; the demonstration of where there is a clearly justifiable need for action/investment; and, the potential improvements in effluent quality that might be offered by various novel treatment processes. Participants and contributors include all water companies in England, Wales and Scotland, Defra, Environment Agencies, Ofwat, Atkins Limited, wca-environment and Plymouth and Cranfield Universities. This poster summarises the scope and aims of this ambitious programme and presents some initial results for Water Framework Directive Priority Substances and Specific Pollutants. Results indicate that compliance status might be at risk at the majority of sites for a limited number of specific substances. This implications of this will be discussed.

MO044 Development of a LC/MS/MS method for the comprehensive assessment of macrocyclic antibiotics in the aquatic environment
I. Soret, I. Krizman, S. Boskovic, Boskovic Institute / Division for Marine and Environmental Research
Macrocyclic antibiotics are among the most popular types of antimicrobials. As a consequence, a number of studies in the literature addressed the issue of exposure to these emerging contaminants in municipal wastewaters and ambient waters. Macrocyclic antibiotics are released from the human body after therapeutic application of these antibiotics, however, the assessment methods reported that macrocyclics can be transformed in the environment either via
microbial or photochemical degradation, yielding a number of transformation products. Nevertheless, most of the existing exposure data refer only to the parent compounds, while the information on possible macroide intermediates and/or transformation products in the aquatic environment are very scarce. One of reasons is the lack of analytical methods for the comprehensive assessment of macroide-derived compounds in aquatic systems. Therefore, the aim of this work is to develop a multiresidue method for the simultaneous determination of several prominent macroide antibiotics (azithromycin, clarithromycin, erythromycin and roxithromycin) along with their intermediates and transformation products using liquid chromatography tandem mass spectrometry (LC-MS/MS). The performance of the method was evaluated by liquid chromatographic separation of macroide compounds using a new type of reversed-phase stationary phase, which combines octadecyl and phenyl moieties, and optimization of enrichment of target compounds from aqueous phase using Oasis HLB and mixed-bed stationary phases. The developed method was employed for the analysis of macroide-derived residues in real wastewater and ambient water samples, collected in the area of the city of Zagreb. It was shown that intermediates and/or transformation products significantly contribute to the overall mass balance of macroide-derived compounds. This indicates that the risk associated with the use of macroide compounds in the aquatic environment should include assessment of possible effects caused by their transformation products and intermediates from their production.

MO045  Comparison of pharmaceutical discharges and treatment from hospital and domestic sources

L. Test, Institut des Sciences Analytiques / TRACES; A. Bergé, R. Baudot, Institut des Sciences Analytiques / TRACES Team; M. Séret, É. Vulliet, Institut des Sciences Analytiques / UMR Équipe Traces rue de la Doua Villeurbanne France

Chemical and water pollution is more and more studied and documented. Among the most studied pollutants, drugs are special contaminants, due their very low concentrations in the environment and the potential presence of metabolites. Even though effluents from sewage treatment plants are well known to be one of the major sources for introduction of pharmaceuticals in the aquatic system, contributions of significant sources such as hospitals or agriculture are still unclear. Furthermore, despite their high solubility in water, their partition can be significant in the suspended solid phase, although this fraction is not always analysed. In this context, this work consists in a long-term study of hospital and urban effluents and their treatment. Target substances include analgesic, anti-inflammatory, antihypertensive, hormonal contraceptive, antibiotic, anti-fungal, anti-convulsant products and metabolites. Thanks to the use of advanced analytical techniques, limits of quantification consistent with ultra-traces detection were achieved. All the pharmaceuticals analysed were detected. Two molecules, econazole and ethinylestradiol were only detected in the suspended solid phase, which shows the importance of analysing this fraction. Three pharmaceuticals were present at significantly higher concentration in the hospital influent: ketoprofen and two antibiotics: ciprofloxacin and sulfamethoxazole...

MO046  Selection of the most environmentally benign ionic liquids option by coupling OECD standardized tests and fish leucocyte mortality

A. Bado-Nilles, INERIS; L. Chabot, INERIS / EXES; G. Marlier, INERIS; S. Jonquet, Université de Rennes 1 / Environnement Conception et Performance; J. Porcher, INERIS / INERIS; UMR SEBIO ECOT; P. Pandard, INERIS

To reduce health and environmental risks of chemicals and to minimize the environmental footprint of human activities, green chemistry searches for alternative, environment-friendly reaction media and at the same time strives to increase reaction rates and milder reaction conditions. A growing area of research is devoted to ionic liquids (ILs) often termed as the green solvent. However, the extreme tunability of sought functional properties. As the aftermath of earlier misleading messages reporting ILs generically as intrinsically "safe and risk free" whatever ILs considered, their increase in potential or emerging applications in key domains (like bio-refining, energy storage, nano-material processing, pollution remediation, etc...) is now more a matter of actual risk profiles of ILs, including both physico-chemical, health and environmental risks that might be triggered by inadequate selection of use of ionic liquids. In this context, we presented a advanced approach serving comprehensive evaluation of environmental hazard by combined use of OECD standardized tests (Daphnia magna acute immobilization test; algal growth inhibition test) and fish ex vivo leucocyte mortality analysis. This innovative approach integrating multicriteria assessment has been tested on a first set of commercial ionic liquids based ([BMIM][Cl], [EMIM][Cl], [EMIM][DCA], [EMIM][MeSO3], [EMIM][EtSO3], [EMIM][BF4], [HMIM][Cl]) and four phosphonium-based ionic liquids ([P6,6,6,14][Cl], [P6,6,6,14][DCA], [P6,6,6,14][i(c8)P202], [P4,4+,4+,4+][TOS]) and is expected to be further used to examine other ILs (bioactive, ILs, pyrrolidinium, ...) in soon future. Results of ecotoxicity tests obtained so far have revealed a clear potential to rank clearly the families of ILs according to the studied risk and even to identify influencing structural factors within a same family of ILs and compounds within the same family.

MO047  Emerging pollutants in reclaimed water. Is there some concern about crop irrigation?

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Reclaimed water is a strategic effective way to help solve the problem of water scarcity and droughts in the EU. In 2009 in Spain, the current level of water reuse was estimated to be about 500 Mm³/year (Spanish Ministry of the Environment). It is predicted that wastewater reuse in Europe will be 3,222 Mm³/year by 2025, with Spain in first position (over 1.200 Mm³/year). Agricultural irrigation is the main application in many EU countries and accounts for around 33% of total water use (EEA, 2012), although this figure comes close to 60% in certain regions of Spain. In this work, reclaimed water samples supplied by a Spanish WWTP were used to analyse the presence of 44 emerging pollutants (pharmaceuticals, parabens and endocrine disruptors). Different microcosms and semi-field studies were performed with three crops (lettuce, corn and radish) irrigated with spring water, reclaimed water and fortified (atenolol, carbamazepine and tricosan) reclaimed water (at 10 and 100 higher levels than the mean observed in reclaimed waters). The aim was to assess possible adverse effects of emerging pollutants on soil-plant systems caused by reclaimed water. The experimental results evidenced no variations in soil functionality, pigments content in leaves or accumulation of pollutants in both plant roots and shoots. However, using a specific tool to measure the genotoxic effects (in terms of % cytostasis and frequencies of micronuclei formation), an effect (albeit slight) was observed in lettuce, for the triclosan. Based on this observation, the risk of crop irrigation with reclaimed water is not expected to be high, but ongoing research is needed to assess the long-term effects of this kind of wastewater reuse on food safety.
consequently on the ion pair dissociation can also be proposed. Further investigation is in progress. To our knowledge, the present study showed for the first time that the toxic potency of imidazolium-based ILs, such as [onim][BF_4], greatly depend from the carrier organic solvents, such as acetone, thus providing significant information for their development, usage/application and safety.

MO049
Butylparaben and propylparaben: Acute effects on Ceriodaphnia silvestrii M. Spadaro, Sao Paulo University / Water Resources and Applied Ecology Center, A.E. Sueit, Federal University of Sao Carlos / Chemistry; E.M. Vieira, Sao Paulo University / Departamento de Quimica e Fisica Molecular. Emerging contaminants are compounds of natural or anthropogenic origin that can be found in different environmental compartments. They constitute potential risk to biota, affecting the growth and development of organisms exposed to them. Among the compounds classified as emerging contaminants, butylparaben and propylparaben preservatives are often used together in formulations of cosmetic and personal care products, although they may cause several deleterious impacts on biota. Hence, the goal of this study was to evaluate the acute toxic effects of butylparaben and propylparaben, using as test organism the species Ceriodaphnia silvestrii, which is a native Brazilian representative of zooplankton. Toxicity tests were carried out using the concentrations: 0.500; 7.50; 1.125; 1.700; 2.550; 3.800 μg L^-1 of butylparaben and 1.00; 1.50; 2.25; 3.37; 5.00 and 7.60 μg L^-1 of propylparaben. Acute bioassays lasted 48 hours and they were repeated five times. Results from all acute toxicity tests were subjected to probit analysis using the PriProbit Program V1.6.3 to determine EC_{50} value. The average for the EC_{50}48h/butylparaben was 1.410 ± 0.024 mg L^-1 and EC_{50}48h/propylparaben was 0.18 ± 0.11 mg L^-1. The results indicated that Ceriodaphnia silvestrii was sensitive to the tested emerging contaminants and the mixture of such compounds at realistic environmental concentrations. Based on this observation and considering that parabens can affect the functioning of several species at even lower EC_{50} values, it can be concluded that additional studies are required to assist the discussions on the implementation of more strict regulations regarding the discharge of these pollutants in the environment.

Flame Retardants: Alternatives, Environmental Fate and Toxicity (P)

MO050
New flame retardants: chemical identification and properties A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center Dept of Pharmaceutical Sciences; G. Malavarman, University of Antwerp / Toxicological Center Dept of Pharmaceutical Sciences; G. Poma, University of Antwerp, Toxicological Center / Toxicological Center; P. Kurt-Karakus, Bursa Technical University / Environmental Engineering Department. Flame retardants (FRs) are chemicals used in a wide range of commercial and household products in order to reduce their flammability. Due to recent legislation and restrictions in the use of brominated FRs, a large number of alternative FRs, mostly non-halogenated, have been introduced to the market. The main aim of this study was to investigate the chemical identity of four new flame retardants technical mixtures and to inform about their properties. For each FR mixture, 5 mg were separately dissolved in iso-octane or methanol, and after appropriate dilution, the solutions were analyzed. A combination of analytical techniques was used, such as gas chromatography-mass spectrometry (in electron impact ionization and electron-capture negative ionization modes) and liquid chromatography-mass spectrometry (with positive and negative electrospray ionization). CETAFLAM-DB168 (Avocet) is a phosphorus-based flame retardant containing an organic phosphate (tri-n-butyl phosphate - TNBP) used for textiles and fabrics in aircraft, automobiles, and hotels. It allows durable flame retardant properties to be transferred to polyester yarn and fabrics by simple exhaust techniques. CETAFLAM-DB9 (Avocet) is a phosphorus-based flame retardant containing a new inorganic phosphate and an organic phosphate (trichloropropyl phosphate - TCP) for polymer. It is used in the automobile, clothing, workwear, furniture, carpets, curtain materials and public transport textile materials. RUCO-FLAM PS-E (Rudolf Group) is an organic phosphorus flame retardant with high percentage phosphorus content used for polyester (PES) and possibly also polycrylate (PA) polymers. PEKOFLAM PES (Clariant) is an organophosphorous-based non-halogenated flame retardant for use on PES and PA textiles and in many (synthetic) polymer systems with ‘wash resistant’ effects. It consists of a mixture of cyclic di- and tri-phosphonates.

MO051
Analysis of organophosphorus flame retardants by GC-MS/MS with EI and APCI ionisation techniques W. Halloum, LUNAM Université / Oniris Laboratoire d’étude des Résidus et Contaminants dans les Aliments; R. Cariou, G. Dervilly-Pinel, Oniris-LABERCA / Oniris Laboratoire d’Etude des Résidus et Contaminants dans les Aliments; F. Jaber, Université Libanaise / Laboratoire d'analyse des composés organiques LACO; B. Le Bizec, Oniris-LABERCA / Oniris Laboratoire d’Etude des Résidus et Contaminants dans les Aliments. Flame retardants (FRs) are human-made chemicals added to consumer and industrial products for the purpose of reducing flammability. Among FRs are the halogenated FRs that may be sub-divided into brominated (BFRs) and chlorinated FRs as well as phosphorus-containing FRs (PFOS). Some halogenated FRs have proven to be persistent, bioaccumulative and toxic to environment, animals and humans. This led to their regulation. Among other things, in the frame of the current European Union regulations for some other groups, a new kind of brominated flame retardants (BFRs) and organophosphorus flame retardants (OPFRs) are proposed as alternatives for historical BFRs and their use is in continuous increase. Most OPFRs are introduced as additives and not chemically bound to the polymer; hence they are slowly released in the environment. Although there are already available or even in-use data were available on their toxicity, adverse effects were reported for some of these alternative compounds. The focus of this work was to develop and to optimize an analytical strategy enabling the identification for 18 OPFRs of which 13 are non-halogenated (TEP, TPB, TBP, TnBP, TBEP, TEHP, TPP, HEDPh, CTCP, mEEtBCP, dEEtBCP) and 5 are halogenated (TCEP, DTCP and TCPP for chlorinated; TDBP and TTBNP for brominated) in fish muscle. The investigated preparation procedure based on pressurised liquid extraction with the in-cell use of Florisil as a first cleanup step (~65% of lipids removal). Gel permeation chromatography was then used as a second cleanup step to maximize the elimination of lipids (~95% of lipids removal). Particular attention was paid to procedural contamination management. Two instrumental methods were developed and optimised in terms of spectrometric and chromatographic conditions by GC-MS/MS operating in positive Electron Impact (EI) or Atmospheric Pressure Chemical Ionization (APCI). The main advantage of ‘soft’ APCI over ‘hard’ EI technique was the reduced fragmentation which increases the detection for the OPFRs in the 2 brominated compounds. The perspective of this work will be to evaluate the method performances and hence the reliability of its application.

MO052
Organophosphate Flame Retardants in the Indoor Environment: A Comparison of Central Europe and North America M. Krätsch., Vojta, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; M. Venier, Indiana University / SPEA; J. Becanova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; K. Romunak, Indiana University; L. Melymuk, R. Prokes, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; J.O. Okeme, University of Toronto / Physical and Environmental Sciences; A. Saini, University of Toronto / Department of Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences; J. Klanova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment. Flame retardants (FRs) have been used for several decades to prevent fire and reduce the flammability of a wide range of materials. These properties are offset by their environmental persistence, toxicity and bioaccumulative potential. Worldwide restrictions on the use of old types brominated FRs have resulted in the increased use of alternate compounds, such as organophosphate flame retardants (OPFRs), produced in increasing volumes in recent years. Since in most applications OPFRs are used as additive chemicals and therefore are not conveniently bound to the polymeric materials, they can be released easily. Due to their presence in common household products, humans are exposed to the released OPFRs in the indoor environment, especially as people spend most of their time indoors. Air, dust and window film samples were collected from a total of 63 houses and apartments in the Czech Republic, Canada and USA during a sampling period of 28 days in May-August 2013. Samples from one room, usually the bedroom, were collected in each home while a second room, usually the living room, was sampled in 10 randomly chosen homes in each country. In addition to the sampling, data about the building and the household were also collected, i.e. information on electronic equipment and furniture in the sampled rooms, occupants, cleaning, ventilation habits, etc. The objectives of the present study were to examine the concentration of 18 OPFRs found in different matrices in the same room and compare individual rooms in the same home, identify possible sources of these compounds in indoor environments and study regional differences in Central Europe and North America. The OPFRs measured in the highest concentrations were tris(2-butoxyethyl)phosphate (TBOEP), tri-n-butylphosphate (TNBP), tris tributylphosphate (TBP), tri(2-chloro-2-propyl)phosphate (TCP), tri(2-ethylhexyl) diphenyl phosphate (EHDPh) in all countries, however the dominant congeners in each matrix differed slightly between North American and Czech samples. For example, 66% of the total OPFR mass in American samples was TCIPP and 17% was TNPB, while in Czech air samples 53% was TCIPP but the second highest concentration OPFR was TBP, that was 20% of total mass. These data suggest there are general similarities in the use and composition of OPFRs between Europe and North America, but differences in individual OPFRs which may be indicative of differences in use between the two continents.

MO053
Halogenated Flame Retardants in Spanish air: a human exposure study C. Corellas, IDAEA / Department of Environmental Chemistry; A. Alastuey, M.

SETAC Europe 26th Annual Meeting Abstract Book 141
years of ship-based observations

R. Suehring, University of Toronto; M.L. Diamond, University of Toronto / Department of Earth Sciences; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering; M. Pucko, University of Minnesota; G. Stern, Fisheries and Oceans Canada; L.M. Jantunen, Environment Canada / Air Quality Processes Research Section / Centre for Atmospheric Research Experiments; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering

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MO054

Distribution of organophosphate ester flame retardants between gas and particle phases - model predictions vs. measured data

R. Suehring, University of Toronto; H. Wolschik, Helmholz Centre Geesthacht; M.L. Diamond, University of Toronto / Department of Earth Sciences; L.M. Jantunen, Environment Canada / Air Quality Processes Research Section / Centre for Atmospheric Research Experiments; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering

The use of organophosphate esters (OPEs) has increased due to their use as replacements for the restricted polybrominated diphenyl ethers (PBDEs). That OPEs are a suitable replacement for PBDEs is being debated, especially since recent studies suggest that a number of OPEs have a persistence similar to PBDEs and can be found in remote environments such as the Arctic. The gas-particle partitioning is a key factors to predict where and how chemicals are distributed. The majori...
analyse adsortion as well as sample and extract stability. The developed method was applied to investigate the occurrence and distribution of these flame retardants both in the liquid and solid waste streams of a typical secondary wastewater treatment system employing activated sludge treatment. The method’s performance in this study demonstrated its applicability for monitoring these emerging contaminants in the environment.

MO058 Indoor Sources of Tris(chloropropyl) phosphate (TCP) and Concentrations Outdoors in Tributaries, Rain, and Wastewater Treatment Plant Effluent I. Truong, University of Toronto / Chemical Engineering and Applied Chemistry; L.M. Jantunen, Environment Canada / Air Quality Processes Research Section; P.A. Helm, Ontario Ministry of the Environment & Climate Change / Environmental Monitoring and Reporting Branch; G.M. Webster, Simon Fraser University / Faculty of Health Sciences; P. Kurt-Karakus, Bursa Technical University / Environmental Engineering; M.L. Diamond, University of Toronto / Department of Earth Sciences
Organophosphate esters (OPE’s) are commonly used as plasticizers, lubricants and as flame retardants. OPEs are a concern because of recent reports of high concentrations indoors, in surface waters, herring gull eggs and fish, and their potential toxicity to aquatic biota and humans. Since OPEs are typically employed as additives that are not chemicallybonded, they are released from products into the environment via volatilization, dissolution and abatement. Here we first investigated one indoor source of an OPE and second, documented tris(chloropropyl) phosphate (TCP) concentrations in multiple outdoor media. The indoor source of TCP to indoor air and dust was linked to spray polyurethane foam (SPF). SPF is used in a heavily insulated home using the isomeric fingerprint signature of TCP. High levels of TCP were measured in the highly insulated house in dust (85 ± 47 µg/g) and air (23 ± 8.4 ng/m3). The isomeric fingerprint of TCP 1:2 (first and second eluting isomers) were statistically indistinguishable from the SPF insulation used (4.0 ± 0.2, vs 4.1 ± 0.1), whereas the ratio of TCP 3:1 differed significantly from other environmental samples (ANOVA, p< 0.05). This evidence suggested that the SPF was the source of the TCP to the highly insulated home. In comparison, the ratio of TCP 1:2 was 3.2 ± 0.9 in dust from homes in Vancouver Canada and 3.9 ± 0.5 in Istanbul, Turkey. Second, we documented concentrations of TCP in three Toronto tributaries during high and low flow periods, in the final effluent from three wastewater treatment plants (WWTP), and in bulk rain water. TCP in WWTPs had the highest average concentrations, followed by tributaries during high flow periods, and tributaries during low flow periods (ANOVA, p< 0.05). TCP in WWTP final effluent was of 6.6 ± 11 µg/L (0.054 -18 µg/L). Tributaries differed from each other only during high versus low flow periods and not from each other, with TCP concentrations averaging 4.2 ± 3.9 µg/L (0.22-11 µg/L) and 0.86 ± 0.95 µg/L (0.11-6.8 µg/L), respectively. TCP concentrations in tributaries correlated with water discharge (m3/s), with a Pearson Correlation Coefficient of 0.65. No correlations were evident between TCP concentrations and suspended solids or turbidity in tributary water, unlike PBDEs. Tributary concentrations of TCP correlated with other OPEs analyzed in these water, notably TCEP (r = 0.89) and TCDPP (r=0.88).

MO059 Evaluation of sediment contamination by historical POPs and alternative flame retardants in the Gironde estuary M. Lauzert, LPTC-EPOC, University of Bordeaux / UMR EPOC LPTC; K. Le Menach, H. Budzinski, University of Bordeaux / UMR EPOC LPTC; P. Labadie, CNRS
Human activities exert different types of pressure on ecosystems, including the chemical pressure associated with the release of numerous synthetic organic compounds such as halogenated organics. A number of these chemicals may be persistent, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyl ethers (PBDEs) which are officially classified as persistent organic pollutants (POPs) and are subject to regulations and restrictions. To address these limitations, several alternative flame retardants (FRs) have been and are increasingly used. A comparison was performed on sediments from the Gironde estuary, between the levels of legacy POPs (PBDEs, PCBs and organochlorine pesticides (OCPs)) and those of emerging FRs: 11 brominated, 7 phosphorus and 6 chlorinated alternative flame retardants including bis-(2-ethylhexyl)-tetrabromophthalate (HEHTBP), 2-ethylhexyl 2,3,4,5-tetrabromobenzene (ETHBTP), pentabromotoluene (PBToT), tetrabromofluorenehexocyanophane (abTBEHCN), octabromotrimethylhexylphenyl (OBIND), pentabromobenzylbenzene (PBEB), 1,2-bis(2,4,6-trimethylphenoxo)ethane (BTBE), decabromobiphenyl (DBB), hexabromocyclododecane (HBCD), hexabromobenzene (HBB), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), tris(2-chlo-roethyl) phosphate (TCEP), tris(chloropropyl)phosphate (TCP), 1,3-Dichloro-2-propanol phosphate (TCPD), Triphenyl phosphate (TPH), Trisphenylphosphate (TDP), tribromophenol (TBPH), diphenyl chloride (DPC), Tri-n-butylphosphate (TBP), Dechlorane Plus (anti, syn), dechlorinated DP products (Ac200, Ac100, DBDPE, 604, and 606) and 1,3,5,7-tetramethylnonane (TMTN).
Sampling was conducted in September 2014, on 23 points in the subtidal zone and 13 points in the intertidal zone, from the confluence of the Dordogne and the Garonne River to the river mouth at the Pointe du Verdon. This study provides a first mapping of the contamination of the Gironde estuary by legacy POPs and alternative FRs. Controlling factors of POPs/alternative FRs will also be discussed. Acknowledgements – This work come from a FOG0LBEG campaign carried out on the research vessel Le Côte de la Manche CNRS-INSU. This study has been carried out in the frame of the Investments for the future Programme, within the Cluster of Excellence COTE (ANR-10-LABX-45). Aquitaine region is acknowledged for PhD grant funding. CPER A2E (Aquitaine region and FEDER) is acknowledged for financial support for instrument acquisition.

MO060 Halogenated flame retardants in eggs of Spanish birds - Aznar-Alemany, Dept of Environmental Chemistry; M. Mallan, IAEDA-CSIC / Dept of Environmental Chemistry; G. Blanco, MNCC-CSIC / Dept of Evolutionary Ecology; E. Barón, IAEDA-CSIC / Department of Environmental Chemistry; D. Barceló, ICRA
Flame retardants (FRs) are compounds applied to materials to increase their fire resistance. Polybromodiphenyl ethers (PBDEs) are the most used FRs and can be found in plastics, furniture or electronic devices. They are classified according to their level of bromination. Since PBDEs are not bonded into plastics but blend they can leach out and are found in different environmental matrices. PBDEs are persistent and toxic, bioaccumulate and suffer long-range transport. They are considered persistent organic pollutants in the Stockholm Convention and their production has been banned in Europe and North America. Hence new brominated FRs, are used as substitutes; as well as chlorinated FRs, including halogenated novelties. The present study describes the occurrence of halogenated FRs in unhatched eggs of 7 bird species from central Spain as well as liver tissue from disseased individuals. Samples include 30 eggs and 11 livers from years 2009-2015. The analytical method monitored 8 PBDEs, 3 emerging brominated FRs (DBDPE, HBB and PBDE) and 5 dechloranes (syn- and anti-Dechlorane Plus and 5 DBCD) using a dual instrument platform (LC-MS/MS and GC-MS/MS). The use of organophosphate esters (OPEs) has increased due to their use as replacements for persistent organic pollutants by inhalation and dust consumption. The exposure was correlated with other OPEs analyzed in these water, notably TCEP (r= 0.89) and PBDEs were detected in all samples, with total concentrations below 319 ng/g lw, except for one sampling spot where they reached 4,292 ng/g lw. BDE-47, -99 and -100, which correspond to the PentaBDE commercial mixture, were the main contributors to the profiles. Dechlorane levels were in the same order of magnitude as PBDE levels in all the spots. Even without taking the most polluted spot into account, the rest of the results are still higher than those found in Dofia Natural Park (south of Spain), maybe because the latter is better protected [1]. Acknowledgments – This work has been funded by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2009-SGR-965). [1] E. Barón, M. Málhez, A. C. Andreu, F. Sergio, F. Hiraldo, E. Eljarrat, D. Barceló. 2014. First report of pyrethroid accumulation in river fish: A case study in Iberian river basins (Spain). Environ Int 68: 118-126.

MO061 Occurrence of polybrominated diphenyl ethers and emerging halogenated flame retardants in food items G. Huma, University of Antwerp, Toxicological Center / Toxicological Center; N. Symons, G. Malarvanan, University of Antwerp Toxicological Center; S. Goschinsky, Scientific Institute of Public Health Belgium; S. Voorspoels, Flemish Institute for Technological Research VITO NV; S.V. Malyheva, J. Van Loco, Scientific Institute of Public Health Belgium; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center Dept of Pharmaceutical Sciences. Brominated flame retardants (BFRs) are chemicals used in a wide range of commercial and household products in order to reduce their flammability. Because most BFRs are not chemically bonded to the products which they are added to, they can easily leach into the environment. People are mainly exposed to BFRs via diet and diet. In particular, the lack of data on the occurrence of BFRs in food prevents an accurate estimation of the human intake. The main aim of this project was to follow up the European Commission Recommendation 2014/118/EU on the monitoring of BFRs in various food items on the Belgian market and to provide data on their occurrence and levels in the main food categories. The presence of polybrominated diphenyl ethers (PBDEs) and novel BFRs in e.g. hexabromobenzene (HBB), bis[tribromophenoxy]ethane (BTBPE), tetrabromobenzene (TBB), tetrabromophthalate (TBPH), trimboxanoisole (TBA), and dechlorane plus (syn-DP and anti-DP) in various food items was assessed using an analytical method developed and validated in this project. This novel method uses low sample amounts and solvent volumes, and involves a two-step clean-up, including florisil and acid silica. The determination of BFRs was performed using gas chromatography-mass spectrometry operated in electron-capture negative ionization mode (GC-ECNI/MS). The validated procedure was first used for the determination of BFRs in 20 individual food items purchased from Antwerp supermarkets (including fish, meat, chicken eggs, dairy products, fruits, vegetables and sauces). BFRs were found in the analysis of more than 200 food samples in the frame of the Belgian food
MO062 Brominated flame retardants in Belgian foodstuffs - recent evaluation by a novel UPLC-MS/MS method

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The study was undertaken as consequence of the Commission Recommendation 2014/118/EU on the Europe-wide monitoring of brominated flame retardants (BFRs) in food. BFRs are anthropogenic chemicals that are added to a wide variety of consumer products in order to improve their fire resistance. BFRs may leach from the products into the environment. Due to their persistence and potential to bioaccumulate in the food chain, BFRs may cause toxic effects in humans and animals. There is a lack of information on the occurrence of BFRs in foodstuffs, which has hampered accurate completion of intake assessments. The main objective of this work was to evaluate the presence and to measure the levels of BFRs, namely brominated phenols (BPs), hexabromocyclododecanes (HBCDs), tetrabromobisphenol A (TBBPA) and its derivatives in foodstuffs consumed by the Belgian population. Quantitative measurements were performed using ultra-high performance – tandem mass spectrometry (UPLC-MS/MS) on an ACQUITY UPLC system coupled to a Xevo-TQ-S mass spectrometer. Sample preparation protocol consisted of a QuEChERS-based extraction followed by two parallel clean-up procedures. Column chromatography with acidified silica gel and dispersive solid-phase extraction with C18 and carbon sorbents were used to eliminate lipids, pigments and eventually other matrix components from the extract. The method is applicable to a wide variety of food matrices and was in-house validated. Representative portions of food samples belonging to different categories, such as fish and seafood, meat and meat products, chicken eggs, oils and fats, milk and dairy products, were collected from Belgian supermarkets in 2015. The samples were pooled per food category according to the consumption data of the Belgian Food Consumption Survey, after which they were lyophilized and subjected to the multi-analyte LC-MS/MS analysis. In this work, data on the occurrence and levels of BFRs in different food commodities consumed in Belgium will be presented. The results will be discussed with regards to the frequency of occurrence and highest detected concentrations of the BFRs, and compared to existing data for other (European) countries. This data will be decisive in the subsequent calculation of intake levels. Acknowledgments: Financial support from Federal Public Service (FPS) Health, Food Chain Safety and Environment

MO063 Gas chromatography/atmospheric pressure chemical ionisation/tandem mass spectrometry to perform non-polar brominated flame retardants monitoring in food items at ppt levels

E. Bichong, I. Guiffard, P. Marchand, LUNAM Université / Oniris Laboratoire d’Etude des Résidus et Contaminants dans les Aliments; B. Le Bizec, Oniris-LABERICA / Oniris Laboratoire d’Etude des Résidus et Contaminants dans les Aliments

Brominated Flame Retardants (BFRs) have been analyzed in food items at ppt levels and Environment Financial support from Federal Public Service (FPS) Health, Food Chain Safety and Environment. Among the BFRs, the DecaBDE was determined to have its primary natural origin from the marine environment. For the other compounds, the results showed a generally low contamination of all analyzed food samples, with values generally below the quantification limit (LOQ).

MO064 In vitro bioaccessibility of plasticisers present in indoor dust using simulated human lung fluid

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ACES Plasticizers are additives imparting durability, elasticity and flexibility in the manufacture of polymeric products such as PVC flooring, retailing and packaging materials. The lack of migration stability has resulted into their classification as major indoor contaminants. Despite their extensive everyday use, the process of assessing their human exposure and possible health effects arising from indoor air contamination only began the past decade with limited results so far. This study forms a part of the EU Marie Curie Initial Training Network “Advanced Tools for Exposure Assessment and Biomonitoring” (A-TEAM) aiming to develop and establish novel methods on human exposure biomonitoring of pseudo-PoPs through alternative matrices, such as saliva, urine, etc. As an example, the study group indoor dust originating from N=61 participants’ houses in Norway. In the present study, we investigate the in vitro bioaccessibility (i.e. uptake/absorption) of plasticisers present in indoor dust collected from vacuum cleaner bags with respect to potential routes of exposure including inhalation and respiration. Simulated artificial human body fluids are used regarding the relevant exposure route, i.e. lung fluids. Indoor dust is sieved at 63 μm using a methanol-washed metallic sieve. 200 mg of sieved dust samples are incubated with 20 mL of artificial lung fluid (ALF) at 37°C for the artificial lung fluid in order to set up a realistic exposure scenario. All media incubations are conducted continuously for 96h. After the incubation step has finished, the samples are centrifuged at 3000rpm for 10min and the supernatant is subjected for a liquid-liquid extraction (LLE) using MTBE:Hexane 3:1. A selective and sensitive method to determine the analytes of interest is employed by using triple quadrupole gas chromatography mass spectrometry (GC-MS/MS) in multiple reaction monitoring (MRM) mode. The method validation was performed using Standard Reference Material for indoor house dust (i.e. SRM 2585) in replicate analysis and the results show that low molecular weight phthalates (e.g. DMP & DEP) are more than 85% bioaccessible through the simulated lung fluids, while the high molecular weight phthalates and alternative plasticisers (e.g. DEHP and DEHT) present bioaccessibility values less than 5%.

MO065 Are some "safer alternatives" of Flame Retardants hazardous as PBTs? Screening of FRs by the cumulative PBT Index in Qsarins

A. Sampson, P. Gramatica, S. Cassani, University of Insubria / DSTA Flame Retardants (FR) are chemicals widely used to enhance fire safety of industrial and commercial products. However chemicals like PBDE, largely used in the past, demonstrated to have unintended negative effects to human health and wildlife due to their properties of Persistence, Bioaccumulation and Toxicity (PBT). Nowadays such chemicals are replaced by New FRs (NRFs), as New Brominated FRs (NBFRs) and Free FRs (FRFs). However, Organophosphorus FRs (OPFRs) supposed to be "safer alternatives". Nevertheless, the information about the chemical properties of these substitutes are often not available and these substances were commercialized without complete information regarding their PBT properties, that are based on long-term behaviors and would require complex and expensive experiments. However, the PBT assessment is required in the context of REACH regulation and PBT chemicals require an authorization. In recent years many monitoring studies have identified these alternative FRs in various environmental compartments and in wildlife. In this study, several new compounds, proposed and used as "safer alternatives" to PBDEs, were analyzed using the recently developed within the framework of an EU H2020 project, a MLR QSR model that allows to identify PBT chemicals directly from their molecular structure, implemented in QSRINS (QSR INSubria) software. A rigorous check of the chemicals that are included in the model Applicability Domain (AD), and for this reason with the most reliable predictions, has been done. The results, obtained directly from the chemical structure for the three preselected NBFRs, were compared with those obtained from the ST-RECHA PBT Profiler and a good agreement (73%) between the two different approaches.
events were mainly performed on the molecular ion, giving a high specificity to using atmospheric pressure chemical ionization (GC/APCI/MS/MS), which allow deca-BDE in the second one. Since some PBDEs products have been replaced by in food items at ppt levels decisive in the subsequent calculation of intake levels. Belgium will be presented. The results will be discussed with regards to the occurrence and levels of BFRs in different food commodities consumed in and fats, milk and dairy products, were collected from Belgian (super)markets in and were performed of BFRs, namely brominated phenols (BPs), hexabromocyclododecanes and OFRs in temperate North America is used to derive emission rate estimates using the Signal. The quality of BFRs introduction in the mass spectrometer being the robustness of the GC/APCI/MS/MS method and its applicability for routine analysis at ppt level in food. A compromise was established on the basis of the optimal HETP and resolution calculation and gradient of temperature (always on 5MS stationary phase type). A compromise was implemented on the national control plan samples and methodology was proposed, highlighting that some compounds, proposed and as PBT Index, could be valid tools to evaluate appropriate and safer substitutes, as an integrating concept for chemical risk assessment. The Risk Assessment produced and used by society. The extensive exposure data gaps hinder the normal functioning of the endocrine system is crucial and this study demonstrates that the use of in silico and in vitro techniques can be used as a fast and reliable method of identifying compounds with unknown biological properties. MO066 Identification of species differences in the effects of androgonic compounds using in silico and in vitro methods S. Asnaghi, A. Pradhan, J. Kharrington, C. Modig, P. Iverson, P. Olsson, Orebro University / The Life Science Center Europe, Stockholm, Sweden (ECS) are compounds that cause reproductive and developmental disturbances. Brominated flame-retardants (BFRs) are one group of ECS that has been shown to affect the normal function of endocrine, behavioural, reproductive, metabolic and neurological systems. BFRs are used extensively in commercial and industrial appliances to meet fire safety regulations and reduce the flammability of materials. Because of their low cost and high efficiency the use of BFRs has increased rapidly. These, in turn have resulted in an increased release to the environment and these have been detected in aquatic, and mammalian species. In this study we included two groups of BFRs, TBECH diastereomers (α, β, γ, and δ) and derivatives of 2,4,6, tribromophenol (TBP), allyl 2,4,6-tribromophenyl ether (ATE), 2- bromomethyl 2,4, 6-tribromophenyl ether (ATRE) and 2- bromomethyl 2,4,6-tribromophenyl ether (DPTE) that were studied for their effects on androgen receptor (AR) functioning. To investigate whether the TBECH diastereomers and TBP derivatives bind to the androgen receptors of the chicken, zebrafish and human in silico molecular docking and dynamics procedure was performed by using the MOE and ICM modelling software. These showed that both sets of BFRs can bind to the ARs in the different species. To confirm the in silico results, in vitro activation assay studies by using chicken LMPH, zebrafish ZFL and human HepG2 cells were performed. This demonstrated that the TBECH diastereomers acted as agonists and the TBP derivatives acted as antagonists to the ARs. It also showed that TBECH diastereomers are stronger inducers of the human AR followed by the zebrafish and chicken ARs. This reveals that because of the sequence difference between the species the same compound can function differently. The TBP derivatives set acted as strong antagonists with comparable potencies between the species. In conclusion, the majority of chemicals that are being used in products have still not been tested and their effects on the endocrine system have therefore not been studied. The rapid identification of compounds that can disrupt the normal functioning of the endocrine system is crucial and this study demonstrates that the use of in silico and in vitro techniques can be used as a fast and reliable method of identifying compounds with unknown biological properties. MO067 Applying the RAIRD model for ecological risk assessment: A case study for 10 organic flame retardants J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical Environmental Science, A. Falls, ARC Arnot Research & Consulting; J.M. Arnot, University of Saskatchewan - Scarborough / Environmental Sciences; T. Gouin, Unilever / Safety and Environmental Assurance Centre; L. Toose, ARC Arnot Research and Consulting; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division Chemicals are being evaluated to determine if they pose unacceptable risks to humans or the environment. Measured (monitored) concentrations in environmental media are non-existent or limited for the vast majority of chemicals produced and used by society. The extensive exposure data gaps hinder the application of risk-based methods for chemical prioritization, screening and comprehensive assessments. The chemical activity approach has been proposed as an integrating concept for chemical risk assessment. The RAIRD Assessment Identification And Ranking (RAIRD) model includes multimedia mass balance fate calculations for organic chemicals in air, water, soil, sediment and representative aquatic and terrestrial species and food web bioaccumulation models comprising various trophic levels (e.g., plants, invertebrates, fish, birds and mammals). In order to test the model, we conducted a case study for measuring-level ecological risk assessment using measured and estimated exposure and toxicity data and the RAIRD model for 10 organic flame retardants (OFRs). The 10 OFRs include chlorinated, brominated and organophosphate flame retardants. The chemicals cover a diverse range of chemical properties for partitioning, reaction, persistence, bioaccumulation and toxicity. A database of 3,129 measured concentrations of 10 OFRs in temperate North America is used to derive emission rate estimates using inverse modelling and to evaluate the model calculations. Estimates of risk are quantified by comparing exposures and effects expressed in terms of chemical activity. Uncertainty in model input parameters (partitioning properties, degradation half-lives, emission rate estimates and toxicity) is used to estimate uncertainty in the RAIRD risk calculations. A comparative risk assessment is used to rank the OFRs for their relative risks to the environment based on current Microplastics in the environment: Sources, Fate and Effects (P) MO069 Comparison of different procedures for quantifying microplastic particles in freshwater samples I.P. Girardi, German Institute of Hydrology; C. Kochleus, S. Hatzy, C. Theisen, Federal Institute of Hydrology; G. Reiferscheid, Federal Institute of Hydrology / Department Biochemistry and Ecotoxicology; N. Brennholt, Federal Institute of Hydrology / Biochemistry and Ecotoxicology (Micro)Plastics litter is one of the main environmental issues of the 21st century. While marine litter has been recognized by policies (e.g. through MSFD) and society as an important problem requiring the need for action, the knowledge regarding its origins is still limited. There is for example no information on the amount of litter being transported through rivers into marine habitats. Research institutes as well as authorities have recently started to quantify riverine (micro)plastics litter, but this is being done with a range of different methodologies and approaches. On this account no comparative studies or comparable assessments can be conducted. This project aims at comparing and evaluating five different approaches for quantifying microplastic particles in freshwater samples. Water samples from the river Rhine, Germany, were taken using an Apstein plankton net (150 µm mesh size). Water is eliminated via filtration with membrane glass fiber filters, lyophilisation or centrifugation in three of five procedures. Afterwards, there is an enzymatic digestion of organic compounds using cellulase (E.C. 3.2.1.4), chitinase (E.C. 3.2.1.14) and lipase (E.C. 3.1.1.3) in the first step, followed by addition of protease (E.C. unknown) after some incubation time. In the end a density separation using sodium chloride is performed. The fourth procedure does not feature removal of water before the enzymatic digestion. The last procedure begins with a density separation and filtration ahead of the digestion. Recovery experiments are done using four different kinds of colored plastics: polypropylene (PP), polystyrene (PS), high-density polyethylene (PE-HD) and low-density polyethylene (PE-LD). The particles were categorized into three groups of 200 µm – 630 µm (30 particles of each material), 630 µm – 2000 µm (20 particles) and 2000 µm – 4000 µm (3 particles). After going through the different procedures the remaining particles were counted using a digital microscope. First results of our current research indicate that the recovery and feasibility of the procedure employing water removal via freeze-drying is successful. A great concern concerning the effectivity of the enzymatic digestion of the filtered and freeze-dried sample is ongoing and therefore further results will be presented at the conference. MO070 Canada’s regulatory approach for microbeads
Microbeads are released into the environment from a variety of applications, including consumer and industrial products. To evaluate the potential environmental impacts of microbeads, the Government of Canada in 2015 completed a science summary, which involved the review of >130 relevant publications and the development of a framework. The review was recommended that microbeads be added to the list of toxic substances under the Canadian Environmental Protection, 1999. In addition, a mandatory information gathering survey was published for microbeads to gather sector specific data along with a notice of intent to develop regulations for microbeads. This presentation will highlight key findings from the science summary.

MO071 Fate and impact of model microplastics in freshwater

A. Dawson, Griffith University; S. Kawaguchi, R. King, Australian Antarctic InstituteForel

Plastics, originating from voluntary or involuntary release, are considered as an emerging contaminants of global importance and rise concerns about their environmental implication. Although the environmental impact of macroplastic waste is extensively studied, the behavior and the effects of micro-plastics, either unintentionally released in the environment, either formed as a degradation product of the macroplastics is still largely unknown. Owning to their small sizes, microplastics can be readily ingested by the aquatic microorganisms, affect them and accumulate in the aquatic food chain. The present study therefore explores the fate of microplastics in surface water and their effect to water flea Daphnia magna. The stability of two model plastic nanoparticles with similar primary size of 200 nm and different surface functionalization, amidine- and carbboxyl-group functionalized latex beads, were characterized following their hydrodynamic size and surface charge variations in lake and river water, and D. magna test medium. Furthermore the effect on D. magna was quantified using 48h exposure immobilization test and following the uptake in the gut by microscopic observations. Negatively charged carbboxyl-latex beads were stable in all tested media as revealed by the unchanged negative z-potential and lack of agglomeration. Interestingly the behavior of the amidine-latex beads was strongly affected by the exposure medium conditions characterized by strong electrostatic agglomeration in lake and river water, however were stable in D. magna exposure medium. Both the positively and negatively charged latex particles were consumed by zooplankton. The amount of the microplastic particles detected in the D. magna gut increase with their concentration in the exposure media. The 48h-immobilization tests demonstrated toxicity both micro-plastic particles to the water flea. The EC50 of 50 mg/L and 80 mg/L for amidine- and carbboxyl- latex beads, respectively were found demonstrating that these microplastics can be considered as harmful for D. magna. Overall the obtained results demonstrated the potential of the microplastics to affect aquatic biota, as well as the importance of the particle surface charge in particle-biota interactions and in their reactivity and stability in the aquatic systems.

MO072 The fate of microplastics ingested by Antarctic krill (Euphausia superba)

A. Dawson, Griffith University; S. Kawaguchi, R. King, Australian Antarctic Institute Forel; C.K. King, Australian Antarctic Division; K. Townsend, University of Queensland / School of Biological Sciences; S.M. Bengtson Nash, Griffith University / Southern Ocean Persistent Organic Pollutants Program

Plastics particles less than 5mm (microplastics) are generally accepted to be ubiquitous within the marine environment. With the concentrations of marine microplastics increasing worldwide and reaching the most remote ecosystems, it has become necessary to develop a greater understand of how marine species interact with this relatively recent addition to the marine environment. Antarctic krill are a key species in the Antarctic ecosystem. They are undoubtedly one of the most important organisms in the southern ocean as almost all species residing in the Antarctic depend on krill either directly or indirectly. They also exhibit great dietary flexibility which may leave them vulnerable to the adverse effects of plastic ingestion, including false satiation, leaching of chemical additives and reduced body condition. In order to determine the capacity of Antarctic krill to ingest microplastics, we have performed ingestion experiments. The ingestion experiment was conducted by feeding a diet of microplastics and algae with a range of concentrations. The diet consisted of 10, 20, 40 and 80% plastic per dry weight of algae, which ranged from approximately 14.5 to 116.2 plastics ml⁻¹. The model microplastics were fluorescent polystyrene beads with a size range of 27-32 µm and density of 1.026g/cm³. Krill were kept in 24h darkness in 0°C filtered seawater for ten days. Feeding occurred daily for four hours and water changes occurred before and after feeding. Faecal pellets were found to contain whole beads and small fragments of plastics, indicating that the mechanical forces of the gastric mill were able to triturate microplastics particles. To investigate this finding further, histological analysis was performed on the digestive tract and on whole krill. The findings are outlined in this presentation.

MO073 Occurrence of microplastics in the stomach content of red mullet (Mullus barbatus) from the Western Mediterranean

C. Martinez-Gomez, A. Martinez camara, V.M. Leon, Instituto Español de Oceanografía / Marine Contamination and Biological Effects; D. Vethaak, DELTARES / Marine and Coastal Systems

In this study the occurrence of microplastics (MPs) (<5 mm – 300 µm) in the stomach content of red mullet (Mullus barbatus) from three areas along the Spanish Mediterranean coast was investigated. Potential relationships between MP ingestion and physiological and reproductive parameters in this species were also investigated. Fish stomachs were collected during routine monitoring programs for chemical contamination in 2013 (fixed in 10% formalin followed by storage in 95% alcohol) and 2014 (frozen at -20°C). Making use of alkaline digestion methodology (KOH 1M, 21 days, ambient temperature) and microscope-based counting (stereo microscope) it was found that percentage of fish with MPs in its stomach was 24%, with a predominance of fibers (66%) and soft spheres (33%), representing between 1 and 8% of the total stomach content weight. We found no relationship between plastic abundance in the stomachs and gonadosomatic index and condition factor of the fish (p>0.05). Our preliminary results generally agree with several previous investigations on plastic content in fish stomach. The identity of the removed particles and fibers await further confirmation using Fourier transform infrared (FTIR) spectrometry. These are the first observations on ingested plastic in a benthic fish species from the Western Mediterranean Sea, contributing to the knowledge about the magnitude of this environmental problem.

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MO074 An Optimised Extraction Method of Fluoranthene from Microplastics using Accelerated Solvent Extraction


Microplastics (MPs) are a group of anthropogenic contaminants with a high persistence in the natural environment. MPs that become brittle or fragmented may release plastic additives like plasticizers, which are known to have toxic effects on the biosphere. In addition, hydrophobic toxic pollutants like polychlorinated biphenyl (PCBs) or polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment may be accumulated on the surface of MPs. In order to determine the ecotoxicological impact of MPs, it is necessary to understand the nature and extent of the chemicals that travel a long distance with MPs in the environment. However, currently no harmonized analytical methods are available to extract MPs from the environmental matrix as well as to extract pollutants from the MPs. Here, we propose a reliable method to determine the mass of contaminants adsorbed onto MPs using an accelerated solvent extractor (ASE).

First, a series of batch experiments were carried out in the laboratory to charge fluoranthene, a 4-ring PAH, onto plastic pellets (ID < 5 mm) made of three different polymers: polyethylene (PE), polystyrene (PS) and polypropylene (PP). Then, fluoranthene adsorbed on the pellets was extracted using an ASE @ 300 (Dionex, Idstein) under a fixed pressure of 100 bar. The ASE extraction was carried out either in one or two cycles (approx. 5 minutes per cycle) at 50, 70 and 100°C depending on the physicochemical persistence of the pellets. Methanol and isopropanol were tested as solvents in this study. The ASE conditions were optimized for the extraction of fluoranthene on the surface of PE-, PS- and PP-pellets to yield a high recovery rate. First results showed that the best ASE extraction conditions for PE were at 100°C for 5 minutes in two static cycles using isopropanol, leading to an extraction efficiency of approx. 70-80%. The similar extraction efficiency was determined for PS at 70°C, which is the maximum possible temperature for the pellets, for 5 minutes in two static cycles using isopropanol. The highest recovery rate of 110 -115% was obtained for PP under the ASE condition at 100°C in 1 static cycle using isopropanol.

MO075 Alternative pathways of microplastic exposure - microplastics and associated POPs on zebrafish gills (Danio rerio) and zebrafish eggs

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We here present an alternative pathway for the transfer of microplastics and associated pollutants by exposing zebrafish (Danio rerio) gills and eggs to very small microplastic particles (<20 µm) loaded with the polycyclic aromatic hydrocarbon (PAH) benzo[α]pyrene (Bp). So far, the toxicity of the transfer of associated pollutants has mainly been studied by the ingestion of microplastic particles into various organisms. Potential effects of microplastics via adherence to and accumulation on gills has only been studied in bivalves and crustaceans, however without analyzing the transfer of adsorbed substances. Likewise, effects by microplastics, as well as the transfer of polycyclic aromatic hydrocarbons from exposed zebrafish eggs has been neglected. Adult zebrafish were exposed to 1-5 and 20-µm
Microplastics loaded with BaP for 6 and 24 h and analyzed for effects by both virgin and BaP-loaded microparticles on gills in the ehtoxyresorufin-O-deethylase assay (EROD) on gills and visual fluorescence tracking of BaP. Similarly, zebrafish eggs were incubated for 96 hpf with virgin and BaP-loaded microparticles and analyzed via the fish embryo toxicity test (OECD 216), visual fluorescence tracking of BaP after 48, 72 and 96 hpf and the in vivo EROD assay after 96 hpf. Results show that microparticles mostly adhere to the mucus of fish gills and are thus subsequently washed out during exposure times. Nevertheless, there was a trend to EROD induction in gills of fish groups exposed to BaP-loaded microparticles. The fluorescence analyses located BaP on gill arches rather than on filaments, if compared to positive controls. In contrast to fish gills, microparticles readily accumulated on the chiton of fish embryos. Fish embryos showed sublethal effects following exposure to BaP-loaded microparticles, whilst virgin microplastic particles had no effect. The present study illustrates that microplastics do not only cause effects by intestinal ingestion, but also by adherence to surface structures such as gills and the egg shell. This represents an alternative exposure route to food-borne consumption of microplastic associated pollutants.

**MO076**

**Adsorption of four endocrine disrupting compounds on polyethylene microplastics**

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Scientific studies have shown that plastics greatly contribute to the littering of the environment. Micro-sized plastics, termed as microplastics (MPs), bisphenol A, estradiol, dienestrol, hexestrol) on polyethylene MPVs (40–40 μm) was investigated. Solution parameter effects of pH and natural organic matter (NOM) were also considered. Our results demonstrate that the adsorption isotherms of all the pollutants to MPPs complied well with the langmuir model. Q, values decreased with pH increase (5–10), mainly due to the different speciation of compounds under different pH values. It is noteworthy that an overall trend of higher Q, values increased with NOM increase, probably due to covalent effects. This result also implies that the presence of NOM may increase the contaminants’ burden on MPPs, and subsequently increase MPPs’ carrier potential for contaminants to aquatic organisms and lead to greater risks to the ecological system.

**MO077**

**Microplastics effects and bioavailability of co-contaminants in the blue mussel Mytilus edulis**

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Microplastics (5 mm–1 μm) are the most numerous pieces of plastics reported in marine environments and concerns exist regarding their potential to affect organisms. Potential negative effects of microplastics (MPs) include disruption of gut physiology after ingestion, release of substances (co-contaminants) sorbed to MPs into organisms, and occlusion of tissue surfaces by accumulation of MPs. MPs are partially hydrophobic and thus they tend to sorb and have potential to accumulate MPs on gills and/or within the gut lumen. Co-contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and metals, can sorb to MPs and desorption after MP ingestion is of toxicological concern. The objective of this study was to assess the bioavailability of associated co-contaminants to MPs in mussels, as well as to study MPs physical effects. Mytilus edulis mussels (one per beaker) were exposed for 24h to polyvinyl chloride (PVC) pristine particles (125–250 μm diameter), at 10 different concentrations (0-1 g/L). Co-contaminants, cadmium (Cd2+), and the PAHs anthracene and pyrene, were allowed to be sorbed to MPs (24h) prior to exposure to microplastic particles to assess accumulation of co-contaminant while in aqueous phase was also established for mussels. Gene expression was assessed in gills and digestive gland using pi-Glutathione S-transferase (pi-GST) as a biomarker of PAHs bioavailability and Metallothionein-20 (MT-20) for Cd2+. Gene expression was measured using quantitative reverse transcription PCR (RT-qPCR) with β-Actin as a reference gene. MT-20 increased linearly with increased particle concentration in the digestive gland, as for mussels exposed to Cd2+ in aqueous phase, but not in gills. Males that spawned during the experimental procedure did not exhibit MT-20 expression in digestive gland tissues. We anticipate that our results, together with ongoing data analysis, will contribute to a better understanding of the fate and effects of MPs and their co-contaminants in the marine environment.

**MO078**

**Microplastic may increase uptake and bioaccumulation of organic pollutants in aquatic crustaceans**

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Microplastic is causing an increasing environmental problem in aquatic environments where it has been shown to enter into the food chain. Maybe even more important, microplastic may act as a contaminant trap and carrier of organic pollutants that could enhance bioaccumulation of these compounds in aquatic food chains. The uptake of microplastics and associated pollutants was investigated using different concentrations and size fractions of microplastic particles. The amount of plastic taken up by the animals was determined by microscopy and the sublethal effects of microplastic on Moina salina were determined using video tracking and enzymatic assays. Moina salina ingested microplastic particles at various rates in these experiments. Microplastic particles similar to the ones used for the feeding experiments were allowed to adsorb 14C-phenanthrene, before these were fed to Moina salina. The radioactivity of the animals and of the microplastic particles was measured by liquid scintillation counting. The data obtained from these experiments were used to determine to which extent microplastic might act as a carrier of organic pollutants in aquatic environments.

**MO079**

**Microplastics: Investigation of different effects on sorption behaviour of organic pollutants in freshwater systems**

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Nowadays synthetic polymers are an essential part of everyday life and an introduction of synthetic materials in the environment cannot be completely avoided. One part of this discharge are microplastic (MP) particles with diameters < 5 mm. Due to a slow degradation, MP, as well as macroscopic plastic, has a high impact on ecosystems. Freshwater systems are a revealing field of research, as these systems play an important transport route of MP in sediments and oceans world-wide. Many aspects of the influence of MP on the environment are still under investigation and thus their effects on ecosystems are yet not fully understood. One aspect of the research is the determination of sorption properties of MP, which are based on different polymer materials such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC) and polyethylene terephthalate (PET). Environmental organic pollutants such as pesticides or pharmaceuticals can act as sorbate and accumulate onto MP due to hydrophobic interactions. Thus, MP may provide a transportation route for organic pollutants in aquatic systems or the food chain. Sorption processes for various organic pollutants are still under research and seem to be dependent on various parameters such as polymer material, age of the particles, biofilm formation and further environmental parameters. The aim of this study was to evaluate different synthetic polymers regarding their potential to act as sorbent for organic pollutants. Therefore, synthetic polymers were investigated in synthetic fresh water that was spiked with different micropolllutants. The utilized polymer materials were polybutadiene, PS and PVC, which were each spiked with pesticides such as e.g. difenacoum and bromadiolon. The MP samples were extracted with hexane or dissolved in tetrahydrofuran and underwent purification via column chromatography. The content of organic pollutant was determined via GC-MS or LC-MS/MS. All polymers showed pesticide sorption and sorption isotherms were calculated for the different polymer/pesticide combinations applying Henry, Freundlich and Langmuir models. As the sorption isotherms were determined for an ideal system (synthetic polymers, synthetic fresh water), the importance of further parameters have to be determined, such as the formation of biofilms and “old” MP samples.

**MO080**

**Incorporation of environmental microplastics causes biomarker responses and behavioral changes in three-spined stickleback**

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Microplastics (MPs) raise great environmental concerns due to the wide distribution in aquatic environment and dreadful capacity to absorb and concentrate environmental contaminants. As potential threats of MPs are greatly unknown, interdisciplinary research is being accelerated towards increasing understanding of biological fate of MPs in organisms, their toxicity and inherent ecological consequences. An initial study was conducted to study vector effects of MPs following environmentally relevant exposure scenarios. Unplasticized polyvinyl chloride (uPVC) microscopic pellets were used as model particles, which were exposed for 30 days to the effluent of sewage treatment plant and the outflow from industrial harbor in Göteborg city, Sweden. Consequently, marine animals such as stickleback fish were feed diets containing exposed particles. Fish were fed with experimental diets for
24 days and then potential chemical uptake and toxicity was assessed using gene and biochemical biomarkers. Additionally, behavior of exposed fish was investigated with open-field and aggression tests. Results revealed that environmental MPs can cause changes in mRNA expression levels of several genes in liver, for example, expression of metallothionein (MT) a known biomarker for metal toxicity and vitellogenin (VTG) an established biomarker for estrogenic exposure. Some changes in behavior were also observed. Further investigations are conducted to fully assess the dietary toxicity of MPs and to elucidate the composition of environmental pollutants adsorbed to MPs.

**MO081**

**PET microplastics have no long-term effects on the freshwater amphipod Gammarus pulex**

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Microplastics (MP) are abundant in most of the global marine and freshwater ecosystems and can interfere with their biota. Potential adverse effects have already been studied in situ as well as in laboratory studies for several marine species, while effects on freshwater organisms remain so far largely unstudied. The present study provides first results on the short-term uptake and the long-term effects of PET MP on the freshwater amphipod Gammarus pulex. As part of an uptake study (24 hours) as well as an effect study (48 days) juvenile (6-8 mm) and adult (12-17 mm) individuals were exposed to fluorescent PET MP with a size range of 10-150 μm. Both studies covered MP concentrations from 0.4-4,000 particles per ml. The rate of MP uptake throughout 24 hours was determined by enzymatically lysing the exposed individuals and analyzing the ingested particles with a fluorescent microscope. Feeding activity, energy reserves (glycogen, lipids, proteins), moult periods and mortality were investigated in the 48-days chronic toxicity study. The results of the short-term uptake study indicate that MP ingestion by G. pulex is not size-selective and increases with the particle concentration. Comparing the absolute and relative uptake of adults and juveniles at same particle exposure concentrations, juveniles ingested more particles than adult individuals. In the chronic toxicity study no significant changes in feeding activity, energy reserves, moult period duration and mortality were observed in any of the treatment groups. In conclusion, this study demonstrates that a common freshwater amphipod is not affected by microplastics. Further studies will be necessary to fully assess the dietary toxicity of MPs and to elucidate the composition of environmental pollutants adsorbed to MPs.

**MO082**

**Uptake and toxicity of methylmethacrylate-based nanoparticle aggregates in aquatic organisms**

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The uptake and toxicity of methylmethacrylate (MMA)-based particles with different surface chemistries (medium and hydrophobic) were assessed using aquatic organisms selected for their relevance and based on the environmental behaviour of the MPs. Pure poly(methylmethacrylate) (medium; PMMA PNPs) and poly(methylmethacrylate-co-stearylmethacrylate) copolymer (hydrophobic; PMMA-PDMA PNPs) of 86-125 nm were synthesised using a mini emulsion polymerisation method. Fluorescent analogues of each NPN (FPNPs) were also synthesised using monomer 7- [trifluoromethyl] -coumarinacrylamide and studied. Daphnia magna, Corophium volutator and Vibrio fisheri were employed in a series of standard acute ecotoxicity tests, being exposed to the PNPs at three different environmentally realistic concentrations (0.01-0.1, and 1.0 mg L\(^{-1}\)) and a high concentration 500-1000 mg L\(^{-1}\). In addition, sublethal effects of PNPs in C. volutator were determined using a sediment reburial test whilst the uptake and degradation of FPNPs was studied in D. magna. The FPNPs and FPNPs did not exhibit any observable effects (VTG at 1000 mg L\(^{-1}\)) in any of the tests except for PMMA-PDMA PNPs and FPNPs following 48 h exposure to D. magna (EC50 values of 879 and 887 mg L\(^{-1}\), respectively). No significant differences were observed between labelled and non-labelled PNPs, indicating the suitability of using fluorescent labelling for tracking of the NPs. Significant uptake and rapid excretion of the FPNPs was observed in D. magna. A. Spurgeon, Centre for Ecology & Hydrology

Studies have shown that microplastic particles in the environment can associate with persistent organic pollutants (POPs) due to the hydrophobic nature of plastics and organic chemicals. It is likely the case wherever microplastics are present, that microplastics in the environment will rarely exist in the absence of other environmental pollutants and that the gut microbiome of an organism may favour desorption of these adsorbed chemicals due to gut condition including pH and ionic strength and the presence of surfactants. Therefore ingestion of microplastic particles has implications for uptake and bioaccumulation of these chemicals. PBDEs (polychlorinated diphenyl ethers) are widely used as flame-retardants in products as textiles and soft furnishings, with the potential to leach into the environment, so are one of the likely POPs to be associated with microplastics. We conducted bioassays using freshwater midge larvae Chironomus sancticoralli and the pond snail Lymnaea stagnalis to observe the effects of microplastic particles (nylon < 50 μm) and PBDEs both individually and in combination, including mortality, effects on the gut microbiome, stress gene expression and PBDE bioaccumulation. Microplastic particles were mixed with quartz sand sediment at 1% concentration (13g total substrate per replicate). A PBDE mix (containing BDE-47, 99, 100, 153 and PBB-153) was added to the sediment-microplastic mix in glass vessels at six environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g\(^{-1}\)). Artificial freshwater was added to each vessel once the solvent had evaporated. Given that POPs will preferentially bind to plastics over sediment, it was assumed that all PBDEs would adsorb to the nylon particles rather than the sediment or the glass vessel. Exposures ran for 96 hours during which there was no mortality effect on either species. Following the exposures RNA, microbiome DNA and tissue chemical analyses were carried out. A parallel study showed ingestion of microplastics by chironomids over a 48 hour period, with microplastics still present in the gut following a 48 hour depuration period, demonstrating that microplastics are ingested and retained. Similar observations have been made on microplastics within a pond snail gut. The results of this study will help develop our understanding of the interactions between microplastics, organic chemicals and organisms in the environment and the implications for the food chain.

**MO084**

**Influence of microplastics on the short-term toxicity of mercury to juveniles of the sea bass (Dicentrarchus labrax)**

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Studies have shown that microplastic particles in the environment can associate with mercury and two concentrations of microplastics were tested in a full factorial experiment design. Effect criteria were swimming performance and sub-individual biomarkers. If any, the gut microbiome, alterations in biotransformation, energy production, and anti-oxidant defences, and lipid peroxidation damage. For several parameters, including the swimming velocity, significant differences among treatments were found, with fish exposed to mercury alone and in the presence of microplastics showing a reduction of the predatory performance (p < 0.05), and impairment of several important physiological functions (p < 0.05). Moreover, for some of the parameters, interactions between microplastics and mercury were found. These findings highlight the need of more research on the interactions between microplastics and other common contaminants on marine organisms.

**MO085**

**Effects of microplastics on benthic macroinvertebrates in freshwater ecosystems**

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Microplastics represent a widespread contamination in the global environment, associated with microplastics. We conducted bioassays using freshwater midge larvae Chironomus sancticoralli and the pond snail Lymnaea stagnalis to observe the effects of microplastic particles (nylon < 50 μm) and PBDEs both individually and in combination, including mortality, effects on the gut microbiome, stress gene expression and PBDE bioaccumulation. Microplastic particles were mixed with quartz sand sediment at 1% concentration (13g total substrate per replicate). A PBDE mix (containing BDE-47, 99, 100, 153 and PBB-153) was added to the sediment-microplastic mix in glass vessels at six environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g\(^{-1}\)). Artificial freshwater was added to each vessel once the solvent had evaporated. Given that POPs will preferentially bind to plastics over sediment, it was assumed that all PBDEs would adsorb to the nylon particles rather than the sediment or the glass vessel. Exposures ran for 96 hours during which there was no mortality effect on either species. Following the exposures RNA, microbiome DNA and tissue chemical analyses were carried out. A parallel study showed ingestion of microplastics by chironomids over a 48 hour period, with microplastics still present in the gut following a 48 hour depuration period, demonstrating that microplastics are ingested and retained. Similar observations have been made on microplastics within a pond snail gut. The results of this study will help develop our understanding of the interactions between microplastics, organic chemicals and organisms in the environment and the implications for the food chain.
tested in this study ranged from 0.1% to 20% (in order of percent dry weight), including the highest concentration of MPs found in the freshwater environment, which is ~1%. The dose-response relationships were calculated for each species using growth/weight as a sub-lethal endpoint and survival as a lethal endpoint. This data will be used to elaborate a Species Sensitivity Distribution (SSD), showing the difference in susceptibility for MPs between species. The SSD can also be used in the future to determine the maximum allowable concentration of MPs in the freshwater environment.

**MO086**

Decreased feeding or food assimilation is a likely physiological mechanism of microplastic effects in the filter-feeding crustacean Daphnia magna

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Marine and freshwater contamination with microplastic (MP) has received increasing focus during recent years. For example, ingestion of MP by various filter-feeding organisms including the freshwater cladoceran Daphnia magna has been documented. However, we still have limited knowledge of MP effects in freshwater environments. In theory MPs may affect exposed organisms by four different mechanisms: 1) ingestion of MPs decreases food intake by interfering with feeding or filling up the gut; 2) MPs may in other ways interfere mechanistically with the physiology of the organisms, 3) toxic additives (e.g., phthalates) may leach from ingested particles; 4) environmental chemicals may adhere to particles leading to changes (i.e., increased or decreased) uptake of the chemical in question. The purpose of the present study was to test the hypothesis that MPs interfere with feeding or food assimilation leading to effects at the organism level and eventually to potential population-level effects. Two experiments were carried out according to a slightly modified D. magna reproduction test (OECD 211). Experiment 1 tested potential effects of particle size (1-10 and 10-106 μm), using 0.96 g/cc polyethylene (PE) beads. Experiment 2 tested effects of particle density using PE beads in the same size range (10-90 μm) but with different densities (ca. 1.00 and 1.10 gc). In both experiments survival, growth and reproduction was measured during a 21 days study. The study was a proof-of-principle study using particle concentrations (i.e., 1000 and 10,000 particles/ml) above likely realistic environmental concentrations. There were no significant effects of MP exposure on D. magna survival regardless of particle size or density. Growth (after day 15) and reproductive output were decreased significantly by the MP particles with the highest density, but not by any of the other particle types. The fit of a dynamic energy budget model indicated that the observed effect was caused by either a reduction in feeding or food assimilation or by increased maintenance cost. The study shows that impacts of plastics depend crucially (though not surprisingly) on plastic properties such as density, and that certain properties (e.g., size) are unimportant if particles end up in a different environmental compartment than the organism. The next step should be to test other combinations of particle size, density and potentially shape at environmentally realistic particle concentrations.

**MO087**

Microplastics in rivers: Focus on short-term temporal and spatial variabilities

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Microplastics in freshwater is an increasing issue. These plastics defined as a particle with a size smaller than 5 mm have been widely reported in marine environments. This study aims at investigating the homogeneity of microplastic distribution in rivers and determine if the short and mid spatial variability of microplastics in rivers. The Marne river was considered. The samplings were performed with a plankton net (mesh size 80 μm). Two separate campaigns were carried out in order to assess the short-term variability of the microplastic concentrations. Six samples were collected per campaign. On the first campaign, each sampling was performed during 1 minute while it was performed during 3 minutes on the second campaign. For the variability through the cross section, triplicates in five different points were sampled. The sampling duration was 3 minutes. Three points of sampling were on the surface with one in the right bank, one in the left bank and one in the center. The two remaining points were in the center with respectively 1 and 2 meters of depth. The depth of the water at the moment of the sampling was 2.6 m. During short-term temporal variability tests, concentrations ranged between 38.2 and 101.6 particles/m² in the first campaign with a coefficient of variation around 45% and between 18.7 and 38.6 particles/m² during the second with a coefficient of variation of 26%. The water flow was different. In fact, the second campaign was carried out after a month of particularly dry weather leading to a very low water flow. Microplastic concentrations seem to be more variable in high water flow conditions. A lateral variability (n=9) of 53% is found. The higher concentrations observed near the banks might be related to the effect of the intense fluvial traffic in the Parisian agglomeration. In fact, it can be expected that a barge passing generates waves that drive microplastics and floating debries towards the banks. Considering all samples collected in the middle of the river, a coefficient of variation of 21% (n=9) is found which varies that the vertical variability is more than twice the lateral variability. This result is not surprising knowing that in river conditions, a constant water mixing is induced by the currents and reduces the chances of stratification. Moreover, the passing of the barges can easily contribute to this mixing.

**MO088**

Microscopical investigations of polystyrene microparticles colonization by Vibrio cassostreae

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Microplastics represent a widespread contamination in the global environment, particularly documented for the oceans. Recent studies of microorganisms’ diversity associated with microplastics show a large presence of bacteria of the genus Vibrio. Particularly, some species pathogenic for marine organisms were identified associated to microplastics collected at sea. These discoveries raise questions about the potential role of microplastics as a vector for pathogenic organisms in the marine environment. A protocol to study the colonization of polystyrene (PS) in controlled conditions was developed, with the aim to understand factors modulating the adhesion on these microplastics. The pathogenic bacteria Vibrio cassostreae 12-9 was used in this study owing to its pathogenicity for oysters Crassostrea gigas, and constitutive mutants expressing GFP (Green Fluorescent Protein) were constructed to follow the colonization using fluorescent microscopy. Colonization processes and dynamics were investigated via confocal fluorescent microscopy and scanning electron microscopy for different types of polystyrene microparticles (smooth, irregular, fluorescent and non-fluorescent) over 24h to 96h. Incubations were performed in different media to assess the influence of some abiotic (presence of nutrients) and biotic (presence of multi-species microorganisms associated with natural seawater) parameters. The results of this study highlight complex processes of colonization/decolonization: for all particles types the percentage of colonised particles was higher in a Zobell culture media rich in nutrients as compared with filtered synthetic seawater. Roughness of the particles led to a better colonization than what was observed for smooth PS microbeads. In natural seawater, presence of natural aggregates formed by debris and microorganisms around the particles led to a strong and long lasting colonization, suggesting that V. cassostreae could play a role as a second colonizer in the marine environment. The knowledge developed during this study opens the way for further studies addressing this topical issue. **Key words**: microplastics, polystyrene, Vibrio, colonization

**MO089**

The effects of microplastic on fresh water Hydra attenuana morphology & feeding

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Microplastic refers to small pieces of plastic Hydra attenuata. Microplastics (polyethylene) used were sourced by vacuum filtering a face wash product containing microbeads. Hydra were then exposed to different concentrations of these microplastics (Control, 0.01, 0.02, 0.04, 0.08 g/ml) and morphology was recorded at 24, 48, 96 hrs and 1 week. Feeding tests were also carried out using the same concentrations as the exposure by recording the amount of Artemia ingested over a period of 120 min. Hydra were found to be able to ingest the microplastics (22 - 444 μm) and feeding was affected by the presence of microplastics even at the lowest concentration used (0.01 g/ml). After 60 min of exposure the Hydra ingested a lower amount of Artemia at all concentrations of microplastic compared to the control. This became particularly evident at the 90 and 120 min mark. Exposure to the microplastics did cause changes to the morphology of the Hydra. These changes were more than twice the size of the preliminary results of this study show that Hydra attenuata are capable of ingesting microplastics. The presence of microplastics disrupted the feeding behaviour of the Hydra thus negatively impacting its ability to feed. Continued research in this area will provide valuable insight in determining the potential impact microplastic can have on the health of freshwater biota.

**MO090**

Quantification of microplastics emissions from cosmetic products to freshwater ecosystems

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Recently, a lot of attention has been put on small pieces of plastic materials
commonly called “microplastics”. There are two sources of microplastics: microplastics generated through fragmentation of larger plastic material called secondary microplastics and primary microplastics – particles manufactured at a specific size range usually used as abrasives (microbeads) in cosmetic products. After usage of these products, microbeads are released into sewage and travel across wastewater treatment plants and are finally discharged into surface waters. However, the amount of microbeads released into the environment has not been yet assessed. Hence, the aim of our study was to estimate amount of microbeads that can daily be released from cosmetic products to surface waters. The study initially characterized the microbeads from cosmetic products with an emphasis on physical properties and particle size distribution and consequently quantified the daily emission of microbeads from consumers by a questionnaire survey. Amount of microbeads in five selected scrubs varied across products, but the highest amount of microbeads was 11% of the cosmetic product. All microbeads were made of polyethylene with the major particle size up to 100 μm. Survey participants (n=178) were estimated to release microbeads per person per day. If wastewater treatment plant efficiency (~ 70% microbeads removal) is taken into account the daily emission of microbeads into surface waters is 4.5 mg of microbeads per person being potentially more than 2 tons of microbeads per day released in the European Union. Results of our study showed, that microbeads from cosmetic products can represent a potential threat for freshwater ecosystems, because they are continuously introduced into surface waters and due to their small size (up to 100 μm), they can be easily ingested by a range of aquatic organisms.

**Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stressors in Aquatic Animals (P)**

**MO091**

Impact of the antimicrobial drug metformin on brown trout S. Jacobs, L. Blust, L. Bervoets; University of Antwerp / Animal Physiological Ecology With a focus on two groups of emerging micropollutants in aquatic environments, food additives and pharmaceuticals, the project Eff-Net crosses the borders between (natural and socio-political) science and public interest by providing an analytical network, which gives insight into the cross-linking of consumer behaviour and ecological impact. In a multi-disciplinary approach, anti-diabetics (e.g. metformin), artificial sweeteners (e.g. saccharine) and antidepressants (e.g. fluoxetine) as well as their metabolites and transformation products are studied from the mode of action at the molecular level up to the effects at the community level. In the present study, we investigate possible effects of metformin, an anti-diabetic drug, in brown trout (Salmo trutta L. fjari). The influence of different temperatures is also addressed. Starting with the exposure of fertilized eggs, developing eggs and juvenile fish are continuously exposed to different concentrations of metformin (1x, 10x, 100x and 1000x the environmental concentration). The experiment is conducted at two different temperatures, regarding possible interactions of chemical toxicity and temperature. To show influences on embryo development, mortality, hatching success, and time of hatch are recorded. At the end of the sac-fry stage, several endpoints indicating fish health are investigated: these include the histological condition of liver, glycogen storage in this metabolic organ and alterations in the level of the stress protein Hsp70. Additionally, animal behaviour (swimming velocity and predator-prey-interaction) is investigated. The information gained from this experiment at the organismic level is used to validate results of experiments addressing effects at the molecular and community level (generated in parallel by other partners of Eff-Net) with respect to their ecological relevance.

**MO092**

Sublethal effects of the combined exposure to metal mixtures and predator stress on Asellus aquaticus M. Van Ginneken, University of Antwerp, Dept. Biology / Biology SPHERE; R. Blust, L. Bervoets, University of Antwerp / Systemic Physiological and Ecotoxicological Ecology; L. Jacobs, Department of Aquatic Sciences and Assessment

In aquatic ecosystems, trace metals often occur in mixtures in which they can strongly interact with each other, creating synergistic, additive or antagonistic toxic effects. Moreover, natural stressors such as temperature fluctuations or predators are present, which can increase the animals’ vulnerability to these contaminants. However, classic toxicity tests are performed using single compounds without additional stressors and could thus lead to an over- or underestimation of the impact of metal exposure in the natural environment. This study investigates the effects of the combined exposure to predator stress and metal mixtures of copper, cadmium and lead on the freshwater isopod Asellus aquaticus. The effects of the single contaminant were evaluated against the European Union aquatic environmental quality standards (EQS): 7 and 70 μg L⁻¹ for Cu; 0.15 and 1.5 μg L⁻¹ for Cd; and 7.2 and 72 μg L⁻¹ for Pb. Animals were exposed to the different metals separately as well as to binary and tertiary mixtures. These metal treatments were combined with two predator treatments in which cues of both fish and invertebrate predators were absent or present. After ten days, various sublethal endpoints were assessed. Besides determining the metal accumulation and growth rate of each animal, we mainly focused on behavioral endpoints such as swimming performance and feeding rate. Furthermore, the energy reserves (glycogen, protein and lipid concentrations), oxidative stress (SOD, CAT and TBARS) and respiration were measured. We hypothesized that metal accumulation, respiration and oxidative stress would be higher in the animals of the (trophic) treatments with stressors. However, we hypothesized the animals of the treatments without predator cues to have higher feeding and growth rates, higher energy reserves and to be more active. This study will help to understand how anthropogenic and natural stressors interact and contribute to the development of ecologically relevant EQS.

**MO093**

Sublethal Effects of Glyphosate and Glyphosate-Copper Complexes to Daphnia magna Determined by Video Tracking and Behavior Analysis L. Rykar Hansen, P. Rosles; Aalborg University / Biology and Environmental Sciences

 Glyphosate (N-(phosphonomethyl)glycine) is the active ingredient in a range of popular broad-spectrum herbicide formulations. Glyphosate is a chelating agent (ligand), and can form complexes with divalent metal ions including copper (Cu). The toxicity of glyphosate to non-target organisms has been evaluated in previous studies, however, little is known about the bioavailability and ecotoxicity of glyphosate/copper complexes to non-target organisms. We have used video tracking to quantify behavioral changes in Daphnia magna after exposure to glyphosate and glyphosate-copper (Cu(II)) complexes. Behavioral responses were quantified for individual juvenile D. magna after exposure for 24 h and 48 h. Sublethal concentrations of glyphosate:Cu (II) resulted in decreases in swimming velocity, acceleration speed, and an increase in the inactive time. In comparison, visual observation of immobility resulted in an EC50-48 h of 25.5 μM. The video tracking results indicated that exposure of D. magna to binary mixtures of glyphosate and Cu(II) attenuated acute metal toxicity but increased apparent glyphosate toxicity due to complexation. The results suggest that glyphosate is a likely mediator of environmental metal toxicity, and that video tracking provides an opportunity for quantitative studies of sublethal pesticide effects to non-target organisms.

**MO094**

As we mainly looking at the bright side of life? The role of food as an exposure pathway M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; J. Zubrod, M. Bundschuh, Department for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; D. Engliert, Institute for Environmental Sciences / Institute for Environmental Sciences; A. Feckler, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment; R.B. Schafer, R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences

The ecotoxicological testing of chemicals is mainly focusing on waterborne exposure pathways. Considering the phytochemical properties but also the mode of action of some chemicals, however, their impact via the ingestion of contaminated food or their implications for the nutritious quality of food may have serious influences on the behavior and physiology of aquatic organisms. We will demonstrate that chemicals with a high affinity towards organic carbon (i.e., lambda-cyhalothrin) adsorb to food, which ultimately potentiates the effects caused by the waterborne effect pathway. Moreover, systemic pesticides such as neonicotinoids but also insecticidal proteins, produced by genetically modified plants, showed a higher toxic effect when organisms fed on related plant material compared to the water-borne toxicity of leached chemicals. This suggests that the compounds are bioavailable to the test species during test passage, a process not reflected in standard toxicity tests. Moreover, substances targeting autotrophic (e.g., herbicides) or heterotrophic microorganisms (e.g., antibiotics and (in)organic fungicides) can affect the quality of the food for the next trophic level, namely grazing and leaf shredding invertebrates. Experimental efforts have largely focused on heterotrophic microorganisms and shredders. These studies suggest on the one hand that shredders can sense toxic effects on heterotrophic biofilms associated with organic matter because they showed preferential feeding on non-contaminated food with a presumably higher nutritional quality. On the other hand, the organisms’ energy processing and physiological fitness can be substantially influenced when dependent on food with a modified heterotrophic biofilm. This may ultimately cause bottom up directed effects in heterotrophic food webs. We discuss the relevance of the food-related exposure pathway with respect to empirical evidence and the broader ecological context.

**MO095**

Spontaneous Locomotor Changes in Zebrafish (Danio rerio) when chronically exposed to Chemical Warfare Agents (CWAs) found in the Baltic
Sea.

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After the 2nd World War the CWAs were prohibited by law and 11,000 tonnes of toxic agents were dumped in the Bornholm Basin east of Bornholm. The dumped chemical munitions have not reached attention from politicians and scientists until recently. During earlier projects, such as MERCW (2005-2008) and CHEMSEA (2011-2014), the area has been screened for the presence of parent compounds and metabolites in sediments and in the water column. These detected compounds have been found in the sediment and a minor part in the pore water. The (eco)toxicity of these compounds remain to be illuminated in which this study hopefully will contribute. Especially, chronic toxicity needs to be described as this mimics a more environmentally realistic situation. One or two compounds will be accessed based upon various factors such as detection frequencies, found concentrations in both sediment and pore water, acute toxicity and physicochemical properties. Besides the mentioned evaluation factors, Sulphur mustard (Yperite) degradation products will have emphasis as the majority of the dumped CWAs is the sulphur mustard gas. The chronic toxicity will be described by spontaneous locomotor changes in Zebrafish (Danio rerio). The CTDB database and Zebrafish gene collection reveals that genes associated with locomotion interacts with some of the found parent and degradation compounds of CWAs; even though the locomotion is a complex out of an array of genes. This study intends to draw lines to the commercially important cod (Gadus morhua). The cod migrates down to the sea floor – even crossing the oxicline – with theCWAs. In the2nd World War the CWAs were dumped east of Bornholm, this study will use the novel ecotoxicity data on recently discovered degradation products and assess the potential threat to the commercially important cod (Gadus morhua). This study is a part of the NATO Science for Peace and Security (SPS) project “Towards the Monitoring of Dumped Munitions Threat” (MODUM).

MO096 Waterborne toxicity and altered food quality affect the food-processing behavior and physiology of Asellus aquaticus - implications for food webs in streams

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The breakdown of terrestrially derived leaf litter is a key ecosystem process for energy provisioning in headwater streams mediated by microbial decomposers and detritivorous, leaf-shredding macroinvertebrates. Stressors like fungicides, however, can impact the functions of these decomposer-detritivore systems via indirect and direct pathways. To disentangle the relevance of each pathway, a 2x2-factorial test design was applied: leaf litter was conditioned under exposure to the model fungicide epoxiconazole (0 and 15 μg L-1) and fed to the shredder Asellus aquaticus, kept under control conditions or exposed to the fungicide epoxiconazole concentration over 28 days. Endpoints were chosen related to the conditioning status of leaf material as well as the food-processing and physiology of A. aquaticus. When fed with fungicide-affected leaf litter, Asellus increased consumption (5%) and depleted excretion (20%) compared to the control, indicating compensatory feeding and enhanced utilization of the ingested food. This behavior may have been triggered by a lower leaf-associated microbial biomass (fungi: 25%; bacteria: 15%) after conditioning under fungicide exposure. Reduced growth (30%) and lipid content (8%) of assells, however, suggest these processes to be insufficient to fully compensate for the energy requirements. Hence, dietary fatty acids (FA) essential for assells’ growth might have been additionally used as an energy source, given the lower essential FA contents (10%) compared to control organisms despite a higher availability of FA on the leaf material. Waterborne exposure led to an increased assimilation of the ingested food, judged by lowered consumption (5%) and excretion (10%). Due to reduced growth (5%) and lipid content (21%), this compensational mechanism was judged insufficient to compensate for mobilization of internal energy reserves to cope with chemical stress. For the combined (worst-case) scenario an additive action of both pathways was found: compared to the control, assells’ consumption and excretion were reduced by 17% and 15%, respectively. Similarly, depletions were found for growth (44%) and lipid content (28%). These results suggest that fungicides have the potential to affect the ecosystem functioning through a decomposer-detritivore systems via several pathways. A more comprehensive understanding of each pathway’s relevance seems therefore fundamental to accurately predict effects on energy provisioning in heterotrophic stream food webs.

MO097 Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (Anguilla anguilla)

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This study investigate the relationships between the estuarine migratory behavior, Methylmercury (MeHg) concentrations, oxidative stress response and detoxification processes in glass eels, collected in marine and estuarine waters at the end of the fishing season (April). Glass eels migratory behavior was investigated in an experimental flume according to their response to dusk. Fish responding to the decrease in light intensity by ascending in the water column and moving with or against the flow were considered as having a high propensity to migrate (migrant). Glass eels still sheltering at the end of the 24 hrs catching period were considered as having a low propensity to migrate and were called non-migrant. Our results provide some evidences that estuarine glass eels present a higher propensity to migrate and lower indications of oxidative stress than marine glass eels. This might reflect a selection process, non migrant marine glass eels progressively settling in the estuary and/or a change in feeding behaviour. Indeed, in April, glass eels restart feeding in the estuary which might decrease the oxidative stress related to starvation, and enhanced migration. There was no difference in MeHg concentrations between migrant and non migrant glass eels. However, it is suggested that non migrant glass eels might present a higher vulnerability to stress (at least contamination and/or starvation), although the underlying mechanisms remain to be elucidated.

MO098 Behaviour as a Tool to Assess the Effects of Environmental Contaminants in Crustaceans: A Review

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Behavioural effects have been used in toxicology testing since the 1960s as they provide a link between biochemical and ecological consequences of environmental contaminants. A review was conducted, totalling over 70 papers on behavioural ecotoxicology in crustaceans to determine the main behavioural endpoints developed and any gaps in the current literature. Studies on behavioural effects are known to be fast and highly sensitive, with reported effects at concentrations up to 1000 fold lower than lethal concentrations. Despite this, standard ecotoxicology testing using acute lethality and chronic effects on growth, development, and reproduction have historically been used to define environmental quality standards. Behavioural endpoints have received far less attention largely due to a lack of the necessary tools to facilitate these studies. Recent computational advances have facilitated an increase in behavioural ecotoxicology due to a capacity to quantify a range of sensitive endpoints. This review has highlighted that most of the work in crustaceans has focused on swimming and avoidance behaviours (49% and 21% respectively). However, there are a large number of other quantifiable endpoints including reproduction, aggression, feeding, anti-predator and types of taxis. With closely related endpoints such as phototaxis and anxiety-like behaviours there is a need for clear definitions and carefully planned experiments for appropriate interpretation of effects. While there have been a wide variety of species used in these studies, endpoints were often species-specific and associated with the life history of the crustacean (e.g. mate guarding). Most studies have focused on traditional contaminants, such as heavy metals (37%), pesticides (27%) and PAH’s (20%), with a recent increase in novel compounds. Behavioural assays have been used extensively in pharmacological testing. Despite being translated to environmental toxicology of fish, they are yet to be fully optimised to other taxa. With advances in the technology to facilitate behavioural studies in ecotoxicology, there is the potential for developing high-throughput techniques that measure multiple behaviours in crustaceans. However these are yet to be fully optimised and validated.

MO099 Behavioral and morphological responses of crucian carp (Carassius carassius) exposed to the antidepressant fluoxetine under predation risk

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Due to their potential for affecting the modulation of behavior, the effects of selective serotonin reuptake inhibitors (SSRIs) in the environment are particularly interesting with regard to interspecies interactions, and the non-consumptive effects induced by predators in prey organisms. Here, we tested the effects of the SSRI fluoxetine on fitness related behavioral and morphological traits of the crucian carp (Carassius carassius) under the presence of the predatory river pike, the northern pike (Esox lucius). The crucian carp is known to change behavior (i.e. boldness and sociability) and morphology (body shape) in the presence of predators. Effects on crucian carp boldness, sociability, body weight and shape were recorded after short-term (two weeks) and long-term (three months) exposure to fluoxetine at 1 and 100 μg L-1, and interactive effects with the added stress of predator presence were evaluated.

SETAC Europe 26th Annual Meeting Abstract Book
The effluent of sewage treatment works (STW) is a complex mixture of domestic and industrial compounds, including pharmaceuticals, which have the capacity to disrupt the normal functioning of aquatic organisms. For the first time, the impact of these effluents on the activity of a freshwater amphipod has been quantified. *Gammarus pulex* were exposed to the effluents of two similar STW at levels of 50%, 100% and controls for 7 days. Measurements of distance travelled (mm) and velocity (mm s⁻¹) of individual shrimp were taken with the Noldus Danio Vision Behaviour system after 0h, 2h, 24h and 7d intervals. Readings were taken every 0.5s for 15 minutes and the resulting sequences of 60 seconds lighted dark. There was no significant difference found between the velocity nor distance between any test groups. However, there was significant differences seen in the ‘acceleration’ and ‘deceleration’ responses of *Gammarus*: individuals exposed to both effluents moved further and faster in the first 5 seconds the lights were switched on (P< 0.01). Typically, *Gammarus* maintained in 100% effluent increased (in both velocity and distance travelled) by ~40% compared to controls after 7 days. Significant differences between treatment groups developed after 24h exposure, were more pronounced after 7 days, and the effect was greater with increasing concentrations of effluent. Similarly, the activity of effluent-exposed animals did not diminish after the lights were switched off. For example, in the first 5 seconds of our experiments in the first light on the following day, the activity was 150% higher compared to controls after 7 days. In general, at 7 days, there was a cumulative effect of effluent-exposed animals and the activity was more pronounced after 7 days, and the effect was greater with increasing concentrations of effluent affecting the activity of amphipods. This study suggests that STW effluents can have a cumulative effect. A negatively phototactic species, any influence on the response of *Gammarus* to light could have wider ecological repercussions. Ongoing studies are investigating the behavioural response to specific pharmaceuticals.

**MO101 Temporal profiles of behavioral alterations in zebrafish embryos and larvae exposed to pharmaceuticals**

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The occurrence of neuroactive chemicals in aquatic environments is of growing concern due to the risks posed to humans and other animals. Aquatic organisms are particularly vulnerable due to their continuous exposure to variable mixtures of pollutants. In fact, recent studies demonstrated that environmentally relevant concentrations of neuroactive pharmaceuticals can affect population-relevant fish behavior such as aggression, feeding and reproduction. There is a need to develop and improve bioassays that can support the evaluation of neuroactive environmental chemicals. In this study, we investigated the temporal profiles of behavioral effects in zebrafish *Danio rerio* early life stages after static exposure to neuroactive pharmaceuticals. Zebrafish embryos and larvae up to 5 days post fertilization (dpf) were exposed to venlafaxine (serotonin noradrenaline reuptake inhibitor antidepressant), carbamazepine (voltage-gated sodium channel inhibitor and GABA receptor agonist anticonvulsant) and oxazepam (benzodiazepine derivative anxiolytic) at concentrations in the μg/L range (1 nM -10 μM). The selected compounds are relevant aquatic contaminants, and concentration ranges are representative of environmental situations. Embryos and larvae were exposed for short (during 24 h) and prolonged (starting at day 0) exposure periods, simulating acute and chronic exposure scenarios. Behavior was assessed at embryonic (2 to 3 dpf) and larval (5 dpf) stages. The following behavioral endpoints were measured: photomotor and startle responses (both embryos and larvae), and phototaxis and thigmotaxis reactions (larvae only). Exposure concentrations were measured at the beginning and at the end of exposures by LC-MS/MS. Results of this study are discussed regarding their ecotoxicological implications and environmental relevance. In addition, the methodological aspects for the application of behavioral assays in future ecotoxicological studies are considered.

**MO102 EFFECTS OF NATURAL DYE ERYTHROSTOMINONE AND SYNTHETIC BASIC RED 51 ON ZEBRAFISH EMBRYOS DEVELOPMENT AND LARVAE BEHAVIOR**

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Organic compounds present in natural sources have been used in several experimental conditions aimed to develop safe human health dyes as alternative to replace synthetic dyes, since these are precursors of mutagenic compounds. Nonetheless, in addition to a safe human health dye, an eco-friendly dye is desired to be developed by industries, mainly those involved in the pharmaceutical and cosmetic industry. This study aimed to evaluate the effects of two natural dyes on zebrafish (Danio rerio) embryos and larvae. The selected dyes are Erythrostomine (ERY) and Basic Red 51 (BR51). A preliminary study on the development and growth of embryos and larva was conducted. For embryos, the effect of ERY and BR51 on hatching and total larval number and survival was assessed. For larva, the effects of ERY and BR51 on swimming velocity (mm s⁻¹) and distance travelled (mm) were evaluated, as well as photomotor (random phototactic responses) and startle (startle response) behaviors. The results showed that ERY affected the hatching rate of embryos and ERY and BR51 affected the swimming velocity of larva. In particular, ERY and BR51 affected the swimming velocity of larva, as ERY caused an increase in swimming velocity, while BR51 decreased swimming velocity. Additionally, ERY and BR51 affected the total larval number and survival, as ERY decreased the total larval number and survival, while BR51 increased the total larval number and survival. These results indicate that ERY and BR51 have different effects on zebrafish embryos and larvae, and that ERY and BR51 could be potentially used as an alternative eco-friendly dye.
for application to the continuous and real-time in situ monitoring of water quality. The Asian clam, Corbicula fluminea, a filter-feeding bivalve living in the upper layers of sediments, is an economically important species with a wide distribution throughout the world. Because of their wide distribution, ease of collection, environmental sensitivity, and ability to accumulate high concentrations of heavy metals, C. fluminea has been used as a model species for biomonitoring purposes. The aim of this study was to determine the dose-response relationship of C. fluminea exposed to waterborne copper (Cu), which would enhance one’s ability to design biomonitoring systems for continuous, in situ monitoring of Cu levels in water. Both valve closing and siphon extension were chosen as the behavior under different levels of Cu exposure concentrations, taking into account local variables such as experimental parameters. We linked proposed valve daily rhythmic models and toxicodynamics-based Hill model to predict valve dynamic responses under different Cu exposures with a circadian rhythm. We found Cu detection thresholds of 5.6 (95% CI: 2.1 – 9.3) and 19.5 (14.6 – 24.3) ug L⁻¹ for response times of 300 and 30 min, respectively. We showed that when exposure Cu concentrations exceed 50 ug L⁻¹, the differential degree of valve rhythmic response has a noticeable sensing ability within 30 min. Our study implicates an early warning dynamic biomonitoring system that provides potential in situ detection of waterborne Cu by circadian valve activities in C. fluminea. We conclude that the proposed daily valve rhythm model can be utilized to predict valve dynamic responses under different Cu exposure concentrations, taking into account circadian valve rhythm endpoints, while using a precise valvometry system to validate the simulation.

MO105 Why are mammalian focal species unaffected by chlorpyrifos applications? Integrating data on foraging-behaviour, exposure, and toxicokinetics M. Fouldoulakis, Dow Agrosciences / SRSA ERS; S. Norman, RidgewayEco; C. Wolf, B. Giessing, TierSolutions GmbH; R. Dittrich, TierSolutions GmbH / Wildlife Ecology; N.N. Polsetik, Dow Agro Sciences LLC / Field Exposure and Effects Department; G. Weyman, ADAMA Agricultural Solutions Ltd. Tier 1 wild mammal risk assessment indicates high risk from use of chlorpyrifos (CP) due to its high toxicity to mammals when tested in standard laboratory studies. However, when aiming to conduct a realistic risk assessment, it is important to understand the status and ecology of the mammalian community present in the same ecosystem. In this respect, it is important to know the basic population parameters of species breeding within the treated fields (long term survival and reproductive performance), their foraging behaviour and feeding ecology, and the spatial & temporal movement of the focal species to evaluate factors (both natural and anthropogenic) which may influence reproductive performance. In addition it is equally important to collect exposure and mechanistic data to support and help explain findings in the field. Several field studies have been conducted towards this aim. To gain information on the mammalian communities within the CP-treated fields a variety of methods were used depending on the goal of the study and the species in focus, which allows measurement and ranking of any factors influencing the local mammalian community, including the application of CP itself. The work undertaken over consecutive years gave a good insight into exposure of mammals utilizing treated fields/fruit orchards. In order to support field findings and provide a higher tier risk assessment for mammals, mechanistic information were also provided based on a body burden model describing Toxicokinetics (TK) and Toxicodynamics (TD). This presentation will describe the comprehensive and holistic approach which was followed, the new approaches for mammalian studies in risk assessment over consecutive years of data collection, and will explain why the high risk of mortality predicted by the Tier 1 risk assessment is not seen in the field.

Cost effective and ecologically relevant approaches in environmental toxicology using invertebrate species (P) MO106 Disruption of hypopharyngeal gland function in honey bees exposed to a dietary neonicotinoid (imidacloprid) E. Collison, TSGE Consulting Ltd. / Biosciences College of Life and Environmental Sciences; H. Hird, Fera Science Ltd; C. Tyler, Biosciences College of Life and Environmental Sciences; J.E. Cresswell, The University of Exeter / Biosciences College of Life and Environmental Sciences. The neonicotinoid imidacloprid impairs development of the honey bee (Apis mellifera) hypopharyngeal gland (HPG), but whether HPG function is correspondingly disrupted has been unstated, which undermines the ecological relevance of HPG-related assays in pesticide risk assessment. To determine whether imidacloprid indeed disrupts HPG function, we therefore implemented laboratory and field studies, using enzymatic, molecular and behavioural endpoints. Specifically, we investigated whether dietary exposure affected temporal polyethism, using Radio Frequency Identification (RFID) technology to track hive traffic in free-flying bees. In the laboratory, we investigated whether dietary exposure affected the enzyme systems that support larval nutrition (major royal jelly proteins, or MRJPs) and social immunity (glucose oxidase, or GOX).

Overall, we demonstrated that dietary exposure to imidacloprid is potentially capable of causing generalised disruption of HPG function, which implies that imidacloprid-induced changes in HPG development can lead to detrimental effects on colony function. We believe this provides some overall support for the ecological relevance of HPG-related assays in pesticide risk assessment involving sublethal effects on honey bees. It is evident that further work is needed to test the field-relevance of some of our findings (namely imidacloprid-induced disruption to nutrition and social immunity), as in some cases (temporal polyethism) transcriptional responses that we observed did not translate into functionally significant impacts on bee health, highlighting the need to move from focus gene assays to transcriptomics with ecologically relevant behaviour. Our work highlights the potential utility of new enzymatic, molecular and behavioural ecotoxicological endpoints for risk assessment of sublethal effects on honey bees, if further work can clarify their ecological relevance to colony-level impacts on bee health under more field realistic exposures.

Deciphering mechanisms of malathion toxicity under pulse exposure of the freshwater cladoceran Daphnia magna L.N. Trac, Danish Technical University; O. Andersen, Roskilde University; A. Palmquist, Roskilde University / Department of Environmental Social and Spatial Change

The organophosphate pesticide (OP) malathion is highly toxic to freshwater invertebrates including the cladoceran Daphnia magna, a widely used test organism in ecotoxicology. To assess whether toxic effects of malathion are primarily driven by exposure concentration or by exposure duration, Daphnia magna was pulse exposed to different integrated doses (duration x concentration), i.e. 3h x 16 µg/L, 24h x 2 µg/L and 48h x 1 µg/L. Following recovery periods of 3h, 6h, 24h and 48h the toxicity of malathion on different biological levels in Daphnia magna was examined by analyzing the following endpoints: survival and immobilization; enzyme activities of Acetylcholinesterase (AChE), Carboxylesterase (C Esth) and Glutathione S-transferase (GST); and Acetylcholinesterase gene expression at the transcriptional level measured by qPCR. The results showed no difference in survival among equivalent integrated doses. Adverse effects were driven by exposure concentration rather than duration. Specifically, short pulse exposure to high concentration of malathion (3h x 16 µg/L) resulted in more immobilized daphnids, lower AChE and C Esth activities and higher transcript level of AChE gene compared to long pulse exposure to low concentration (48h x 1 µg/L). The expression of the AChE gene was up-regulated, indicating a compensatory mechanism to cope with inhibition of the enzyme. In addition, for this OP insecticide, the severity of the effects increased during the postexposure recovery period. The results of this study indicate that Environmental Risk Assessment based on the standard OECD immobilization test may underestimate OP risk in certain situations, and emphasize the relevance of employing a realistic exposure test scenario. The study has helped elucidate the response mechanism at the molecular level of the target enzyme for OP pesticides, which is still questionable in previous studies. More work on enzyme P450 (CYP), C Esth and GST regulation, and the duration after exposure could further improve the understanding of molecular responses of these enzymes to toxicity of OPs in Daphnia magna.

MO108 Does long-term fungicide exposure affect the reproductive performance of leaf-shredders? P.T. Baudy, University of Koblenz-Landau / Institute of Environmental Sciences; J. Zuberb, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; M. Konschak, University Koblenz-Landau / Institute for Environmental Sciences; M. Weil, ECT Oekotoxikologie GmbH / Institute for Technology Assessment and System Analysis ITAS; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment Lead. Coordinating amphiplay a critical role in the ecosystem function of leaf litter breakdown, a key process in detritus-based aquatic ecosystems. However, these organisms’ fitness and functioning may be adversely influenced by exposures to fungicides, a group of pesticides targeting conserved sites of toxic action, while the effects on shredders’ reproductive performance have not yet been assessed. To fill this knowledge gap, a semi-static 56-day partial life-cycle bioassay with Hyalella azteca (ten animals/rePLICATE; n=30) was performed. Two field relevant levels of a fungicide mixture (at a low and a high sum concentration of 5 and 25 µg/L, respectively) were tested, whereby the single fungicide concentrations in the low sum concentration (1 µg/L each) corresponded approximately to the fungicides’ regulatory acceptable concentrations (according to the European Union’s environmental risk assessment; EU-ERA). Endpoints related to the energy processing (leaves consumption and feces production), the
growth, and the reproduction (amphipods, crabs, and snails) off the offspring of the amphipods were assessed. While leaf consumption was unaffected, exposure to both fungicide treatments significantly reduced amphipods’ fecundity (∼20%) compared to the control, which may be indicative of a decreased food utilization to compensate for stress-related energy demands. However, this mechanism did not fully compensate for the higher energy demand and might have caused trade-offs in the energy allocation among physiological processes: while growth remained unaffected, amphipods forming amphixs were less abundant in both fungicide treatments (only significant in the high treatment). As a result, time to release of first offspring was delayed in both treatments and the number of offspring was significantly lower in the high treatment, whereas offspring length was unaffected. Moreover, a 5-10% increase in mortality was observed in both fungicide treatments compared to the control, which may suggest a reduced allocation of energy to maintenance. The results of this study indicate that chronic fungicide exposures can negatively impact shredders’ reproductive performance and fitness by reducing the abundances and thus a contribution to leaf litter breakdown, which may have detrimental consequences for detritus-based food webs. Moreover, this study provides further proof that the EU-ERA procedures for fungicides may not safeguard aquatic ecosystems.

MOI09
Using in-situ bioassays with the aquatic snail Physa acuta to assess impacts of wildfires on water quality
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Wildfire is a common phenomenon in South Europe, namely in Mediterranean countries as Portugal, where over the last decade an average of 144,000 ha burnt per year. It is known that wildfires affect aquatic systems altering both physical and chemical properties through the increased bioavailability and transportation of pyrolytic substances such as polycyclic aromatic hydrocarbons (PAHs) and metals associated to ash/solid loads. Hence, river ecosystems, particularly its biota, require a closer examination regarding wildfire effects. Once in situ assays address more realistic environmental multi-stress situations, an in situ bioassay at a Portuguese recent burnt area (Miranda do Corvo, Coimbra) was performed to test reproductive effects by using the aquatic snail Physa acuta. Reproductive parameters, such as the total number of eggs, egg masses per snail, average of eggs per egg masses and the average of eggs and masses per snail were the end points tested at the end of the 8 days of exposure. The assay was performed at the time of the first major rainfall events and comprise four distinct sites: one reference site located in Ceira river upstream the burnt area (RUS); and three other sites, two of them located within the burnt area in tributary streams (SDS and SUS) and one downstream the burnt area in the Ceira river (RDS). Test chambers were elaborated accordingly to the needs of the pulmonate snail, allowing flowage and luminosity entrance. All the endpoints assessed indicate that RUS (the reference site located upstream the burnt area) was the site where reproduction was less affected, while SDS (a stream within the burnt area) was the site with lower reproductive data. The downstream the burnt area was the worst affected, requiring a closer examination to evaluate their impact in many aspect of lives. MCI/MI have been used extensively and effectively as a preservative since the 1980s with a broad spectrum of activity against fungi, yeasts and bactera at low concentrations. Since the millennium, MI has been used as an individual preservative alongside the MCI/MI mixture and other biocides and to some extent as a replacement of MCI/MI. The reason may be that MCI/MI is on the list of dangerous substances (i.e. R43/H317) and has to be declared when present in concentrations >15 ppm in cosmetics. No such requirements were specified for MI up until 2005 where the maximum concentration in cosmetics were set at 100 ppm, a 25-fold increase from when it was in combination with MCI in the 3:1 ratio having a maximum concentration of 3.75 ppm. However, in 2015 EU declared a limit of 15 ppm MI in rinse-off cosmetics and no safe limit was specified for leave on cosmetics. In industrial products there is also a self-classification system with a limit of 0.1% (1000 ppm), but it is not regulated as the self-classification name implies. Taking all of these sources into consideration the concentration of these compounds in water bodies may be higher than previously assumed and therefore pose a possible risk to the freshwater invertebrates living there. Hyalella azteca is an example of a freshwater amphipod that has well-established model organism in ecotoxicology. This study aims to investigate the effect of MI on H. azteca, as well as to explore MI biotransformation by H. azteca. Effects are assessed as changes in mobility and survival, and cellular reactions to reactive oxygen species (i.e., catalase induction, superoxide dismutase activity and lipid peroxidation) after MI exposure. Biotransformation is investigated by CYP induction and GST activity as well as disappearance rate with and without H. azteca present. Results from this ongoing study will be presented and discussed in relation to the current regulations.

MOI11
Evaluation and improvements of a mayfly, Neocloeon (Cloeon) triungulifer (Ephemeroptera: Baetidae) toxicity test method.

A recently published test method for Neocloeon triungulifer assessed the survival and growth of larval mayflies exposed to several reference toxicants (NaCl, KC1, and glucose). Results were compared to a previous published toxicity test method (4). To identify potential sources of variability, the authors measured 7-day growth and survival in NaCl, using: (a) 1- vs. 3- vs. 5-day-old larvae, (b) 1- vs. 3-day-old larvae hatched from freshly collected eggs (not stored at cold temperatures) vs. eggs held in long-term storage at 4°C, and (c) 1- vs. 3-day-old larvae from five different laboratory cultures (two U.S. EPA, one USGS, one University of Guelph, and University of Aveiro). Fifty percent lethal concentration (LC50, mg/L) and 25% inhibition concentration (IC25, µm) point estimates were utilized to quantify survival and growth, respectively. Statistically significant differences in NaCl LC50s were observed among different ages of mayflies at test initiation (LC50=615.56, 989.43, 1667.24), with older organisms being less sensitive. No significant differences in growth could be elucidated between different aged treatments. Survival and growth of freshly collected eggs (LC50=701.27, LC50=626.56) differed significantly from eggs stored at 4°C for either three (LC50=594.13, 254.24) or four (LC50=524.18, IC25=361.28) months, though no significant differences were observed between stored cohorts. A comparison of freshly collected eggs of 4 the different laboratory cultures tested demonstrated substantial differences in survival or growth. Additional results will also be presented on larval age, storage, and laboratory source comparisons with KC1 and CuSO4, and comparisons of freshly collected eggs vs 2 month old stored eggs at 10°C. To ensure consistent and sensitive test results we recommend continuing the use of 1-day-old larvae when using this species.

MOI12
Toxic effects of wildfires on aquatic systems: in situ bioassays
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Wildfires constitute a diffuse source of contamination of aquatic systems affecting water quality, through the production and transport of pyrolytic substances such as polycyclic aromatic hydrocarbons (PAHs) and metals associated to ash/solid loads. However, the effects of these compounds from recently burnt areas on the aquatic biota have been poorly studied. Hence, the objective of this study was to assess the ecotoxicological effects of wildfires in aquatic systems. A sampling area, recently burnt, was selected near Miranda do Corvo (Portugal). Five sites were selected: two in the main water course of the watershed - Ceira river, being one located upstream (RUS) and the other downstream (RDS) the burnt area ; two tributary streams within the burnt area (SURE and SUS); and finally one in a stream at a remote located area (CS) (Figure 1). First rainfall events after the wildfire, distinct groups of organisms, including the water flea Daphnia magna, the freshwater shrimp Atyphaera desmarei, the freshwater clam Corbicula fluminea and the mosquito fish Gambusia holbrooki were exposed in situ, in all five sites, using test chambers. With exception of D. magna, all other tested organisms can be found in Ceira river. After an experimental period of two days with a post-exposure feeding inhibition were evaluated. Feeding depression after exposure time was selected as a sub-lethal endpoint because it is a quick, sensitive and ecologically relevant indicator of toxic stress. The results showed a very low mortality for all the species and sites, thus this lethal parameter was not sensitive to discern differences among the assessed sites. Conversely, the sub-lethal post-exposure feeding inhibition, revealed a low feeding rate in streams within the burnt area (SURE and SUS), that seemed to be the most affected
MO113 Influence of surfactants in the bioaccumulation kinetics and metabolism of selected pharmaceuticals in benthic invertebrates
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Nowadays, it has been widely demonstrated that elimination of many pharmaceuticals during conventional wastewater treatment is incomplete. Therefore, despite at low concentrations, these bioactive compounds are being discharged in natural waters on a regular basis and are consequently considered as semi-persistent contaminants. The structural and functional equilibrium of freshwater ecosystems could be compromised by their environmental presence. However, data on this regard is still insufficient for a full risk assessment, particularly on their direct or indirect effects in the first trophic levels. Surfactants, on the other hand, are used in much higher quantities and also released into sewage systems or directly in surface waters. Their environmental presence and potential ecotoxicity have been thoroughly studied. Benthic invertebrates are key in the functioning of freshwater ecosystems, and they metabolise a broad range of pollutants (in nutrient recycling and represent a relevant food source for other invertebrates and fish). The amphipod Gammarus fossarum is frequently used as sentinel species of water quality, as it is widespread in western Europe and its physiology is well known. Recent works have shown the bioaccumulation of selected pharmaceuticals in gammarids, which has been widely addressed either their bioaccumulation kinetics or their capacity to metabolise the toxicant. The main goal of this work is to study the role of a mixture of frequently detected detergents in the bioaccumulation kinetics of selected pharmaceuticals in G. fossarum, evaluating their potential synergic or antagonistic effects. Laboratory exposure experiments for both pharmaceuticals and detergents (concentration ratio 1:25) were set up for three individual compounds: the anxiolitic oxazepam, the anti-inflammatory ibuprofen and the antibiotic sulfamethoxazole. The feeding rate was selected as end-point of the experiment. Gammarids samples were processed using microQuEChERS extraction and pharmaceuticals bioaccumulation and metabolism was followed-up by means of nanoliquid chromatography coupled to MS/MS (nanoLC-MS/MS) and high resolution mass spectrometry (nanoLC-HRMS), aiming to elucidate potential transformation products. Kümmerer, K. Springer Ed. 2008 Ying, G. et al. Environ. Int. 32(2006), 417-431 Besse, J.P. et al. Water Res. 47(2013) 650-660 Berloz-Barbier, A. et al. J. Chrom. A 1367 (2014) 16-32 Miller, T. et al. Sci. Of the Total Environ. 511(2015) 153-160

MO114 Eliminating Stressors in Laboratory Tests with Chaoboridae and Corixidae
F. Joyce, S. Taylor, Cambridge Environmental Assessments

The sensitivity of organisms tested under laboratory conditions may differ significantly to that observed in their natural environment. Standard procedures for laboratory invertebrate bioassays operate under optimal laboratory conditions specified in OECD guidelines, which are generally ‘sterile’, e.g. void of natural refugia and food. However, when using feral organisms, the change from field to laboratory conditions can cause additional stress, thus potentially affecting the outcome of acute toxicity tests for use in regulatory risk assessments of plant protection products. The need for test modifications for some taxa based on individual species life-histories and habitat preferences are important to consider for successful testing of non-standard invertebrates. For example, the acclimatisation period and testing conditions in the laboratory can be modified depending on the requirements of different species in their natural environment and their response to stressors such as light levels and temperature. Here we will present examples taken from two invertebrate taxa (Chaoboridae and Corixidae) for which control mortality during acute toxicity tests performed by Cambridge Environmental Assessments was anomalously high in comparison to standard organisms. We will discuss the subsequent research and trials carried out to mitigate non-chemical stress and increase control survival and reliability, and also provide recommendations for the potential development of standard operating procedures for non-standard invertebrate work.

MO115 Cultivating and Testing Early Life-History Stage Larvae of Chaoborus crystallinus and Cleopatra dinemor
M. Allen, S. Taylor, A. Howells, F. Joyce, F. Radford, Cambridge Environmental Assessments

The juvenile stage of freshwater invertebrates are regarded as the most sensitive life stage to the toxic effects of exposure to Plant Protection Products (PPPs). Standard invertebrate testing approaches e.g. Daphnia Magna(OECD 202), Chironomid (OECD 235) use Here we will present the results of the development of testing approaches for two non-standard invertebrates, the mayfly (Cloeon dipterum) and the phantom midge (Chaoborus crystallinus) using specific life stages. Organisms were collected from outdoor mesocosms at Cambridge Environmental Assessments (CEA) and cultured in the laboratory. Mayfly and Chaoborus cultures were maintained through the laboratory with mean cages so that adults would emerge and re-lay their offspring within the cultures. As this method was unsuccessful we attempted a range of alternatives to breed these species under controlled conditions. For example, breeders of de-ionized water were added to the Chaoborus culture cages and a period of low light at dawn and dusk was introduced to encourage oviposition by both species. This proved temporarily successful for Chaoborus, however continued breeding did not occur. Mayflies failed to breed under the different conditions. In order to standardise our higher tier tests, larval stages 1 and 2 for mayflies were identified and Chaoborus within the size range 5-10 mm were used within the acute toxicity test. Although the results of this work did not meet the objectives that we set to achieve (i.e. organisms of a known age were not able to be used), this presentation will show our experiences and also recommend next steps for further refinement of culturing and testing methods.

MO116 Magnetic particles' effects on survival and hatching of the rotifer Brachionus calyciflorus and the growth of the Chorrella sp. alga. M. Salcedo, I. De Vicente Alvarez-Manzana, University of Granada / Ecology

The main objective of this research is to study the possible toxicological effects of iron magnetic microparticles on the rotifer Brachionus calyciflorus and the freshwater phytoplankton Chlorella sp. These magnetic microparticles have recently been proposed for the restoration of eutrophic aquatic ecosystem, for remove the phosphorus located in them. With the aim of carrying out the toxicological test on B. calyciflorus, it was followed ASTM protocol. During 24 hours the organisms were in contact with different concentrations of magnetic particles, analyzing the mortality of the organisms. On the other hand, a test about the rotifer s eggs hatching in contact with dissolved iron from the particles was carried out. In the case of the test with the alga Chlorella sp., OECD’s standardized protocol was followed. This test last 72 hours and they were used different concentrations of magnetic particles, after these 72 hours the inhibition growth of the alga was calculated. The results obtained were a CE50 of 1.63 g/l of iron for the rotifers and 0.085 g/l for Chlorella sp., the alga was more sensitive to the particles than planktonic organism being 100% of algal growth inhibition from 0.5 g/l. Hatching of Brachionus calyciflorus eggs in contact with the dissolved iron was total.

MO117 Effects of Chernobyl-derived radionuclides on fluctuating asymmetry and fecundity in Asellus aquaticus: 30 years on N. Fuller, University of Portsmouth / Biology; J.T. Smith, University of Portsmouth / School of Earth and Environmental Sciences; A. Ford, University of Portsmouth / Biological Sciences

The effects of long-term, environmentally relevant doses of radiation on biota remain unclear owing to a lack of studies following chronic exposure in contaminated environments. Such studies are necessary to elucidate and mitigate the impacts of both planned and accidental releases of anthropogenic radioactivity on the environment. This field study aimed to determine the effects of chronic exposure to environmental radionuclides using an aquatic crustacean as a test organism. The effects of radionuclides on the freshwater amphipod that is a well-established model organism in ecotoxicology. As the self-classification name implies. Taking all of these sources into account, the freshwater animal was cultured in the laboratory. Results from this comparison show that Asellus aquaticus is frequently used as sentinel species of water quality, as it is widespread in western Europe and its physiology is well known. Recent works have shown the bioaccumulation of selected pharmaceuticals in gammarids, which has been widely addressed either their bioaccumulation kinetics or their capacity to metabolise the toxicant. The main goal of this work is to study the role of a mixture of frequently detected detergents in the bioaccumulation kinetics of selected pharmaceuticals in G. fossarum, evaluating their potential synergic or antagonistic effects. Laboratory exposure experiments for both pharmaceuticals and detergents (concentration ratio 1:25) were set up for three individual compounds: the anxiolitic oxazepam, the anti-inflammatory ibuprofen and the antibiotic sulfamethoxazole. The feeding rate was selected as end-point of the experiment. Gammarids samples were processed using microQuEChERS extraction and pharmaceuticals bioaccumulation and metabolism was followed-up by means of nanoliquid chromatography coupled to MS/MS (nanoLC-MS/MS) and high resolution mass spectrometry (nanoLC-HRMS), aiming to elucidate potential transformation products. Kümmerer, K. Springer Ed. 2008 Ying, G. et al. Environ. Int. 32(2006), 417-431 Besse, J.P. et al. Water Res. 47(2013) 650-660 Berloz-Barbier, A. et al. J. Chrom. A 1367 (2014) 16-32 Miller, T. et al. Sci. Of the Total Environ. 511(2015) 153-160

MO118 Effects of Chernobyl-derived radionuclides on fluctuating asymmetry and fecundity in Asellus aquaticus: 30 years on 155
Culturing and Testing Early Life-History Stage Larvae of Asellus, Crangonyx, Lymnaea
F. Pickering, S. Taylor, A. Howells, F. Radford, Cambridge Environmental Assessments

The conduct of acute aquatic toxicity testing in the laboratory provides an efficient means of predicting the effects chemicals may have in the environment. Standard invertebrate testing approaches use age specific organisms e.g. Daphnia magna (OECD 202, 2004) and Chironomus (OECD 235, 2011) to eliminate potential differences in sensitivity at varying life-stages. However, there is an increasing need for the development of standard culturing and age recommendations for non-standard invertebrates as higher throughput of testing is required. The results of recent work carried out by Cambridge Environmental Assessments (CEA) in this area for culturing age specific non-standard invertebrate species for use in acute laboratory studies. A range of freshwater species were selected for age specific culturing and testing namely: Lymnaea stagnalis, Crangonyx peregrinipes, and Asellus aquaticus. Organisms were collected from outdoor mesocosms and bred in the laboratory and juveniles were isolated periodically to ensure age specific juveniles for testing. We present the methods used for collecting, acclimating and culturing these freshwater invertebrates and also make recommendations based on the suitability of organisms selected and recommended ages for use in testing. In addition, we will highlight the need for any further research and development required.

MO119
Redoing a simple and precise methodology to assess filtering rate quantification for bivalve: Neutral red dye
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Neutral red is a weak cationic dye soluble in water, with a low toxicity for almost all classes of organisms, for which has been used as histological and vital stain since early twentieth century. Estimating the amount of water filtered by bivalves is one of the earliest applications of neutral red. However we found less than twenty studies applying this methodology since its description in 1954. The aim of our study was to optimize the methodology of filtering rate based on the solution of neutral red by addressing the effect of i) acidifying pH; ii) holding temperature before spectrophotometric reading; and time iii) before and iv) after acidification of solutions of neutral red used for filtering assays. Furthermore, as a case study we fine-tuned the filtering assay for the estuarine bivalve, Cerastoderma edule, as potential species to be used for estuarine monitoring purposes. Results showed not statistically differences in absorbance of neutral red solutions holding at 4 or 20°C, neither in solution acidiﬁed between the range of pH 4.5. However, absorbance at 545 nm decreased signiﬁcantly as pH of solutions increase at pH 6. The time before acidification has not a signiﬁcant effect on absorbance. However, once the neutral red solution is acidiﬁed, the absorbance of the solution tends to decrease over time for which is recommended read the absorbance in the first 24h. Finally, our results showed that the sensitivity of this technique decrease at low concentrations, for which is recommended used neutral red concentration enough to allow, after the filtering period, final concentrations above 0.5 mg/L. For C. edule the filtering condition the individual was exposed to ≥100 mg/mL of neutral red dye during 30 min period in dark conditions. The Cd 48-h EC50 postexposure filtering for C. edule was 3.4 fold lower than the 48-h LC50 (0.33 vs 1.13 mg/L, respectively), confirming filtering rate as a more sensitive and cost-effective endpoint for this bivalve than mortality. The bioassay using the bivalve C. edule based on postexposure filtering rate based on this simple colorimetric technique appears to have potential as a tool for environmental estuarine assessment. Future development of field assays based on postexposure filtering rate (namely, in situ assays) with this species is promising to use in monitoring programmes. We expect that the new harmonized protocol became in a widely used cost-effective tool for monitoring filtering rates.

MO120
Earthworms (Eisenia fetida) response to short-term exposure to triclosan
J. Zaltauskaite, Vytavas Magnus University / Department of Environmental Sciences; D. Miskelyte, Vytavas Magnus University / Department of Environmental Science.

Triclosan (TCS) is a broad-spectrum antimicrobial and antifungal agent and it is a common constituent of household and personal care products. TCS widespread use has resulted in its introduction into environment and has raised concerns regarding possible adverse effects on terrestrial and aquatic organisms. Earthworms Eisenia fetida were exposed to 10-730 mg/kg of triclosan for 14 days. Mortality, behavioral changes including the appearance of catatonic clumps, superoxide dismutase (SOD), glutathione reductase (GR) and malondialdehyde (MDA) were examined after 4, 7 and 14 days. None of the tested TCS concentrations evoke earthworm mortality during the experiment. Triclosan exposure reduced the growth of earthworms. Short time exposure (up to 14 days) showed that highest concentration induced cataloplate accumulation and by increasing TCS exposure time cataloplate activity was decreasing. Highest superoxide dismutase and glutathione reductase activity was detected after 7 days exposure of triclosan.

MO121
Passive dosing of hydrophobic organic chemicals in toxicity tests with Caenorhabditis elegans

The standardized toxicity test with the nematode Caenorhabditis elegans (ISO 10872) is widely used for toxicity testing of chemicals. Chronic toxicity endpoints such as reproduction and survival are very sensitive as well as first sublethal sensitivity and ecological relevance compared to acute mortality. Due to its simple, cost-efficient cultivation and short generation time, C. elegans is particularly suitable for high-throughput toxicity testing. Testing sub-lethal parameters such as reproduction requires feeding with Escherichia coli cells during the test. However, the bacteria can act as sorptive sink and, therefore, reduce the freely dissolved, bioeffective concentration (C_{ELE}) of hydrophobic organic chemicals (HOCs). This results in poorly defined exposure conditions and reduced test sensitivity when actively spiking the HOCs into the test medium by using an organic solvent as solubilizing agent. Passive dosing is a promising technique for controlling and maintaining constant C_{ELE} in small-scale toxicity tests. Hence potential decreases of C_{ELE} are compensated by continuous partitioning of chemicals from a saturated reservoir. This study investigated the applicability of passive dosing of HOCs from silicone O-rings in the chronic C. elegans toxicity test. 10 polycyclic aromatic hydrocarbons (PAHs) that cover a broad range of hydrophobicity were tested as model chemicals. During all experiments, C_{ELE} was reduced by a significant amount as compared to the test chemical concentration in control treatments. Apart from C_{ELE} the SPME proved to be suitable for verifying C_{ELE}.
food influences) and to provide benchmarks to define acceptable pollutant concentrations in water, sediment, and diets. Field confirmation of benchmarks predicted by laboratory toxicity tests is important in determining the significance of pollutants and in design of ameliorative measures.


Total Dissolved Solids (TDS) dosing studies representing different sources of ions were conducted from 2011-2015. Emergence responses in stream mesocosms were compared to single-species exposures using a whole effluent testing (WET) format and an ex-situ method (single species tests in containers receiving mesocosm water). The first 4 years used a dose-response design for each year’s TDS recipe, which differed based on the relative dominance of major ions. The 2015 experiment tested three of the previous year’s recipes dosed simultaneously but at only one level of moderate specific conductivity (ca. 850 μS/cm). A mayfly, Neocloeon triangulifer, was incorporated for testing alongside the standard WET test organisms. Other ex-situ tests corroborate results sensitive to other standard test organisms, but there were differences in sensitivity between the ex-situ method and WET tests. N. triangulifer’s sensitivity in the ex-situ exposure reflected that of mayfly emergence from the mesocosms. Caddisfly emergence was generally as sensitive to TDS exposures as mayflies in 2011-2014 tests, although less sensitive than the tests when TDS was dominated by Ca salts. However, caddisfly emergence was not significantly different from the controls and therefore, not as sensitive as mayfly emergence for the 2015 constant conductivity study. Chironomids were less sensitive to excess TDS than Ephemeroptera and Trichoptera taxa when TDS was dominated by Cl and NaHCO₃, but appeared more sensitive when the TDS was a mixture of SO₄²⁻ and HCO₃⁻. Mayfly emergence was the only emergent endpoint that demonstrated a clear and consistent adverse response to excess TDS. Emergence in the control was always the highest and sulfite the lowest. Cl and HCO₃ were intermediate and similar in response, but overall significantly lower than the control. In the WET format, mayfly growth in bench tests was: control > Cl > SO₄ > HCO₃. For the 2015 experiment, when Cl, HCO₃, and SO₄ were adjusted to establish a conductivity of approximately 850 μS/cm, the 20% hazardous concentration (HC20) indicated that deleterious effects to mayflies occurred at this conductivity regardless of ion composition. These results suggest that stream benthic communities are significantly more sensitive to excess TDS from coal mining activities that leave leachable spoils rich in carbonate and sulfate than to produced waters emanating from deep oil and gas wells that tend to be dominated by sodium and calcium chloride.

MO124 Comparative toxicity assessment of lead on two Daphnid species G. Araujo, Universidade de Aveiro / Biology; C. Pinheiro, Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; D. Abessa, Unesp / Marine Biology and Coastal Management; S. Loureiro, Universidade de Aveiro / Biology

Water is an essential resource for humans, economy and ecosystems and often can be under risk of contamination. Chemical contamination of anthropogenic source is generally related to industry, agriculture and urban activities. Lead is a naturally occurring metal, but high levels can be found in the environment due to anthropogenic activities. Thereby, this study aimed to compare the toxicity of lead to two Daphnids species from different climate scenarios. To accomplish this goal three toxicity tests were performed with both species. To predict the lethal concentration (LC) of lead to these organisms an acute immobilisation test (OECD 201) was carried out. Regardless sublethal effects, Pb effects on the reproductive output and female growth was achieved through the reproduction tests (OECD 211). In addition, and looking at one of the functional traits of daphnids as filter feeders, feeding inhibition tests were carried out based on the protocol developed by McWilliam & Baird (2002). Comparing lethal concentrations for D. magna and D. similis it is noteworthy that the LC₅₀ values were similar, with D. magna showing a slightly higher value (LC₅₀=0.45mg/L) than D. similis (LC₅₀=0.36mg/L). Regarding the chronic assay, D. magna was more influenced by Pb on the reproductive output and D. similis on growth. A similar pattern was also attained in the feeding inhibition tests. According to the obtained results we can see that Pb affects both daphnids in different ways, as also shown for other chemical compounds.

MO125 The application of the Weight of Evidence Approach as an integrative tool for ecotoxicological risk assessment in Iberian rivers N. De Castro-Catalá, Universitat de Barcelona / Ecology; M. Kuzmanovic, CSIC Spanish National Research Council; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; C. Barata, CSIC / Environmental Chemistry, I. Muñoz, University of Coimbra / Department of Life Sciences; P. Acevedo, Instituto de Investigación en Recursos Cinegéticos / SETAC Europe 26th Annual Meeting Abstract Book

Passive dosing of hydrophobic organic chemicals in toxicity tests with Escherichia coli and Cerastoderma edule is a promising technique for controlling and maintaining constant Cfree in small-scaled toxicity tests. However, caddisfly emergence was not significantly different from the controls and therefore, not as sensitive as mayfly emergence for the 2015 constant conductivity study. Chironomids were less sensitive to excess TDS than Ephemeroptera and Trichoptera taxa when TDS was dominated by Cl and NaHCO₃, but appeared more sensitive when the TDS was a mixture of SO₄²⁻ and HCO₃⁻. Mayfly emergence was the only emergent endpoint that demonstrated a clear and consistent adverse response to excess TDS. Emergence in the control was always the highest and sulfite the lowest. Cl and HCO₃ were intermediate and similar in response, but overall significantly lower than the control. In the WET format, mayfly growth in bench tests was: control > Cl > SO₄ > HCO₃. For the 2015 experiment, when Cl, HCO₃, and SO₄ were adjusted to establish a conductivity of approximately 850 μS/cm, the 20% hazardous concentration (HC20) indicated that deleterious effects to mayflies occurred at this conductivity regardless of ion composition. These results suggest that stream benthic communities are significantly more sensitive to excess TDS from coal mining activities that leave leachable spoils rich in carbonate and sulfate than to produced waters emanating from deep oil and gas wells that tend to be dominated by sodium and calcium chloride.

MO126 Effects of Copper-Chromium-Borate wood preservative at different level of biological organization of the earthworm E. andrei S. Campiche, M. Visse, E. Grand, Swiss Centre for Applied Ecotoxicology; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; B. Ferrari, Ecocentre Centre CH

Earthworms are commonly used organisms in ecotoxicology to assess soil quality or effect of chemicals on the soil fauna. In this study, the effects of a biocide, used for outdoor wood preservation and containing a mix of chromium, copper and borate (CuCrB), were evaluated at different levels of biological organization using the earthworm Eisenia fetida. Reproduction and behavioral endpoints were assessed using standardized ecotoxicological tests. Additionally and considering the important role earthworms play in organic matter breakdown, functional impacts were evaluated by measuring the feeding activity of the earthworms exposed to the biocide. For this purpose, the usefulness and feasibility of using the batch laboratory method, which may be seen as a partial compromise between the “hurt” (biotest) and “hurt” (bioassay) approaches, was evaluated. Results indicated that earthworms were not significantly different from the controls and therefore, not as sensitive as mayfly emergence for the 2015 constant conductivity study. Chironomids were less sensitive to excess TDS than Ephemeroptera and Trichoptera taxa when TDS was dominated by Cl and NaHCO₃, but appeared more sensitive when the TDS was a mixture of SO₄²⁻ and HCO₃⁻. Mayfly emergence was the only emergent endpoint that demonstrated a clear and consistent adverse response to excess TDS. Emergence in the control was always the highest and sulfite the lowest. Cl and HCO₃ were intermediate and similar in response, but overall significantly lower than the control. In the WET format, mayfly growth in bench tests was: control > Cl > SO₄ > HCO₃. For the 2015 experiment, when Cl, HCO₃, and SO₄ were adjusted to establish a conductivity of approximately 850 μS/cm, the 20% hazardous concentration (HC20) indicated that deleterious effects to mayflies occurred at this conductivity regardless of ion composition. These results suggest that stream benthic communities are significantly more sensitive to excess TDS from coal mining activities that leave leachable spoils rich in carbonate and sulfate than to produced waters emanating from deep oil and gas wells that tend to be dominated by sodium and calcium chloride.

MO127 Planarians in ecotoxicology: Effects of phenanthrene on Dugesia tigrina F. Simão, Aveiro University & CESAM; C. Gravato, A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; J. Pestana, CESAM & University of Aveiro / Department of Biology and CESAM

Freshwater planarians are important models for developmental and regeneration research and are being redeemed as useful animals in neuropharmacology and
ecotoxicology. Such interest is based on their unique characteristics, such as a stem cell population that originates all other cell types and provides them with the ability to regenerate complete body parts including their nervous system which has similarities to that of vertebrates. Furthermore, a plethora of endpoints can be easily measured and quantified in these flatworms, including behavioral, regeneration, reproduction, and single biochemical parameters. Therefore, planarians have the potential to provide new insights on the effects of some well-studied and ubiquitous compounds, such as polycyclic aromatic hydrocarbons (PAHs). The sexual planarian Dugesia tigrina, a common freshwater predator, was used to study the effects of phenanthrene. The estimated 96-hour LC50 for phenanthrene was 830.62 μg/L, while acute LC50 for head loss was 483.06 μg/L. Sub-lethal endpoints such as locomotion, feeding and regeneration were measured over 8-day chronic exposures. After 8 days, behavior of animals exposed to the highest phenanthrene concentrations was affected, with exposed animals travelling smaller distances and showing toxic anoxia in comparison to controls. This study demonstrates the need for in vitro toxicity assessment on the peripheral olfactory system and the sexual behavior, we studied the impact on the female sex hormone system. In the frame of the ANR DISCO project (endocrine disruption), we then focused on its effect on the female sex hormone system. In the frame of the ANR DISCO project (endocrine disruption), we then focused on its effect on the female sex hormone system.
that exposure to this PAH can result in adverse effects to phenanthrene concentrations was affected, with exposed animals travelling chronic exposures. After 8 days, behavior of animals exposed to the highest concentrations would reduce the percentage of males at 1ng DEHP. In the frame of the ANR DISCO project (endocrine control in our species) and could be potentially disrupted by DEHP. DEHP at population scale, we then focused on its effect on the female sex regeneration, reproduction and biochemical parameters. Therefore, planarians have high quality toxicity data for substances which are less stable under OECD conditions. In the natural environment, metals are not renewed during the 72h exposure. Even if this method is sufficient for evaluating soil algal bioassay. Growth zone, biomass, and photosynthetic activity of representative soil alga were measured and quantified in these flatworms, including behavioral, ability to regenerate complete body parts including their nervous system which was 830.62 g/L, while acute EC50 for head loss was 483.06 g/L. Sub-lethal exposure to this PAH can result in adverse effects to phenanthrene concentrations was affected, with exposed animals travelling chronic exposures. After 8 days, behavior of animals exposed to the highest concentrations would reduce the percentage of males at 1ng DEHP.

MO135
Effect of heavy metals on invasive knotweed (Fallopia spp.) and the combined effect of metal pollution and plant colonization on MultiDrug Resistance (MDR) phenotypes in soil bacteria

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The presence of heavy metals in the environment, which is largely due to anthropic activities, represents a stress for all living organisms, impacting ecosystem structure and function. At the microbial scale, the cellular toxicity of metals could alter microbial communities by enhancing MDR bacterial phenotypes through mechanisms of resistance co-selection [1]. Some plant species are able to grow on heavy metal polluted soil; this is the case of the invasive complex Fallopia which is able to accumulate metals, leading this plant to be dominant in high polluted urban environment [2]. On the other hand plants are known to be major drivers of soil microbial communities structure and functioning through root exudation [3], and Fallopia has been shown to exert significant effects on soil bacterial communities through its secondary metabolites content [4]. In this context we wanted to evaluate the effect of metal pollution on Fallopia below-ground secondary metabolism, and the combined effect of metal pollution and plant colonization on soil bacterial MDR resistance phenotypes. In this aim, we undertook mesocosm experiments where rhizome fragments of Fallopia spp. were grown in greenhouse in soil pot artificially polluted or not with heavy metals (Pb, Zn, Cd, Cr). Our results show that (i) heavy metals delay plant growth at high concentrations, but did not affect plant height after 3 months; (ii) plants were able to be contained in the range of water soluble metal concentration; (iii) each plant genotype could be discriminated on PCA plots at each collecting time, but the effect of metals was only detectable for both below-ground plant parts (roots and rhizomes) at 1 month and not at 3; (iii) the antibiotic resistance profiles of soil bacteria were affected by plant or metals but the combined effect of plant and metals was not explained by addition effects, but rather by interaction effects, which indirectly confirms the importance of metal-induced change on plant metabolism. [1] J Berg et al. Environmental Science & Technology 44 (2010) 8724-8728. [2] J. Soltyssak et al. Acta Botanica Slovaca 7 (2011) 209-218. [3] S. Michelet et al. Plant Physiology & Biochemistry 72 (2013) 169-177. [4] C Bardon et al. New Phytologist 204 (2014) 620-630.

MO136
Higher tier testing with microalgae: do light irradiation and species interaction count?

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Single species tests are generally performed under standardized conditions. Although very important to provide first information about the pollutant toxicity, the results of these tests lack to provide information on the potential influence of environmental factors that are often used to evaluate environmental risks. The natural environment multiple variables play a role in determining the effects of pollutants on microbial species. Contaminants and physical-chemical factors might act as multiple stressors that simultaneously influence algal cells whereas algae species, besides having different sensitivities to the stressors, might positively or negatively interact. In this work copper tolerance was studied in the secondary cultures of Chlamydomonas reinhardtii and Synecochytris sp. was investigated under three different light levels, ranging from low to saturating light. The use of flow cytometry allowed the simultaneous measurement of multiple parameters (growth, chlorophyll fluorescence and oxidative stress) after 24 and 48 hours incubation in monovalent and mixed cultures. The obtained EC50 values were used to compute Species Sensitivity Distributions (SSD) in order to verify to what extent parameters such as HCS change in case higher tier tests that take into account high light stress and species interactions are performed. Synecochytris sp. resulted to be more sensitive to copper than C. reinhardtii under low light intensities.

MO137
Impact of copper (Cu) and arsenic (As) on the biomarkers of Myriophyllum alterniflorum in an open recirculated system

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For several years, heavy metals have attracted attention due to their abundance in the environment and their toxicity to human health, animals and plants. Myriophyllum alterniflorum DC. (Haloragaceae) is an aquatic macrophyte found in the Limousin river (France) notably in the Vienne River whose potential for biomonitoring of metal pollution has been well demonstrated in particular for copper, cadmium and arsenic pollutions. The objective of this study was to identify in this species sensitive biomarkers for the early detection of a river pollution taking into account the hydrodynamic conditions. Oligotrophic synthetic medium with similar composition to the river Vienne was prepared. Watermilfoils were cultured for 21 days with or without contamination by 100 μg/L copper or arsenic in aquaria systems (150 L). They were placed in three hydrodynamic zones: quiet, turbulent and laminar flow zone at a rate of 3 cm/s. During this period, physiological biomarkers (respiratory and photosynthetic activities and osmotic potential), biochemical biomarkers (chlorophyll a, b and carotenoids pigments and H2O2 content), and morphological biomarkers were recorded. Both contaminants caused an oxidative stress characterized by the generation of hydrogen peroxide, a decrease in the pigments content, osmotic potential, photosynthetic and respiratory activities whatever the hydrodynamic conditions. In addition, a decrease in root length occurs with both contaminations, more developed in the shoot length when plants were grown in the presence of arsenic during contact contamination. This decrease in root and shoot lengths was higher in turbulent than in laminar zone. Therefore, we can consider that biochemical biomarkers as H2O2 and pigment content, physiological biomarkers as respiration, photosynthesis and osmotic potential and morphological biomarkers especially as root length were the sensitive biomarkers for Cu and As detection in water. While only morphological biomarkers of watermilfoil were affected by the hydrodynamic conditions. Simultaneously, others biomarkers will be studied like histological biomarker such as micronuclei formation in roots. Keywords: Myriophyllum alterniflorum, copper, arsenic, biomarker, hydrodynamic conditions.

MO138
Interest of using aquatic macrophyte, Myriophyllum alterniflorum, for early stage detection of pollution by copper or arsenic

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Aquatic macrophytes are important to assess the water quality, notably through the Macrophyte Biological Index. In River. They can also be used to fight against pollution. For several years, GRESE laboratory studied watermilfoil to determine its potential for use in contaminated waters. In this work, Myriophyllum alterniflorum, presents potentially useful biomarkers for the early detection of pollutants but also a significant ability to bioaccumulation. The aim of this study is to identify, in alternate watermilfoil, the most sensitive biomarkers for an early detection of surface water pollution by trace elements such as copper (II) and Arsenic (III). An aquatic macrophyte, Myriophyllum alterniflorum, was cultivated for 21 days in a medium contaminated by copper or arsenic at concentrations of 100
and 500 µg L\(^{-1}\). Several physiological (osmotic potential, photosynthesis, respiration) and biochemical (Glucose6PhosphateDehydrogenase (G6PDH), malondialdehyde (MDA)... biomarkers are studied. Simultaneously, the phychochemical parameters of the medium are also investigated and the evolution of the concentration of the pollutant in the watermilfoil tissue is monitored. According to contaminant nature and concentration, biomarkers responses are different. Indeed copper and arsenic reduce differently the respiratory and photosynthetic activities, pigments contents and nitrate reductase activity with a decrease ranging from 10\% with copper at 100 µg L\(^{-1}\) to 60 \% at 300 µg L\(^{-1}\); and from 15\% with arsenic at 100 µg L\(^{-1}\) to 66 \% at the higher concentration (500 µg L\(^{-1}\)). In reverting the DOM activity, MDA, proline and osmotic potential increase from 20\% to 70\% with the increase in copper concentration. While this increase is more important with arsenic ranging from 100 \% at 100 µg L\(^{-1}\) to 85\% at 500 µg L\(^{-1}\). Compared to copper, higher impact of arsenic on the biomarkers of *Myriophyllum alterniflorum* is observed. These differences were due to the proportion of the two DOM, one of the two reagent mixtures used in the experiment, and to the ecological influence the toxic above certain concentrations; the other (As) is a non-essential and toxic element.

Once the most relevant biomarkers were identified in vitro, their responses would be evaluated in in situ-reintroduced watermilfoil on sites impacted by urban, industrial or agricultural activities. This study should allow identification of effective tools for biomonitoring of aquatic environments.

*Myriophyllum alterniflorum*, inorganic pollutants, biomarker, biomonitoring.

**MO139**

Is herbicide toxicity on marine micro-algae influenced by the natural Dissolved Organic Matter (DOM)?

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As primary producers, microalgae can be directly affected by herbicides. These organisms can also be impacted by other environmental elements such as the dissolved organic matter (DOM), that may also interact with pesticides. Indeed, MOD can affect pollutant transport, fate, biodegradation, bioavailability, and toxicity on organisms. Therefore, the present study aimed to investigate whether natural DOM influence the toxicity of three herbicides (irgarol (I), diuron (D) and S-metolachlor (S)), single and in mixture, on two marine microalgae, *Chocloecos calcitran* (Cc) and *Tetraselmis suecica* (Ts). After 6 days-exposure to each herbicide (I0.05 and I0.5 µg L\(^{-1}\); D0.05 and D0.5 µg L\(^{-1}\); S0.5 and S5 µg L\(^{-1}\)) and to mixtures (M1: I0.05+D0.05+S0.5 and M2: I0.5+D0.5+S5), the effects of herbicides were assessed on growth (double time T0), photosynthetic yield (Yac), reactive oxygen species presence and intracellular relative lipid content. For each condition, two sets of triplicates were run: one with natural DOM added to the culture media, another without DOM. Without DOM, I0.5 and M2 significantly affected almost all parameters with similar trends in both species: an increase of T0 by more than twice (+125\% and +129\%) for Cc and Ts respectively, at I0.5, a decrease of Yac (approximately 30\%) and a decrease of the relative lipid content. The M2 impacts observed in Cc were similar to the effects induced by irgarol at 0.5 µg L\(^{-1}\), however a higher toxicity was shown for Ts (+167\% for Ts2). By contrast, a possible synergistic effect of mixture. When DOM was added in the culture, the herbicides were also effective on the two species: for both species: while the effects of I0.5 in Ts2 were decreased (+10\%), they were strongly enhanced for Ts (+178\%), as were the effects of M2 (+298\%). The presence of DOM decreased the herbicide toxicity for Cc whereas toxic effects were increased for Ts. These results may partly be due to the combination between DOM and herbicides, leading to a lower bioavailability of herbicides. However, the difference in toxicity between the two species remains to be explained. Analysis of herbicide concentrations, dissolved organic carbon concentration and DOM, which are still ongoing, will help to improve the understanding of interactions between microalgae, herbicides and DOM. Finally, this study demonstrates the importance to consider DOM as a major factor possibly involved in toxicity modulation in the environment.

**MO140**

Sensitivity of duckweed species in different test designs

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The sensitivity of the test organism and the test design are important issues in planning ecotoxicological studies. The aim of the study was to demonstrate that the test design affects the sensitivity of aquatic plants. In the study three species of duckweed were used as test organisms: *Lemna minor*, and the giant duckweed *Spirodela polyrhiza* were exposed to a reference substance 3,5-dichlorophenol in different test designs. A static and a semi static test compound *Lemna gibba* exposed in the static tests were less sensitive, regardless of whether the system was with or without sediment. However, the presence of sediment in the system affects the sensitivity. *Lemna minor* and *Spirodela polyrhiza* seemed to give a similar response, and in the semi-static test without sediment the most toxic impact of the reference substance was observed. *Lemna gibba* appeared to be the most sensitive species in the static ecotoxic test with sediment. When comparing these three species, the generated results indicate that *Spirodela polyrhiza* is the most sensitive one. Despite that the model organism used is the floating duckweed, the exposure in various test systems may have implications for ecological relevance to generated results.

**MO141**

Tissue damage assessment in the macrophyta *Lemna gibba* exposed emerging contaminants using image analysis

A. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hydrobiologia Laboratorio Alejandro Villalobos; A. Dalah-Sobrino, UCEJ Emergente contaminants are used in recent years and are considered as emerging contaminants. Since these products are mouthwashes (Astringosol, Bexident, Colgate and Listerine) in the macrophytes *Lemna gibba*. Bioassays were performed with a duration of 6 days. Macrophytes (10 colonies) were exposed to 5 concentrations of products (25, 12.5, 6.5, 3.12 and 0.78\%) in duplicate. After the exposure time, photographs were taken of each test and analyzed with the software Motic (ver. 2.3, for to evaluate the degree of changes (chlorosis and necrosis or loss of living tissue) of fronds and relate to their degree of liperoxidación. The product that caused the greatest degree of necrosis in the macrophytes was mouthwash Colgate (95.2\%) and which had the lowest effect was Bexident (24.7%). The levels of lipid peroxidation have a direct relationship with the degree of necrosis. The analysis of the state of the fronds using the traditional image analysis, it allowed to make the evaluation of damage caused by xenobiotics more accurately, quickly and inexpensively so is a good tool for evaluating effects.

**MO142**

Toxicity effects of herbicide mixtures on the eelgrass *Zostera noltei* N. Dispensa, Wageningen University; E. Buffan-Dubau, ECOLAB UMR CNRS UPS INPT; J. KalleroHoff, ECOLAB UMR CNRS UPS INPT / Ecolab Laboratoire déagologie fonctionnelle et environnement; G. Merlin, ECOLAB UMR CNRS UPS INPT; J. Silvestre, ECOLAB UMR CNRS UPS INPT / ECOLAB UMR CNRS UPS INPT; H. Budzinski, University of Bordeaux / UMR EPTC LPTC; I. Auby, IFREMER Arcachon; A. Elger, University of Toulouse / EcoLab Sea grasses play an important role in coastal ecosystems. Worldwide seagrass declines have been observed due to multiple stressors. One of these stressors is the mixture of pesticides used in intensive agriculture in coastal areas. Effects of mixture toxicity are complex and poorly understood. The knowledge about such effects is limited as the main focus of environmental risk assessment is on single compounds. However, mixture toxicity is reality and thus more ecologically relevant. The aim was to gain understanding about short-term effects of realistic herbicide mixture exposure on the physiology of the eelgrass *Zostera noltei*. For that purpose, macrophytes collected in situ were exposed at different concentrations of mixtures simulating the composition of typical cocktails of contaminants in two French coastal lagoons. One experiment was conducted using a mixture of 60\% 2,4-CPA and 40\% bentazone representative of the Vaccarès lagoon, Camargue (Mediterranean coast). Another used a mixture of 55\% S-metolachlor, 15\% atrazine, 15\% diuron and 15\% cybutryn (irgarol), representative of the Arcachon lagoon (Atlantic coast). Three biomarkers of stress were measured: quantum efficiency of photosystem II, photosynthetic pigment composition and enzymatic activity of glutathione reductase at different time points and total concentrations of herbicide mixtures. Data of the Camargue laboratory exposure experiment were compared with field measurements on photosynthesis and pigment contents. Data showed that the results from the realistic mixtures and concentrations had subletal effects on Z. noltei. Photochemical efficiency showed significant decrease at a total mixture concentration of 10 µg L\(^{-1}\) after 6 or 1 µg L\(^{-1}\) after 24 h (Arcachon mixture) and at a concentration of 10 µg L\(^{-1}\) after 24h (Camargue mixture). Effect on pigment composition was detected after 24h. Multivariate analysis of the pigment composition showed primarily a shift in the zeaxanthin:violaxanthin ratio. Field measurements did not show large differences in photochemical efficiency. Field effects were difficult to directly relate to herbicide stress as other stressors were also involved. Understanding effects of realistic chemical mixtures on sublethal endpoints of seagrasses will help to improve ERA and more efficient management strategies to prevent further declines of seagrasses meadows worldwide.
Biomedical Sciences TES Division; K. den Haan, CONCAWE / Petroleum Products Safety; H. Hermes, IBACON GmbH; A. Seeland-Fremere, IBACON GmbH / Aquatic Ecotoxicology; V. Wydra, IBACON GmbH Following submission of REACH dossiers on petroleum substances, CONCAWE decided to extend the ecotoxicity data base with cyanobacteria and a higher plant species, in response to an ECHA observation that test results on these species were not included in the TLM-model training set, as required by REACH Chapter R10.3.1.3. A number of single hydrocarbons (e.g. toluene, xylene, 1,2,3,4-tetrahydrophthalalene, 1,4-diethyl benzenne, methylcyclohexane and 1,2-dimethylcyclohexane) covering a range of hydrophobicity were tested. Toxicity data were generated initially on two aquatic macrophytes (Lemna gibba and Myriophyllum spicatum) for toluene and 1,2,3,4-tetrahydrophthalalene, with the remaining four hydrocarbons tested only on Lemna. Toxicity data were generated on the blue green algae (Anabaena flos-aquae) for all the hydrocarbons. A range of test concentrations of each hydrocarbon were generated by sequential dilution of a saturated solution in test media. Care was taken to minimise the loss of test compounds during test media preparation and transfer of test solutions. Toxicity of these hydrocarbons ranged from ~ 1 – 30 mL/(72 h ECo50) for blue green algae and from ~ 2– 70 mL/(7 day ECo50) for Lemna with expected lower toxicity values with an increase in hydrophobicity. The two sets of data were well correlated (r² = 0.954) Blue green algae were consistently less sensitive (2-3 x) than green algae to these hydrocarbons, and consistently more sensitive (approx. 2-3 x) than Lemna.

MO144 Toxicity screening of pesticides towards two marine phytoplankton species V. Vydrina, IFREMER; RBE-BE-EX; S. Stachowski, IFREMER; D. Menard, IFREMER / Laboratory of Ecotoxicology; F. Akcha, IFREMER; M. Devier, H. Budzinski, University of Bordeaux / UMR EPOC LPTC The PHYTOCOTE project, funded by LabEX COTE, concerns the use of pesticides in Aquaculture vineyards (France), their transfer and impacts on ecosystems. As primary producer, microalgae are at the bottom of the aquatic food web and are of great concern for aquatic ecosystems. In the frame of the “impacts” task of the project, this work aims to develop a screening method to assess and compare the toxicity of several active substances for two marine microalgae commonly used in aquaculture: Tissocystis gilba (chrysophyceae) and Skeletonema costatum (diatom). The tested pesticides are the herbicides diuron (phenylurea) and S-metolachlor (chloroacetanilide) and the insecticides fipronil (phenylpyrazole) and imidacloprid (neonicotinoid). These screening results will allow us to select substances and species in order to evaluate, at the laboratory scale, the trophic transfer of pesticides from microalgae to a primary consumer (oyster), as well as the induced effects. The screening test consists in 5-day exposures of microalgae cultures to contaminants using sterile 24-well microplates in controlled conditions. Microplate wells are filled with 1.5 mL of sterile culture media and inoculated with cultures (initial concentration of 2 x 10³ cell/mL). The two microalgae strains are exposed to 5 concentrations of each substance, (3 replicates per concentration and 6 replicates for the control). Growth inhibition, the endpoint selected, is estimated daily by measuring the chlorophyll fluorescence at 685 nm using a microplate reader. At the end of exposure, each well is sampled to determine i) the cell density by flow cytometry and ii) the effective exposure concentration by chemical analysis. EC₅₀ are then calculated for each substance and species. These tests are currently ongoing. Preliminary results derived a reliable statistical analysis in the microplate wells within the 5-day exposure, due to plastic adsorption and/or degradation. In our experimental conditions, a linear relationship was obtained between the chlorophyll fluorescence at 685 nm and the cell density measured by flow cytometry. The two microalgae species do not react similarly when exposed to the pesticides. Herbicides are more toxic than insecticides. Finally, this study will provide toxicity data about several substances on the first trophic levels of aquatic ecosystems: primary producers in one hand, and primary consumers exposed by their diet in the other hand.

MO145 Determination of Plant uptake factor (PUF) values with two different crops and two different pH levels in uptake solution under greenhouse conditions V. Gourlay, A. Leonhard, G. Fent, R. Kubiak, RLP AgroScience IA Plant uptake of pesticides is an important process limiting their availability for leaching. The project also addresses the possible formation of parent compounds (Pₙₐ) and acid dissociation (Pₐₐ), are the most important properties determining the ratio between the concentration of compound in the plant-root system to that in the pore water adjacent to the roots. The plant uptake factor is used as input parameter in environmental fate mathematical models, such as leaching models (e.g. FOCUS, PEARL). Based on the experimental guideline ELIM@NATE (2014), the plant uptake test is designed to study the uptake factor (Pₙₐ) and the effectiveness of biocides and other chemicals used by households and industry. In arid and semiarid countries, reclaimed water is frequently used for irrigation of agricultural crops. Several studies have reported that a number of EOCs are taken up well by plants. Hence, human exposure to those chemicals can be associated with the use of reclaimed water for crops. Several empirical and process-based models have been developed for the prediction of concentrations of EOCs in plants. Accordingly, adsorption to soil, lipophilicity and the speciation of the chemical (neutral or ionized) are the governing properties for the uptake and translocation to plant parts. A mesocosm study was thus designed to allow the laboratory-scale determination of the effective exposure concentration by chemical and metabolic analysis. The pesticides S-metolachlor, 15% atrazine, 15% diuron and 15% cybutryne were used to determine the uptake of eight EOCs (bisphenol A, caffeine, camazepine, ibuprofen, propranolol, sulfamethazine, tolalide, tricosan). Batavia lettuce (Lactuca sativa cv. Arena) was grown in a perlite/sand mixture with five concentrations of the eight compounds (0, 12, 29, 58 and 117 µg kg⁻¹). After 28 days, the plants were sampled and the concentrations in substrate, roots and leaves were determined using gas chromatography (GC) and ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). Uptake into plants was highest for camazepine. Most compounds were rapidly degraded, as seen from the difference in initial and final concentrations within the substrate. Inverse modeling with the standard plant uptake model was used to estimate degradation. Measured dissipation was contrasted with physico-chemical loss (volatilization, translocation, dilution) and the difference was contributed to biodegradation in plant. This indirect method cannot prove degradation but can help to quantify loss processes. Two degradation kinetics were fitted to experimental data, namely first order and first order with Michaelis-Menten. Highest degradation rates in plants were calculated for caffeine (leaves), propranolol (leaves) and ibuprofen (roots and leaves). Of course this method bears some uncertainties but it is a first step to consider degradation inside plants, which is widely unknown but essential for predictions of human exposure to EOCs.

MO147 Sorptive capacity of rhododendron leaves for organic pollutants measured using passive dosing: Lipid characterization and passive dosing experiments D. J. Bolinius, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; A. H. Ackerman, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; A.H. Kierkegaard, F. Fjadersta, J. Holmblck, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; A. Jahnke, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology Leaves play an important role in the cycling of semi-volatile organic pollutants as a result of their large surface area and lipid rich cuticle. Semi-volatile pollutants that are scavenged from the atmosphere by leaves can be transferred to the soil through the shedding of waxes and litter ¹, where they will either be trapped or re-released to the atmosphere upon decomposition of the plant material. Most multimedia chemical fate models use either an octanol-equivalent model for leaves (e.g., BETR) or reported values from the literature for specific species. Leaf uptake can be considered as a measure of the sorptive capacity of the leaves. The sorptive capacity of the leaves can vary considerably 4. In this study we modified an established passive dosing system to study the sorptive capacity of the leaves. Consequently, we decided to carry out the passive dosing system to determine the sorptive capacity of the leaves. We used a two compartment setup with a fan into the lid of the passive dosing system to introduce the fan into the lid of the passive dosing system to enhance the kinetics so that we reach equilibrium as well for dichlorobiphenyl and hexa-chlorobenzene within the same amount of time. This system is used to compare the sorptive capacities of a wide variety of leaves including Norway spruce, Douglas fir, red oak, common reed, European beech, rhododendron and the European oak with and without biocides. The sorptive capacity of leaves can be compared with existing literature data and that will support
MO148
Physical responses of date palm (Phoenix dactylifera L.) exposed to air and soil pollution near the industrial complex in Sfax, Tunisia
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This study aims to evaluate the impact of complex air and soil pollutions on date palm plants (Phoenix dactylifera), one of the most important commercially valuable crops in Tunisia. These field experiments were performed in the vicinity of the industrial complex in the south of Tunisia (Sfax) for the polluted samples and in unpolluted areas for control date palm plants. Several biomarkers were studied belonging mainly to the antioxidant system like enzymes or antioxidant compounds. Results show that air and soil pollutants increased the stress as demonstrated by an increase in the rate of hydrogen peroxide (H2O2) production, electrolyte leakage and lipid peroxidation in all plant parts. This stress affects chlorophyll and carotenoid contents of leaves. Additionally, the activities of enzymes involved in reactive oxygen species (ROS) detoxification were affected. Air and soil pollution induce an increase in the activities of ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT). Moreover, in polluted date palm plants the contents of antioxidant molecules like polyphenols and proline increase strongly, this is consistent with the role of these potent antioxidants in scavenging ROS. The present study evidenced the deleterious effects of industrial activity on the antioxidative defense system of Phoenix dactylifera, an important crop species spreading in arid region of Tunisia.

MO149
Variation of nectar sugar content in different flowering plants
P. Mack, J. Wong, Eurofins AgroScience Services Ecotoc GmbH; S. Knaebe, Eurofins AgroScience Services Ecotoc GmbH / Ecotoxicology Field Pollinators need nectar for survival and provision of the next generation. They choose nectar with higher sugar content and thus higher energy ratio for consumption. There is a lower limit to sugar content where it is not viable for pollinators to visit the respective nectar source again (US-EPA 2012). The EFSA guidance document “on the risk assessment of plant protection products on bees” (2013), provides default values of nectar sugar content for the risk assessment. These worst-case values are the lowest sugar content which may be foraged by the pollinators. The rationale behind these default values is that higher sugar content will lead to lower foraging efficiency. Air and soil pollution increase the stress in the plants leading to changes in the nectar composition. Nectar composition varies from species to species and depends on environmental parameters like temperature, humidity, chemical exposure (duration, concentration), and the quality of the water used for irrigation. This study was performed on four species belonging to the plant family Fabaceae. The findings demonstrate that with the new method a robust and accurate prediction of nectar sugar content is possible.
MO154 Assessing the applicability of a proposed protocol for determining a plant uptake factor (PUF): preliminary results

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Models for estimating the leaching of plant protection products to groundwater incorporate plant uptake as a mitigating process. Transpiration stream concentrations (TSCF) have been the key parameter used in estimating plant uptake. However, there are no standard methods for determining TSCF and considerable variation for a single chemical is often observed in the literature. A new European Crop Protection Association (ECPA) protocol, designed to determine chemical specific plant uptake factors (PUFs) from simple laboratory studies, has been proposed to replace TSCF values. The protocol is designed to calculate chemical specific PUFs from the changes in chemical mass and hydricropic solution volume measured over an eight-day period. Theoretically, PUF values range from 0 (no uptake) to 1 (complete uptake). The objective of this study was to evaluate the feasibility of the proposed protocol for determining PUFs using 14C-labeled 1,2,4-triazole (log Kow = -0.58), caffeine (log Kow = 0.07) and diethylphthalate (log Kow = 2.4) and Tybalt wheat as the test plant species. Replicate plant uptake studies were conducted for each chemical. Wheat seeds were germinated in perlite then transferred to aerated hydroponic containers prior to chemical exposure. After adding the test chemicals to the hydroponic systems, measurements of chemical concentration and solution volume made over an 8-day period were used to determine PUFs. The final recoveries and the distribution of 14C were also determined by direct measurement. PUF values were 1.24-triazole, 0.07 (caffeine) and 0.83 (diethylphthalate). Volatilization from the hydroponic solution, confirmed in subsequent experiments, was the major loss mechanism for diethylphthalate resulting PUFs much higher than the theoretical maximum. It was also observed in preliminary experiments that high evaporation rate or transpiration make it difficult to determine realistic PUFs. Overall, the proposed PUF protocol is simple to conduct and relatively reproducible. However, additional studies on the impacts of evapotranspiration and chemical volatility are needed before the potential limitations of the protocol can be understood and defined.

MO155 Effect of plant species on the determination of the plant uptake factor (PUF) for use in regulatory fate modelling

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Stakeholders from academia and regulatory authorities have proposed to develop a new study design to determine the plant uptake factor (PUF) for regulatory fate modelling. In order to establish a homogeneous coverage of plant species, establishment of higher tier study sites. In a preceding field study conducted in 2014, it was assessed according to an extended Braun-Blanquet scale in four replicates per species. The obtained results are not sufficient to demonstrate acceptable risk, higher tier non-target terrestrial plants (NTTP) need to be assessed. Routine testing with a substance specific Plant Uptake Factor (PUF) for use in regulatory fate modelling. The updated test protocol was made available at the Piacenza Symposium [2]. For a further implementation of the new procedure with different plant species, various PUF values, more experience is needed to determine PUF values of active substances and their metabolites were conducted. Here the analysis of a broad spectrum of studies is presented that were carried out by applying the new study protocol with e.g. potato, tomato, winter oil seed rape and wheat plants. The evaluation of the obtained results focused on the applicability and reliability of the protocol to establish the new method for the use in regulatory fate modelling with various plant species. The findings demonstrate that with the new method a robust and reliable test can be performed to determine the PUF with a variety of different crops and agricultural relevant pH values [5.5 and 7.5]. References: [1] Hingston, Klunder & Schriever 2013: Report of the EUregPUF Workshop, York, UK; [2] Lamshoefdt et al. 2015: First results with a new test design for the determination of a substance specific Plant Uptake Factor (PUF) for use in regulatory fate modeling. Proc. XV Symposium in Pesticide Chemistry. Environmental Risk Assessment and Management (eds. Luzzani et al.), p. 19-20, Piacenza, Italy. http://www.symposiumpesticide.org/wp-content/uploads/2015/06/Proceedings-SP CXV_rev.pdf?e66407

MO156 The plant uptake factor - a regulatory perspective

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In the pesticides risk assessment, regulatory decisions are often based on results of environmental modeling. The input parameters utilized in the calculation of predicted environmental concentrations need to be reliable in terms of precision and accuracy. To meet these requirements, most input values (degradation rate, adsorption to soil particles) are usually derived from experimental studies performed according to accepted guidelines (e.g. OECD) with well established methods developed and validated by scientific experts. The plant uptake factor (PUF) is a parameter describing the ability of plants to take up substances from the soil pore water via their root systems. This specific amount of substance is not susceptible to leaching processes into groundwater. Consequently, the PUF can be a crucial modeling parameter with a high impact on regulatory decision making. This potential of the PUF for refinements of lower tier groundwater assessments has also been recognized by the regulatory authorities. In the last years a considerable effort has been expended by the industry in developing a standard study design for the experimental determination of the PUF. A preliminary study protocol was evaluated in a ring test in ten laboratories in order to obtain information on the robustness, reliability and practicability of the proposed method. In our contribution we will present an analysis and interpretation of the results of this ring test as well as a comparison with recently published data. In order to compare and reproduce the results, and to assess the quality of the study, this includes information describing the experimental design (number of replicates, appropriate controls), plant growth conditions (light intensity, humidity), chemical exposure (duration, concentration), and the quality control information associated with the methods used for plant and exposure concentration analysis. The plants used are alfalfa, tobacco, cucumber, such as harvest weight, dry matter content, transpiration, water use efficiency, and growth rate. It is rare that these physiological parameters are assessed. Based on a survey of the literature, simulations with physiology-based plant uptake models [1] and the direct experience of over 20 plant uptake experimentalists, we developed a suggested minimum list of data reporting requirements for experimental plant uptake studies. Moreover, we give advice on how such parameters can be obtained with relatively low efforts. The goal is to understand the observed large variations of results seen from different uptake studies, and ultimately the recommendation of a standard uptake test. References: [1] Trapp, S. 2015. Calibration of a plant uptake model with plant- and site-specific data for uptake of chlorothalonil and its metabolites. Environ. Sci. Technol. 49(1):395–402. Acknowledgement - The authors thank the participants of the DTU summer course 12906 in 2015 for the fruitful discussions and contributions.
efluent, algal blooms). Filtration to 0.4 µm and 0.2 µm was undertaken, and sample DOC, nutrient, and chlorophyll-a concentrations determined. Sample DOC was characterised via excitation-emission fluorescence. The total dissolved and (operationally defined) labile Zn and Cu fraction, complexion formation (CC), complex conditional stability constants (log K) and free metal ion concentrations have been measured using time-resolved cathodoluminescence at genus level. Size fractionation reveals metal speciation in the freshwater and low salinity regions is controled via complexation by larger colloids. Total DOC concentrations are not correlated with increased metal complexation, indicating that this parameter may not necessarily be the best indicator of potential bioavailability.

Excitation-emission data suggests that autrophic productivity could play an important role in governing [Zn⁺] in the high salinity zone.

MO158 Particle structure effects on Chemodynamics: a combined electrokinetic, selectivity and detection limit activity model and the biotic ligand model, led to an increasing interest in the link between structure and reactivity in these matrices. The critical importance ascribed to the free ion in models, such as the free-ion equilibrium stripping) technique, which was specifically designed and developed to quantify the kinetics and thermodynamics of the adsorption, internalization and transport of chemical species dynamics in solution as well as the integration of the various properties and their complexation to metal ions. Among the various speciation tool for applications in environmental and toxicological studies.

MO160 Determination of free concentrations of metals with vibrating gold electrodes D. Corea, R.F. Domingos, Institut de Physique du Globe de Paris / Equipe de Géochimie des Eaux, E. Companys, Universitat de Lleida and AGROTECNIO / Dep Química; J. Galceran, Universitat de Lleida / Dep Química; P. Salain, University of Limerick School of Environmental Sciences; J.P. Pinheiro, Universidade de Lleida / LIECENSU UMR CNRS

Speciation is key to understanding availability of metals to biota. Hegemonic paradigms such as the Free Ion Activity Model (FIAM) or the Biotic Ligan Model (BLM) highlight the relevant role of free concentrations of the studied substances in the medium for their toxic or nutritional capabilities. So, there is an urge for developing new engineering chemodynamics platforms, the software ORCHESTRA has the advantages to be accessible and can be modified by users for extensions and modifications. As such, we expect that this new analytical implementation of AGNES will ultimately become an important tool for applications in environmental and toxicological studies.
MO164 Understanding Cr biological fate in freshwater bivalves and identification of biomarkers
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Chromium occurs in the environment mainly in two redox forms, namely Cr(III) and Cr(VI). These oxidation states have contrasting chemical and ecotoxicological features. Cr(VI) is of high ecotoxicological concern because it is readily absorbed into cells via the sulfate uptake pathways. Cr(III) is considered less harmful and the actual mechanisms of its internalization into cells are not well known. However, after entering into the cells, Cr(VI) is rapidly reduced to Cr(III) and only Cr(III) will possibly accumulate inside the cells. For this reason, it is desirable to compare accumulation and effects in biological organisms of the two chromium redox forms. Thus, in a first experiment acclimated specimens of the bivalve Corbicula fluminea were exposed to four concentrations (0.5, 50 and 500µg/L) of either Cr(VI) or Cr(III) via the aqueous route for up to 96 h with renewal of exposure medium every 24 h. Bivalves were then dissected to isolate gills and digestive glands. These soft tissues were then digested and assayed for Cr content by ICP-MS. In a second experiment, organisms were exposed using the same protocol and dissected after 24 h and 96h of exposure. Chromium content was measured as in experiment one and biomarkers of cellular damage, antioxidant, antitoxic defenses and energetic parameters were also assessed using a Konelab 20 Xiti instrument after 24h and 96h of exposure. At 5µg/L, chromium bioaccumulation was comparable to Cr content in control organisms. At 50 µg/L, Cr(VI) accumulated in the gills and digestive glands. Cr(III) preferentially accumulated in the gills. At 500µg/L, accumulated Cr concentrations were higher in the gills than in the digestive glands for both forms. While Cr(VI) concentration was stable in the exposure medium, total and filterable (0.22µm) Cr(III) concentrations decreased by more than 80% after 24 h. Results of biomarker expressions are currently being collected and elaborated.

MO165 The influence of co-contaminants on the bioavailability of arsenic, lead and cadmium
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Incidental ingestion of contaminated soil is often a major pathway for human exposure to inorganic contaminants. However, exposure is influenced by both biotic and abiotic factors that impact contaminant bioavailability. To date, bioavailability assessment of contaminated soil has focused on arsenic (As), cadmium (Cd) and lead (Pb), however, studies have typically assessed contaminant bioavailability individually, even when multiple elements are present in the same matrix. As a consequence, it is unclear whether interactions between these elements occur within the gastrointestinal tract (GI tract) which impact absorption and bioavailability. In this study, As, Cd and Pb bioavailability was determined using an in vivo mouse model whereby mice (n = 12 per treatment) were exposed to the contaminant incorporated into AIN93G feed for 10 days. Initially, mice were exposed to each element individually, at three environmentally relevant concentrations; sodium arsenate (1.5, 5 mg As kg⁻¹), Pb acetate (3, 15, 30 mg Pb kg⁻¹) and Cd chloride (0.2, 1, 2 mg Cd kg⁻¹). Subsequently, binary and tertiary elemental combinations were supplied to exhaust all possible combinations. Contaminant bioavailability was assessed by determining the concentration of As, Cd and Pb in target tissue (liver, kidney, femur) or excreta (urine, faeces). Contaminant relative bioavailability was also assessed in aged (12 years) spiked soils, using individual and tertiary elemental doses. When mice were exposed to As, Cd and Pb incorporated into AIN93G feed, the dose-response varied depending on the contaminant and endpoint assessed. However, for all elements, the dose-response was linear and did not differ significantly for single, binary or tertiary combinations. These results suggest that, at the concentration range tested, there was little or no influence on As, Cd or Pb absorption and bioavailability as a result of co-contaminant interactions. Similarly, when spiked soils were assessed, As, Cd and Pb relative bioavailability was consistent, irrespective of whether the soil contained single or tertiary elemental combinations. Moving forward, research in this area could investigate the potential for interaction occurring between these contaminants at a cellular level.

MO166 Effect of temperature on nickel biodynamics in Daphnia magna as determined with stable isotope experiment
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Temperature has a strong influence on the physiological state of ectothermic organisms like Daphnia magna. The physiological state of the organism can influence metal uptake, detoxification, sequestration and elimination and
consequently metal toxicity. The information available about metal uptake rates in *Daphnia* is limited to studies with only one clone and organisms which were not acclimated prior to testing. An acclimation period is necessary in order to achieve the same performance across the different temperature treatments. The objective of the present study was to assess the effect of temperature on nickel uptake in four *D. magna* clones. The uptake of nickel was studied in four *Daphnia magna* clones at 15, 20 and 25°C. Four *D. magna* clones from the same natural population were acclimated during two generations to the temperature treatments. Nickel exposure concentrations were based on the 21 d 50% effect concentration on reproduction of the four *D. magna* clones at 15°C and 20°C, i.e. 50 and 76 μg Ni/L respectively. Experiments with organisms five days old were exposed to the stable isotope 60Ni during 72h and samples were collected at the time points 0, 8, 24, 48 and 72h. To avoid uptake via food organisms were not fed during this period. Results showed that nickel body concentrations varied among the four *D. magna* clones. After 48h of exposure nickel body concentrations were lowest in *D. magna* clone 15 and the steady state was generally reached within 24h for all clones at 25°C in contrast with 15°C, which generally only reached a steady state after 48h of exposure.

**MO167**

Effect of aging on the toxicity of leached rare earth elements in soil over time

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Rare earth elements are increasingly being mined and used for various technological advances. As such, the risk assessments of new and existing products containing rare earth elements have increased. A 2011 report by the Government of Canada’s Chemicals Management Plan. Particularly of interest, are soil effect data specific to boreal forested regions where metallic compounds can enter into the terrestrial environment through industrial emissions (e.g., metal smelting, mining, etc.), and ecotoxicological data are lacking. Given that boreal regions form a significant portion (~30%) of Canada’s landmass, the effect of rare earth metals were evaluated on boreal species using forested soils spiked with the test substance. To address the effect of leaching and aging on toxicity over time, tests were conducted by simulating deposition and weathering of two earth metals, lanthanum (La) and cerium (Ce), in reconstructed soil columns. Natural soil horizon stratification was maintained throughout the aging and leaching process. Subsequently, the toxicity of the aged soils were evaluated over time on plant growth (*Pinus banksiana*), as well as soil invertebrate reproduction (using the caddisfly, Proisotoma minuta, and the earthworm, *Dendrodrilus rubidus*). The study was designed to mimic the natural environment, at which metal deposition might occur, with mobilization through the soil profile as a result of, for example, precipitation. The effect of the aging process on toxicity will be discussed, along with a discussion of metal availability by profile.

**MO168**

The importance of speciation in Chromium hazardous and risk assessment


In aquatic environments, Chromium exists mainly in two redox forms, namely Cr(VI) and Cr(III). The role and the impact of Cr on the environment and living organisms depend on its chemical form. Cr(III) is considered much less ecotoxic compared to the hexavalent form of the same element. However, some recent studies report higher toxicity of Cr(III) than Cr(VI). Experimental verification of speciation during ecotoxicological tests can facilitate comparison among laboratory experiments or between laboratory and field situations. We used IC-ICP-MS to investigate possible conversion between Cr(VI) and Cr(III) in a standardized algal test medium (ISO692) both in the absence and in the presence of the green alga *Pseudokirchneriella subcapitata*. Aliquots of test medium were spiked with 60 μg L⁻¹ of Cr(III) or 115 μg L⁻¹ of Cr(VI), corresponding to the EC50s at 72h determined in our laboratory for the two forms. No interconversions were observed. After 72h, Cr(III) concentrations in solutions spiked with Cr(III) were not stable over the experiment duration (72 hours). Considering that the total (unfiltered) Cr concentrations in the same solutions remained stable over 72 hours, we surmise that the observed decrease in filterable Cr concentrations was due to particle formation and not to Cr losses onto e.g. walls of the flask. Analysis of Cr(III)-spiked aliquots of test medium by Nanoparticle Tracking Analysis (NTA) confirmed that colloidal particles (approx. diameter 150 nm) quickly formed when Cr(III) was added to the medium. Particle diameter determined by NTA did not change with time even if filterable Cr concentrations measured during the experiment decreased by 70%. Analogous Similar experiences experiments using Single particles Counting (SPC) system suggest that Cr(VI) particles also decrease in time, thus making sense of the observed decrease in filterable Cr concentrations.

**MO169**

Assessing bioavailability of soil arsenic using *E. coli* whole-cell bioreporters

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We determined the bioavailable arsenic in contaminated field soils before and after soil-washing processes to evaluate the arsenic removal efficiency using novel *E. coli* whole-cell bioreporters (WCBs). All of currently available arsenic bioreporters are based on arsenic responsive genes in *ars* operon, but we used general arsenic responsive genes in *nik* operon, which is upregulated by both *ars* operon and *nik* operon in *E. coli*. Unlike other whole-cell bioreporters, the regulatory protein of nik operon, NikR, represses the transcription in the presence of Ni(II). Interestingly, when WCBs were exposed to eight heavy metals and metalloid, it responded only to As(III) exposure and showed the expression of reporter gene (egfp) under nikA promoter. Moreover, the response was proportional to a dose-dependent manner. Thus, this WCBs would be a genuine bioreporter to determine bioavailable arsenic quantitatively.

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**MO170**

A meta-analysis on metal uptake kinetics and the relationship between metal accumulation and soil properties in soil organisms

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Bioaccumulation can be a good indicator of the exposure of organisms to metal polluted soils. Metals are taken up from the aqueous phase of the soil (pore water) which is considered the main exposure route for most soil organisms. Metal bioaccumulation is a complex process that includes uptake, internal distribution, storage, and excretion. Several biomarkers of exposure and effect have been checked in terrestrial small mammals (rodents and insectivores) inhabiting water and soil polluted sites, but still, whether other components of the substances (e.g., organic component in the case of organometals) to which they belong are of concern and elicit an effect. In this study, metal bioaccumulation in soil organisms and the relationships between metal bioaccumulation, metal exposure concentrations, and soil physical-chemical properties were investigated. We used data from the literature on terrestrial organisms such as earthworms, potworms, isopods, beetles, centipedes, snails, and collombolans. We summarized the uptake and elimination rate constants derived from bioaccumulation tests on different soil organisms. For all species and all metals, bioaccumulation followed a specific pattern which could be described by toxicokinetics models applied to calculate bioaccumulation parameters. Non-essential elements, such as cadmium and lead, generally accumulated in the soil invertebrates with relatively high uptake rate constants and most organisms were able to remove them from the body by excretion. For the essential elements, copper and zinc, internal concentrations remained quite constant for almost all test species. Among soil physical-chemical characteristics, soil pH, cation exchange capacity, clay and organic matter content significantly affected uptake rates of non-essential metals in soil invertebrates. For essential metals, kinetics was hardly influenced by soil properties, but rather prone to physiological regulation mechanisms of the organisms. Our analyses illustrated that toxicokinetics could be a valuable measurement to assess bioavailability of metals in soil.
that around 17% of the internal Pb could not be eliminated. The uptake rate constant was 0.14 g atom/g day, and elimination rate constants were 0.96 d⁻¹ (based on internal Pb concentration) and 0.046 d⁻¹ (based on increasing toxicity with time), suggesting that approximately 5% of the internal Pb contributed to the observed toxic effects. Biological 1/s values for Pb elimination were calculated to be 0.72 and 1.51 d⁻¹, which showed kinetics and toxicodynamics, respectively, again indicating that development of toxicity with time was much slower than the uptake of Pb in E. crypticus.

MO172 Bioavailability and ecotoxicity of Cu substances to terrestrial organisms  
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The Government of Canada's Chemicals Management Plan (CMP) is an initiative created to quantify the environmental and human hazards associated with chemicals used in industry and commerce in Canada. Inorganic metal moieties are one group which has been identified as a CMP priority and form components of larger inorganic and organic substances. Given the volume of chemicals requiring evaluation under CMP initiatives, a metal moiety-based approach has been recommended to stream-line the risk assessment process. However, there is uncertainty over the effect of these moieties of concern to soil systems, and further still, whether other components of the substances (e.g., organic component in the case of organometals) to which they belon are of concern and elicit an effect. In order to assess a moiety-based approach, copper-containing substances, representing different forms of elements, were selected for ecotoxicity tests: water-soluble (copper sulfate) and water-insoluble (copper oxide) inorganic metals; a water-soluble organic metal salt (copper diethyldithiocarbamate); and water-soluble (copper D-glucosamine) and water-insoluble (copper glucocan) organometals. Soil ecotoxicological studies were conducted using standardized soil methods to characterize effect on plant growth (Trifolium pratense and Elymus lanceolatus) and invertebrate survival and reproduction (Folsomia candida and Eisenia fetida). Soil chemical analyses were also conducted to evaluate for total metal, total parent compound (in the case of organic metal substances) and ionic activity.

MO173 Exploring the contribution of abiotic and biotic factors in elemental accumulation patterns in the shrew Crocidura russula exposed to air pollution  
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Several biomarkers of exposure and effect have been checked in terrestrial small mammals (rodents and insectivores) inhabiting water and soil polluted sites, but less ecotoxicological information is available in air polluted sites. Moreover, the bioaccumulation patterns of abiotic and biotic factors that interact at “real world conditions” were understudied in small mammals. Here, the influence of abiotic (season) and biotic (age and sex) factors in the bioaccumulation patterns of 17 elements with ecotoxicological interest (Pb, Hg, Cd, Fe, Zn, Cu, Cr, Co, Mn, Mo, V, Ni, Sn, Sr, Ba, Se, S) was explored in soft tissues (liver, kidney, lung, heart, spleen, pelt) of Crocidura russula inhabiting a coal-fired power plant impacted area. The importance of the factors analysed was age>season>sex and the shrews from the polluted site showed highest concentrations of all elements in their tissues, specially Pb, Cd, Fe, Sn and Sr. Among tissues, heart and pelt were primary target tissues for accumulation of metals with toxicological interest such as Pb, Mo, V, and Ni. Differential exposure and detoxification response in shrews from polluted site also produced higher dispersion of data. Overall, the power station emissions increase the entry and/or bioavailability of potentially toxic metals and non-metals in C. russula tissues that bioaccumulate depending on several biotic and abiotic factors. In conclusion, there is necessary the study of such factors to better interpret accumulation data of persistent pollutants and their toxic effects in natural populations.

MO174 Spatial variation of extractable elements in anoxic sediments from the Oslofjord (Norway) and the Gulf of Gdańsk (Poland)  
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Certified reference material (BCR-701, fresh water sediments for sequential extraction) was used for the estimation of the procedure efficiency. Moreover, organic carbon, total nitrogen, stable carbon and nitrogen isotopic composition (δ13C and δ15N), grain size, 209Pb activity and pigments, were determined in the samples. Spatial distribution of the elements was analyzed with principal component analysis (PCA). Finally, the environmentally relevant quality of the sediments was evaluated with contamination factor (CF), pollution load index (PLI) and risk assessment code (RAC). Multivariate analysis (PCA) separated samples from Norwegian fjord and Gdansk Bay. The most significant component was characterized by high loading values of rare earth elements (REE), that can be explained by metamorphic origin of the Oslofjord bedrock. Relatively high proportion of trace elements Cd, Mn, Pb and Zn were found in the acetic acid soluble extract fraction. This indicates presence of exchangeable, weakly bound forms of these elements, prone to be bioavailable to benthic organisms. On the other hand, Cr, Fe and Ni were found mostly in the residual fraction of the sediments indicating their low mobility and low susceptibility to be released from the sediments. The CF showed that all locations were contaminated by Cd and Mn with highest values of CF in the innermost part of Oslofjord (Bunnefjord), where the deeper-water exchange is restricted. This was confirmed by PLI – the highest values of PLI was also found for station located at Bunnefjord. According to RAC, sediments in Bunnefjord and at a station located in a deepest part of Gdansk Bay (Gdansk Depth) were classified as constituting “very high ecological risk” due to high concentration of Mn in weakly bound acid soluble fraction of sediments. This indicates the potential of Mn to be a threat to local ecosystem ability of this heavy metal to enter food web. High and moderate risks were estimated for Sb and As respectively.

MO175 Scenario analysis of metal fate in estuaries  
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Estuaries provide a multitude of ecosystem services such as drinking water supply and water purification. Therefore, estuarine areas have been zones of intense human occupation and industrialisation, with a legacy of historic and current metal contamination. Due to the accumulation of fine-grained sediments, estuaries are sinks for heavy metals which may be problematic pollutants due to their environmental persistence and potential toxicity. Additionally, coastal systems are facing threats associated with climate change that may affect metal mobilization. For example, dredging and coastal siltation may result in the re-working of tidal flat sediments, while annual river discharge may change (decrease in summer, increase in winter) due to climatic shifts. Such effects will interact with tides and baroclinic circulation and thus determine heavy metal distribution in future estuaries. The present study uses the 3D hydrodynamic model Delft3D-FLOW fully integrated with the water quality model Delft3D-WAQ to investigate environmental dynamics, metal partitioning and metal fate (during 1-year period) in an idealized estuary (funnel-shaped). This model was scaled so that processes could be representative of alluvial systems as the Thames Estuary (London, UK). Simulations were run for Cd, Cu, Fe and Pb under three morphological scenarios (normal tidal flats, moderate tidal flats, severe coastal siltation), three discharge conditions (low, medium, high), and a tidal amplitude of 4 m in comparison to no tide. The results highlight that tidal flats play a significant role in the dynamics of dissolved and particulate metals. Similarly, river discharge and the position of salinity front jointly caused non-conservative metal behaviour. This study underlines the sensitivity of metal partitioning and residence time of contaminants to the abundance of tidal flats, and thus indicates that river-dominated tidal estuaries require these environments to achieve good biogeochemical functions on water purification.

MO176 The effect of environmental conditions on the bioavailability of metals from contaminated sediment  
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Abandoned metal mines have left a legacy of sediments contaminated with metals. These sediments may constrain the achievement of the targets set out by the EU Water Framework Directive. A cost-effective approach to dealing with sediments contaminated by metal mining activities should be based on an understanding of how environmental conditions influence bioavailability, and hence the conditions under which they present a risk to achieving Good Ecological Status. Here we
have undertaken a controlled laboratory experiment in replicated mesocosms, where we have incubated a field-relevant biomonitor species (Baetis spp. mayflies) with sediment collected from a river impacted by an abandoned copper mine. The water (conductivity approx. 40 µS) and Baetis were collected from an independent control site of similar geology but not impacted by metal mining. The mesocosms were manipulated to provide three levels of organic matter, three levels of water hardness, and two levels of resuspension, in a fully factorial design. The body burden of metals in Baetis was used as a measure of the bioavailability of metals under the experimental treatments. Once set up the mesocosms were incubated at 10 °C for three weeks, for the sediment and water to equilibrate. After this initial equilibration period, a sample of the sediment was collected from each mesocosm for determination of particle size distribution, organic carbon content, and Fe oxides. After the initial equilibration period, the sediment in those replicates to be resuspended was disturbed and allowed to settle. Then 20 individuals of the biomonitor species (Baetis) were added and the experiment incubated for another period. After incubation for four weeks, the body burden of metals in the Baetis was determined using a Vista-Pro CCD.

Simultaneous ICPOES. At the end of the experiment a second sample of sediment was collected for characterisation as above. Data were analysed using generalised linear models, to establish the effect of the experimental treatments, and any interactions among them, on the body burden of metals in Baetis: our measure of the bioavailability of metals in the experimental mesocosms.

MO177 A turbidity current plume model for submarine tailing disposal on sloping terrain: providing data for risk assessment of the exposure to pelagic organisms J. Skanske, SINTEF / Monitoring and Modelling; R. Nepstad, SINTEF Materials and Chemistry / Monitoring and Modelling; E.J. Davies, SINTEF Materials and Chemistry / Oil Spill Research; T. Nordam, SINTEF Materials and Chemistry / Monitoring and Modelling; B. Hansen, J. Farkas, SINTEF Materials and Chemistry; D. Altin, BioTrix; A. Booth, SINTEF Materials and Chemistry / Environmental Technology; T. Nordtug, SINTEF Materials & Chemistry / Applied Environmental Biology and Chemistry; A. Olsen, Norwegian University of Science and Technology; I.B. Overjordet, SINTEF Materials and Chemistry / Environmental Technology.; Johansen, SINTEF Materials and Chemistry. Submarine and coastal shallow-water disposal of mine tailings has the potential to spread toxic metal into the aquatic environment. However, the extent of spreading, and therefore also the risk of exposure, depends greatly on the initial conditions of the release: the composition of the slurry, the discharge rate, and the depth and bathymetry around the discharge area. Computer modelling of the disposal plume that takes into account these initial conditions has the ability to predict the transport both to the sea bed and the transport of dispersed matter from the plume in the water column. Therefore, computer modelling of the release can estimate the degree of exposure to pelagic and benthic organisms from mining discharges. Plume calculations in the DREAM model currently handle discharges onto a flat sea bed, taking into account radial spreading and the angle of repose of the deposited matter. However, submarine discharges may be placed on sloped terrain, for example in deep fjords, where the release will cause a turbidity current that will settle according to the bathymetry in the discharge area. Here, we present improvements to the DREAM model to handle density currents by taking into account the bathymetry around the release site. Our approach is to calculate the bathymetry around the discharge point along the resulting plane, using an estimated roughness to infer friction supplied by the sloping terrain. By applying the improved model to areas of ongoing tailing disposal, we will use the resulting water column concentrations to inform risk assessments of the exposure of metals to pelagic organisms.

MO178 Influence of humic acids on the bioavailability of metals in sediment samples from the Berg River Catchment, South Africa V.S. Somerset, CSIR South Africa / NRE; U. Skepe, University of the Western Cape South Africa; S. Ntshabele, University of Stellenbosch; J. Peeters, Stellenbosch; C. Walters, CSIR / Natural Resources and the Environment. Heavy metals in the environment pose serious human health risks, since some of these metals such as lead; mercury and cadmium are highly toxic even at trace amounts. Humic acid is known to be the highest variable in the composition along the sub-micron range. Whilst the bulk of tailings will settle on the sea floor close to the release point, the sub-micron fraction is likely to remain suspended for longer periods. We have undertaken a study aimed to quantify Tl concentrations in 150 urine and 297 hair samples from the population of Valdacidal Carducci and Pietrasanta, Italy. A recent study showed the presence of thallium (Tl) at concentrations of concern in groundwaters near Valdacidal Carducci (Italy). Tl contamination was also found in water intended for drinking and bathing purposes. For this reason, we need to further investigate to complete our knowledge on environmental Tl contamination, processes and possible adverse effects to biota.

MO180 Thallium pollution in Tuscany, Italy: a non-invasive population-based control study E. BRAMANTI, Italian National Research Council; B. Campanella, University of Pisa; M. Onor, A. D'Ulivio, Italian National Research Council; M. D'Orazio, R. Gianneccini, R. Petini, University of Pisa. We report the results of a field investigation based on the use of a non-invasive population-based study, which aims to quantify Tl levels in hair and urine samples from the same subjects. Our study aimed to i) quantify Tl with non-invasive sampling procedures; ii) correlate Tl levels found in hair and urine with Tl concentration in the tap waters used by inhabitants; iii) exploit the suitability of saliva as alternative matrix for environmental exposure studies. Tl values found in urine and hair samples were correlated with Tl concentration levels found in tap water in the living area of each citizen and with his/her habits. Tl concentration is higher than 0.6 ng/g L and 20 ng/g in about 50% of urine and hair samples, respectively. As Tl is generally removed from the body via urine, the high concentrations of Tl in the biological samples of the studied area must be ascribed to high accumulation of Tl through the food chain. The kinetics of decay of Tl concentration in urine samples was also investigated, finding that even 60 days after the end of the exposition, mean Tl urinary concentration was still extremely high. Our findings indicate that
MO181 Uptake and potential acute and physical effects of barite on the pelagic zooplankton Calanus finmarchicus (Crustacea: Copepoda)
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Drilling muds used for offshore oil drilling operations contain weighting particles like barite. The present work involved assessing barite uptake and acute (lethality) and physical (sinking speed) effects of barite-containing drilling mud on Calanus finmarchicus. This species is by far the dominating zooplankton species in the North Atlantic. Copepods were treated with an environmentally realistic (10 ppm) concentration of drilling mud up to 168 hrs followed by 100 hrs of recovery. Species were sampled several times during the experimental period, and both microscopic photographs and metal analyses confirmed uptake and accumulation of barite and co-uptake of other metals (e.g. silicon and aluminium). Acute toxicity was assessed, but an LC₅₀ was not determined within the range 5-320 ppm. Physical effects of drilling mud on the neutral buoyancy of copepods were assessed by measuring sinking speed of sedated copepods. The main conclusions from this investigation were that i) copepods do in fact filter and ingest mud particles, ii) barite does not appear to have acute toxic effects, ii) barite particles are withheld within the gut for longer than 100 hrs recovery in clean sea water even though the extractable amounts of Ba, Si and Al were somewhat reduced after recovery, and finally iv) at environmentally realistic concentration ingestion of barite particles affect copepod buoyancy.

MO182 Potential impacts of mine tailing plumes on pelagic filter-feeders
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Deposition of mine tailings in fjords along the Norwegian coast is known to have ecological implications for benthic fauna, but little information is available on the potential impacts on pelagic organisms. Depending on local hydrology, release of tailings can result in plumes where the particle size distribution may extend into the sub-micron range. Whilst the bulk of tailings will settle on the sea floor close to the release point, the sub-micron fraction is likely to remain suspended for longer time periods and for spreading to more extensive. Calanoid copepods, such as Calanus finmarchicus, are important pelagic filter-feeders serving as a crucial link in the food chain between primary producers and fish. They are ubiquitously distributed in the Northern Atlantic including fjord systems in Norway. Their impressive capacity for filtering particles for food suggests that they will readily ingest mine tailing particulates, with subsequent exposure to metal-bearing particles and dissolvable metal ions originating from the tailings. We report experimental data for the purpose of environmental risk assessment of mine tailing deposits in Norwegian fjords, where the acquired data will be used to inform risk models. The physicochemical properties of the mine tailing fraction remaining suspended in seawater for an extended period (e.g. 10 d) is comprehensively characterised (composition and particle size distribution). Copepod filtration rates will be determined for different size fractions of particulate matter, sensitivity of copepods to specific elements in the particulate and dissolved phase, and uptake and depuration rates of those elements. Further, the influence of flotation and flocculation chemicals used in mining processes on particle and element bioavailability and potential toxicity will be studied. Finally, we will determine potential implications of environmentally realistic exposures (particle size distributions) to mine tailing particulates on growth and reproduction. Environmentally realistic exposure experiments will be designed based on data from field surveys in fjords with active submarine tailing depositions using state-of-the-art in-situ particle imaging systems.

MO183 Determining the removal of platinum group metals in industrial effluent during sewage treatment
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Exposure assessment of metallic substances in industrial effluent discharged to freshwater via sewage treatment plants (STPs) requires knowledge of the retention of the metals during sewage treatment. No information could be identified in the scientific literature on the removal efficiency of municipal STPs processing industrial effluent containing platinum group metals (PGMs). A monitoring study was therefore undertaken to measure the removal of PGMs (platinum, palladium, rhodium and ruthenium) at a number of STPs in order to inform the chemical safety assessment of PGM substances for REACH registration. The removal efficiency for metals during sewage treatment depends upon a number of factors including the sorption characteristics of the metal, the treatment technology applied at the STP and the plant’s retention time, which varies according to rainfall and flow rate. A monitoring study was undertaken at three STPs across Europe known to receive effluent containing PGMs. PGMs concentrations (total and dissolved) were measured in influent, effluent and sludge. Analysis was also undertaken for parameters such as pH, alkalinity, hardness and the concentration of total suspended solids. Preliminary analysis of the results indicates differing removal efficiency for the individual PGMs and significant temporal variation at the STPs study sites which is likely to be due to differences in retention time caused by changes in rainfall.
distribution $^{137}$Cs concentration in street dust was the highest at 7.6km south (503.000 Bq/kg) and decreased with distance from FDNNP. The higher $^{137}$Cs concentrations were shown in the north-west and south followed by north and west in the 137Cs concentrations in the 50-150km area revealed south > northwest and southwest > north and west whereas the northeast of Tokyo and the northern part of Gunma Prefecture specifically indicated higher anomaly beyond ≈15km. The spatial distribution is very consistent with the diffusion path of the radioactive plume estimated by SPEEDI and results of locally conducted on-site monitoring of atmospheric deposition. 3.2. Comparison of $^{137}$Cs level with soils and sediments $^{137}$Cs concentrations in street dusts were significantly higher than those in top soils (≤5cm) of forest, paddy, crop, fruits and grassland fields collected in the p<0.05) $^{137}$Cs has been detected in estuarine and bay sediments, and street effluent is a known source of heavy metals entering neighboring water catchment area. These facts indicate a possible washout of $^{137}$Cs from street to aquatic ecosystems.

4. Conclusion Street dust contains high concentrations of $^{137}$Cs transported by heavy metals. Therefore, Street dust can be a media to analyze $^{137}$Cs pollution and a probable channel for $^{137}$Cs to migrate from land to ocean. It may play a considerable role in elucidating $^{137}$Cs dynamics in environment.

Acknowledgement: We would express our appreciation to the local NPO Corporation and financial support by JSPS KAKENHI (No. 26870180).

MO186

Heavy metal Bio-accumulation in different tissues of Euryglossa orientalis, Chirocentrus nudus and Bahrekan Bay in the Northwest of Persian Gulf

M.M. Kournabahani, Islamic Azad University / Department of Environmental Science; A. Hariripour, Concentration of Ni, V, Pb and Cu were determined in both sediment and liver, gills and fillet of Euryglossa orientalis and Chirocentrus nudus along the Bahrekan Bay in the Northwest of Persian Gulf in Iran. Sediment samples and fish species were collected during winter 2013 and spring 2014. Heavy metal analysis was done by atomic absorption spectrophotometry. It showed that mean concentrations of heavy metals were high in liver and gills of Euryglossa orientalis. Also heavy metals had the most accumulation in liver of Euryglossa orientalis. Target tissue for accumulation Ni,V,Cu and Pb are gills and liver in E. orientalis and C. nudus. In tissues of two fish species fillet has the minimum concentration level of trace elements. The concentrations of heavy metals were lower than legal limits in the fillet (edible part), except for Pb that was higher than permitted limits that is a negative point for consumption by human.

Bioaccumulation factors (BAFs) were determined for different tissues of fish species with respect to elemental concentrations in sediment. BAFs results indicated that all BAFs in liver are more than gills and then more than fillet. Also BAF of Cu in liver and gills of k is more than 1.

Toxicity Testing in Sediments - Bioassays As Link Between Chemistry and Complex Benthic Community Testing for Sediment Quality Assessment (P)

MO187

Assessing the predictive ability of Sediment Quality Guidelines (SQG) for fine sediments by using a pollution-sensitive biotic indicator


Fine sediments are often associated with pollution, because they are a major binding phase for many substances inside waterbodies. The appropriate management of polluted fine sediments, whether concerning, for example, dredging activities or river management plans, is thus an important issue. For prioritization of further assessment or management options for fine sediments, we have developed Sediment Quality Guidelines (SQG) that are based on endobenthic organisms living between fine sediment particles throughout their whole life cycle (Nematode). Specifically, we first derived Threshold Effect Concentrations (TEC) and Probable Effect Concentrations (PEC) for 40 single substances and 4 sum parameters. Then, for quantification of the toxic potential and subsequent classification of a sediment sample, an index based on the PECs and the corresponding measured concentrations of the sample can be calculated (mean PEC-Q). However, validation of the predictive ability of the index is crucial and thus, we used and independent data set consisting of simultaneously analysed chemical and biological (nematode community) information for this purpose. For each sample of the data set, mean PEC-Q as well as the NemaSPEAR[%]-index (Nematode SPEcies At Risk), which indicates pollution-induced nema totoxic community alterations (Hörs et al., 2011. Environ. Int. 37: 940-949), were calculated. The results showed a decrease of the NemaSPEAR[%]-index, i.e. a loss of pollution-sensitive species, with increasing mean PEC-Q. However, the correlation is unsurprisingly not a total one and in specific sediments false-negative and false-positive indications might occur (e.g., due to lower or higher bioavailability of some substances or confounding factors influencing the NemaSPEAR[%]-index). Thus, we finally transformed the correlation of the two indices into “incidences of toxicity” (MacDonald et al., 2011. Arch. Environ. Con. Tox. 39: 20-31), from which the probability of toxicity for a specific mean PEC-Q can be deduced. Specifying the probability of toxicity instead of the mere exceedance or not exceedance of a fixed threshold value likely yields valuable information for risk managers and regulators.

MO188

Toxicity screening of sediments from Lake Geneva using the freshwater ostracod Heterocypris incongruens (ISO 14371).

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Lake Geneva is the largest water body of freshwater in Western Europe, draining a watershed of about 8000 km$^2$. The northern end is an important tributary of the Rhone basin using its major tributary. Lake Geneva is subject to anthropogenic pressures due to a large population, agriculture, and industries, and is periodically monitored for contaminant concentrations in water, sediment and biota [Loizeau et al. 2013]. The last monitoring campaign addressing sediment quality of the whole lake dated back to 1988 and addressed mainly metal and nutrient concentrations in sediments [Arbouillé et al. 1989]. More recent studies on the zoobenthos have shown a significant improvement of the quality of sediments over the past twenty years [Lods-Crozet & Reymond 2006]. In 2015, a large sampling campaign supported by CIPEL (International Commission for Lake Geneva Protection) was carried out to provide a comprehensive assessment of micropollutant concentrations in water, sediments and zoobenthos. Sediment samples collected from the whole lake, with a sediment-contact test using the freshwater ostracod Heterocypris incongruens [ISO 14371-2012]. This test offers many advantages over other traditional sediment-contact tests such as requiring a small sample volume and the possibility to obtain test organisms from cysts then culturing them is normal. Results showed a significant correlation of the two indices into “incidences of toxicity” (M$^2$). Thus, we finally transformed the expected to change the biogeochemical characteristics of the sediment, which in turn may modify the bioavailability of contaminants and thus, the biological responses of the exposed organisms. Therefore, sediment freezing may lead to biased extrapolation of results to environmental conditions. In this study, we monitored over a period of one month the chemical behavior of 1 L static microcosms containing freshly sampled sediment versus the same sediment stored frozen. The results showed that the pH increased differently in the water column during the first 17 days of the experiment, and then reached a similar pattern in both microcosm types until the end of the experiment. The nitrogen cycle monitored in the overlying water was slower and more intense with the frozen sediment than with the fresh sediment. Toxic species NH4+, NH3 and NO2- were present until day 14 in the water column of the frozen sediment, whereas they were only present until day 4 in the fresh sediment. The compounds N-organisms were close to the toxic threshold values for pelagic and benthic organisms in the frozen sediment microcosms. We thus assume that higher concentrations, i.e. above ecotoxicological threshold, might be reached with sediment containing high biotic activity or if food for the exposed organisms is added to the microcosm. Finally, despite the expected more sediment died during the freezing period; only bacterial colonies and cyanobacteria grew on the sediment during the experiment. Cyanobacteria, which are known to produce toxins, expanded dramatically between day 14 and 21. In conclusion, we do not recommend stored-frozen sediment for ecotoxicological testing of pollutants under static conditions. However, for method development or bioavailability experiments, when the chemical and biological response need to be conducted over several months, stored-frozen sediment can be used with a
Minimum conditioning period of 10 to 14 days before introducing the test organisms. For the fresh sediment microcosms, we recommend a 7 days conditioning period before introducing the test organisms. Finally, sediment storage and conditioning should be considered when developing standardized ecotoxicological procedures.

MO190

In vitro screening for steroid endocrine disruptive and Ethoxyresorufin-O-deethylase (EROD) inducing activity in surface sediments from Mar Menor lagoon (SE Spain)

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The Mar Menor (Murcia, SE Spain) is the biggest hypersaline coastal lagoon in the Mediterranean Sea, impacted by intensive agricultural activities, urban growth, massive tourism and historical metal pollution. In this study, we investigated the presence of steroid compounds and Aryl hydrocarbon receptor inducers in surface sediments from the lagoon using in vitro bioassays. Fifteen sediment samples from Mar Menor were tested for (ant)estrogenic, (anti)androgenic and EROD activity using the HER-Luc, AR-EcoScreen™ and fibrilbrot like RTG-2 cell lines, respectively. Organic extracts were prepared from total sediments using methanol (for hormones and ER tests) and hexane:acetone (for EROD measurements). Overall, higher in vitro activity was detected in sediment extracts from depositional areas of the Lagoon (central and southern basins) and near main marinas. All but two sediment extracts induced EROD activity (maximum value 0.97 ng Eq-P-Naphtotetraflavone/mg sed).

Anti-estrogenic activity was also detected in eleven samples, of which five of them were able to block up to 80% of the effect caused by EC50 of 17ß-estradiol. Anti-androgenic activity was found in six sediment extracts, of which all but one were able to decrease the effect caused by EC50 of dihydrotestosterone below 80%. None of the sediments extracts induced agonistic estrogenic or androgenic activity. Spatial patterns of in vitro responses obtained in this study were compared with those of in vivo responses of sea-urchin pre-embryos exposed to sediment eluates and with sedimentary organic contaminant concentrations (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, surfactants, pesticides and pharmaceuticals) available from the same sampling cruise and stations. Certain similarities between spatial distribution of chemical concentrations, in vivo and in vitro responses were observed. Further extensive chemical analysis for contaminant identification is underway to improve the interpretation of our findings. Acknowledgement - This work has been supported by Seneca Foundation (Region of Murcia, Spain) through ‘BIOMARO’ project (15398/PI/10) and by IMPACTA project.

MO191

Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Lebanese surficial marine sediments

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Organic contaminants are introduced to the marine environment through coastal oil refineries, shipping activities and accidental spills. The organic contaminants most commonly found in marine sediments are: Polychlorinated Biphenyls (PCBs), Organochlorine Pesticides (OCPs) and Polycyclic Aromatic Hydrocarbons (PAHs). The characterization of PAHs in Lebanese marine sediments is a new field. The objectives of this research are to i) identify and characterize the concentration of PAHs in the sediments of Tripoli, Jounieh, Dora, and Tyre regions in Lebanon and ii) determine the possible sources of PAHs. Pre-sampling, sediment sampling, lyophylization, Soxhlet extraction, rotary evaporation and gas chromatography were performed. The total PAHs concentrations ranged from 2.49 to 731.93 μg/kg dry weight, with the lowest PAHs concentrations found in Tyre and the highest in Dora and Jounieh. The PAHs were mostly from pyrogenic sources.

Natural toxins: an on-growing challenge for environmental research, monitoring, and management (P)

MO192

Harmful algal bloom smart device application: using image analysis and machine learning techniques for early classification of harmful algal blooms

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The Ecological Stewardship Institute at Northern Kentucky University and the U.S. Environmental Protection Agency are collaborating to optimize a harmful algal bloom detection algorithm that estimates the presence and count of cyanobacteria in freshwater systems by image analysis. Green and blue-green algae exhibit different hue-saturation-value (HSV) color histograms in digital photographs. These differences are exploited by machine learning techniques to train a smart device (cellular phone, tablet, or similar) to detect the presence and amount of cyanobacteria in a small surface portion of a freshwater system. The Harmful Algal Bloom Classification Application (HAB APP) has been field tested and has proven to classify blue-green algae accurately. The HAB APP has been tested on several small streams and ponds, correctly classifying green algal blooms and has been tested on the Ohio River, correctly classifying blue-green algae in the recent 636-mile long cyanobacteria bloom. The application is planned to be tested and optimized in Lake Harsha, a 22,000-acre reservoir which supplies six million gallons per day of drinking water to the Ohio county in which it lies and is a source of many recreational activities, including swimming, boating, and fishing. The application will be used on images taken from a freshwater intake facility. The presence and amount (or lack thereof) will be verified by other detection instruments and in vitro by agency scientists and hysteresis techniques will be used to monitor the presence and amount of cyanobacteria on a periodic (e.g. daily, seasonally) basis.

MO193

Global bioaccumulation and biomagnification of Microcystis spp. highlights clonal diversity in natural bloom-forming populations and expands metabolite structural diversity

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Cyanobacteria are photosynthetic prokaryotes that are able to synthesize a wide range of secondary metabolites, with noticeable bioactivity (comprising toxicity) according to the ribosomal and non-ribosomal processes involving numerous NRPS/PKS clusters. Microcystis represents one of the most common cyanobacteria taxa constituting the intensive blooms that arise nowadays in freshwater ecosystems worldwide. These microorganisms produce numerous cyanotoxins (toxic secondary metabolites), which are potentially harmful to Human health and aquatic organisms. With the aim of contributing to better understand the variations in cyanotoxins production, such as microcysts (MC), between clones of the same cyanobacterial blooms, we investigate in the present study the diversity of several Microcystis strains isolated from different freshwater bloom-forming populations from various geographical area. Twenty five strains of Microcystis from the Paris’ Museum Collection of cyanobacteria (PMC) were compared by an integrated approach combining genotyping and metabolites chemotyping. These characterizations comprise analyses on morphology, Internal Transcript Spacer (ITS), microcystin synthesis gene, and global secondary metabolite contents. Complementary metabolomics shotgun analyses by MALDI-TOF and ESI-Q/TOF mass spectrometry reveal clear discriminant metabolite profiles between strains collected from identical or different localities. A global clustering analysis indicates that microcystin synthesis gene presence explains more of the metabolite diversity distribution, than the species or the sample localities do. A global network generated from MS/MS fractionation patterns of the various metabolite detected in all 25 strains performed with GNPS tool indicates that these Microcystis spp. strains produce a wide set of chemically diverse metabolites, comprising only few microcystins, but many aegyrisinos and microginins, together with a large set of unknown molecules that still remain to be investigated and characterized at their structure as well as at their potential bioactivity/toxicity levels. Shih et al. (2013) Proc. Natl. Acad. Sci. U.S.A. 110:1053-8

MO194

Cyanobacterial water blooms dominated by species from different orders produce teratogenic retinoid-like compounds into surface waters

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It is well known that cyanobacteria produce a diverse spectrum of compounds, some of which can cause adverse effects and pose risk to both aquatic organisms and humans. Our study characterizes the presence of retinoid-like compounds in field cyanobacterial water blooms and their production to the surrounding waters. These compounds are important for a lot of physiological processes in living organisms, but also belong to the most potent compounds causing teratogenicity. The first part of research focused on testing various processing approaches using diatom extraction (50% methanolic solution) and cyanobacterial water blooms. The next was to determine the concentration of these compounds from surface waters samples. Extracts of model cyanobacteria were analyzed for the presence of all classes of teratogenic retinoids in various cyanobacterial water blooms and their production to the surrounding waters. These compounds are important for a lot of physiological processes in living organisms, but also belong to the most potent compounds causing teratogenicity. The first part of research focused on testing various processing approaches using diatom extraction (50% methanolic solution) and cyanobacterial water blooms.
cyano bacterial biomasses of common species as well as artificial mixtures of several retinoid-like compounds were used during the optimization process. The total retinoid-like activity was determined by in vitro assessment using transgenic cell line P19/A15, the concentration of individual compounds was analysed by LC-MS-MS. Ten distinct procedures were tested for optimization of the sample processing with laboratory samples and model mixtures. Two of the selected procedures were efficient in concentrating the retinoid-like compounds from field water samples showing comparable results. The water samples from five study sites have shown retinoid-like activity over 20 ng/L equivalent concentration of standard retinoid all-trans retinoic acid (ATRA). The greatest detected retinoid-like activities in water affected by cyanobacterial water blooms reached several hundreds ng ATRA equivalent/L. Study shows that phytoplankton blooms of different composition can produce retinoid-like compounds to aquatic environment. Their production could directly affect the present organisms, especially sensitive early developmental stages, namely in case of massive water bloom development, which is a common problem in many surface waters. The work was supported by the Czech Science Foundation grant No. 14-29370P.

MO195
Influence of natural compounds produced by phytoplankton on the effects of anthropogenic estrogens commonly co-occurring in surface waters

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Adverse endocrine disruptive effects of (xeno)estrogens on aquatic biota have been known for decades. Some of the most potent estrogens like steroidal hormones estradiol, estranol, estriol and ethynylestradiol occur in surface waters world-wide. In addition, other less known chemicals, such as phytoestrogens, can contribute to estrogenic activity. Moreover, overall activity results from complex of both agonists and antagonists functioning of chemicals present in environmental mixtures. Apart from man-made pollutants, wide spectra of chemicals in aquatic environments are produced by harmful algal blooms, including some known phytoestrogens. This study combines results from field sampling with laboratory investigations to evaluate the role of phytoplankton metabolites in estrogenic activity of environmentally relevant mixtures in co-exposure with anthropogenic pollutants. Our research has shown estrogenic activities in biomasses of phytoplankton water blooms of various taxonomic composition from some freshwater reservoirs, where also several anthropogenic estrogens have been detected. The potential influence of the phytoplankton produced compounds on the effects of anthropogenic pollutants has been investigated in detail. The test protocol was characterized for extracts of broad spectra of cyanobacterial and algal species from different orders including several widespread species frequently dominating water blooms. The extracts were tested alone and in co-exposure with relevant anthropogenic pollutants that were shown to commonly co-occur with the water blooms. The study represents an example of combination of chemical and anthropogenic pollutants that were shown to commonly co-occur with the water investigated in detail. The interference with estrogenic receptor has been investigated in vitro. The extracts were tested alone and in co-exposure with relevant metabolites in estrogenic activity of environmentally relevant mixtures in including some known phytoestrogens. This study combines results from field sampling with laboratory investigations to evaluate the role of phytoplankton metabolites in estrogenic activity of environmentally relevant mixtures in co-exposure with anthropogenic pollutants. Our research has shown estrogenic activities in biomasses of phytoplankton water blooms of various taxonomic composition from some freshwater reservoirs, where also several anthropogenic estrogens have been detected. The potential influence of the phytoplankton produced compounds on the effects of anthropogenic pollutants has been investigated in detail. The test protocol was characterized for extracts of broad spectra of cyanobacterial and algal species from different orders including several widespread species frequently dominating water blooms. The extracts were tested alone and in co-exposure with relevant anthropogenic pollutants that were shown to commonly co-occur with the water blooms. The study represents an example of combination of chemical and biological approaches to investigate less known but widespread sources of estrogens and realistic scenario of mutual interactions of anthropogenic and natural compounds acting in mixtures. The study was supported by the Czech Science Foundation within the project P503/12/0553 and EU FP7 project SOLUTIONS (No. 603437).

MO196
Genotoxicity of complex mixtures of compounds extracted from different strains of cyanobacteria

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The occurrence of cyanobacterial blooms has increased significantly in many regions of the world in the last century due to water eutrophication. These blooms are hazardous to human health, ecosystems, and production of cyanotoxins, which can be classified in five different groups: hepatotoxins, neurotoxins, cytotoxins, dermatoxins, and irritant toxins (lipopolysaccharides). There is evidence that certain cyanobacterial toxins, such as microcystins, nodularins and cylindrospermopsins are genotoxic and carcinogenic. However, the mechanisms of the water bloom and revealed dominance of different species. Two of the selected procedures were efficient in concentrating the retinoid-like compounds from field water samples showing comparable results. The water samples from five study sites have shown retinoid-like activity over 20 ng/L equivalent concentration of standard retinoid all-trans retinoic acid (ATRA). The greatest detected retinoid-like activities in water affected by cyanobacterial water blooms reached several hundreds ng ATRA equivalent/L. Study shows that phytoplankton blooms of different composition can produce retinoid-like compounds to aquatic environment. Their production could directly affect the present organisms, especially sensitive early developmental stages, namely in case of massive water bloom development, which is a common problem in many surface waters. The work was supported by the Czech Science Foundation grant No. 14-29370P.

MO197
Toxicity of cyanotoxins to human liver stem cells: effects on 2D and 3D cultures

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Toxicity of cyanobacterial blooms poses a serious threat to human health and environmental human due to the production of cyanobacterial toxic metabolites. Microcystins (MCs) and cylindrospermopsin (CYN) are considered to be the most hazardous cyanobacterial secondary metabolites; since they are not associated with acute toxicity but they can induce also long term adverse effects at chronic exposures. In our study, we investigated hepatotoxic and hepatocarcinogenic effects of cyanotoxins using novel in vitro model of HL1HT1 cell line, which represent hTERT-immortalized adult human liver stem cells. HL1HT1 cells are characterized by expression of stem and liver oval cell markers, high proliferation potential, ability of anchorage-independent growth, and lack of gap junctional intercellular communication (GJIC). Micromolar concentrations of CYN induced strong cytotoxic effects in HL1HT1 grown in traditional monolayer (2D) cultures primarily affecting respiration (Alamar Blue assay) and cell energetic metabolism (Neutral Red uptake) rather than membrane integrity (CFDAAM assay). On the other hand, growth and viability of HL1HT1 cells were not affected by MC-LR up to concentration 10 micromol/L. In 3D spheroid culture, HL1HT1 cells became more sensitive to cyanotoxin treatment, where both MC-LR and CYN inhibited formation, growth and viability of spheroids (Alamar Blue) even at concentrations low as 0.1 micromol/L. The observed increase in the sensitivity of spheroid cultures indicate the ability of liver stem cells to differentiate in 3D microenvironment and to better recapitulate physiological liver-specific gene expression and functions. Microcystins are known to interact with proteins involved in cyanotoxin cellular uptake or bioactivation. This study demonstrates that 3D cultures of adult human liver stem cells HL1HT1 represent a perspective in vitro model which allows studying hepatotoxic, tumor promoting and carcinogenic effects of toxicants and understanding thus the role of stem cells in the development of chronic diseases and toxicities. Support: Project GA15-12408S funded by the Grant Agency of the Czech Republic.

MO198
Uptake and biotransformation of cyanobacterial toxins in cultures of liver progenitor and stem cells

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Cyanobacterial toxins (cyanotoxins) represent important group of aquatic contaminants. Microcystins and cylindrospermopsins are recognized as the most hazardous cyanotoxins with respect to their potential for both acute and chronic effects on human or wildlife health. These substances cause primarily hepatotoxic or nephrotoxic effects, in general, however, microcystins may cause negative effects also to other tissue or organ cells, such as kidneys, gastrointestinal tract, neural or immune systems. Organ- and tissue-specific effects of cyanotoxins are most likely governed by differential expression of genes responsible for cyanotoxin cell uptake, bioactivation/biotransformation and elimination, which might dramatically differ between cell types quickly being investigated in vitro uptake and biotransformation of cyanotoxins microcystin-LR and laboratory cultured cyanobacteria species. The greatest tested concentration of samples was 2 mg/L (DM – dry mass). However, some of the extracts were cytotoxic at this level, thus genotoxic evaluation was done only for non-cytotoxic levels. The genotoxic potentials of cyanobacterial extracts were evaluated using Single cell gel electrophoresis method (Comet assay) and Cytokinesis-block microculture micronucleus assay (Micronucleus test) - both by using human bone megakaryocyte HepG2 cell line. Comet assay reveals single and doublestrand breaks, and other abnormalities on DNA associated with incomplete excision repair, whereas Micronucleus test is a method for the evaluation of genomic instability. Using Comet assay, positive effects were shown for four cyanobacterial extracts: *Cylindrospermopsis raciborskii* at levels 0.2 – 2 mg/L and *Anabaena flos-aquae* at level 0.5 g/L. Benz[a]Pyrene at level 2.5 g/L/mg, and Etoposide at level 1 pg/mL. Results of our research confirmed hypothesis that complex samples of some cyanobacterial species can exert genotoxic effects, similarly to effects of certain pure cyanotoxins. This research was supported by the grant Cyanocost COST-STEM-ESI105-24073 and the Czech Science Foundation grant No. GACR 15/14-29370P.
cylindrospermopsin in experiments with tissue cultures of rat and human liver progenitor or stem cell lines, namely rat liver epithelial cell line WB-F344 which exhibit characteristics of multipotent liver progenitor cells, and hTERT immortalized adult human liver stem cell line HLhIT1. Sensitive and reliable LC-MS/MS method was used to monitor concentrations of parental cyanotoxins as well as their biotransformation products in the cell culture medium collected from monolayer cultures of undifferentiated and differentiated liver progenitor/stem cells, and also from 3D spheroid cultures of WB-F344 and HLhIT1 cells. Western blot analysis using specific antibodies directed against microcystin-LR was used to detect microcystin-protein adducts in the cells. Using our approach, we were able to characterize ability of each cell line to take up and metabolize microcystin-LR and cylindrospermopsin, to characterize effects of cell culture conditions on the kinetics of toxin uptake and transformation, and to correlate these differences with the changes in expression and activity of proteins responsible for the cell uptake of microcystin-LR (organic anion-transporting polypeptides) and metabolic transformation of cyanobacterial toxins (CYP450).

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MO201 Intracellular and extracellular retinoid-like activity of widespread cyanobacterial species

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Cyanobacteria are common and potentially harmful inhabitants of freshwater worldwide. They are producers of various types of bioactive compounds, which are toxic and they may cause animal death, embryotoxicity, teratogenicity and adverse effects on human health. Some studies indicate that certain phytoplankton species can produce retinoid-like compounds that could contribute to some of these effects. Retinoids comprise a family of polyisoprenoid lipids which include vitamin A and its natural and synthetic analogues. These substances act by binding to retinoic acid receptors (RAR), and regulate proliferation, differentiation, apoptosis, cytokine production, cell motility, and cell transformation of the cell. The aim of our work was to evaluate the variability in production of retinoid-like compounds, which could be released into the environment, by selected widespread cyanobacterial species. We tested in vitro potential of extracts and exudates from laboratory-cultured species to induce responses mediated through retinoid acid receptor (RAR) using transgenic murine embryonic carcinoma cell line, which contains reporter luciferase gene under the control of retinoic acid-responsive element. The detailed investigations focused on commonly occurring cyanobacterial species with tendency to form massive water blooms, one coccal (Microcystis aeruginosa) and four filamentous cyanobacteria (Anabaenopsis gracile, Limnothrix redekei, Cylindrospermopsis raciborskii and Plankothrix agardhii). We analysed 18 extracts and exudates of the five different cyanobacteria. We found activation of retinoid receptor after exposure to intracellular samples from all studied cyanobacterial species. The cyanobacterial exudates of all species except of P. agardhii also contained retinoid-like activity reaching up to 0.6 µg/g in case of one cultivation of L. redekei. We have recalculated total produced retinoid-like activity using extract and exudate data for each tested cyanobacterial species. The total retinoid-like activity of the five selected species ranged from 0.1 to several µg ATRA/g dry weight. The production of retinoid-like compounds was recalculated to biomass dry weight as well as to cell density. This enables to estimate the production in environmental biomass and highlight the effects of all investigated species on human health.

The research was supported by the Czech Science Foundation grant No. 14-29370P.

MO202 CYANOCOST: an EU COST Action on Cyanobacterial Bloom and Cyanotoxin Risk Management

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Cyanobacteria are ubiquitous photosynthesizing gram-negative bacteria associated with toxic water bloom in freshwater lakes, frequently serving as source of drinking water. Cyanobacterial production of a wide range of hepatotoxins, neurotoxins and dermatotoxins affects water quality, which is rising environmental and health concern worldwide. One of the most hazardous cyanobacterial toxins is microcystins (MCs). Due to their stability in the environment and high risk to human health, the WHO has established a provisional guideline of 1µg/L for microcystin-LR (MC-LR) in drinking water. Whereas, the hepatotoxic potential of MCs was studied extensively, there is growing lack of studies focused on the effects of cyanobacterial metabolites on immunity. The aim of our study was to determine the effects of MC-LR on physiological functions of macrophages (RAW 264.7), which represent one of the key effector immune cells. In our study, we used the concentrations of MC-LR (1 - 1000 nM), which were reported in human blood and thus are considered as relevant. Importantly, we have shown that even nanomolar concentrations of MC-LR significantly stimulated the production of proinflammatory cytokines, which could negatively affect human health. Interestingly, we found that the MC-LR induced changes were not attributed to the inhibition of protein phosphatases activity, due to the lack of microcystin trans-membrane transporters.

Considering all pieces of evidence gathered in the present study, we hypothesize that MC-LR is able to directly interfere with macrophage surface receptors that trigger the signal transduction pathways and thereby alter immune response. These results suggest that MC-LR have significant immunomodulatory activities even in very low and environmentally relevant concentrations. The research was supported by the projects of the Czech Ministry of Education L01214 and LM2010128, and by the CYANOCOST action (EU COST ES1105).
(3) Prevention, control and mitigation of cyanobacteria; (4) Drinking water treatment and cyanotoxins.

MO203

Behavioural and physiological responses of the oyster Crassostrea gigas exposed to three strains of Alexandrium minutum producing different toxin types

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Biologists of the dinoflagellate Alexandrium minutum, producers of Paralytic Shellfish Toxins (PST), are regularly detected on the French coastline. PST accumulate into harvested species, such as the Pacific oyster Crassostrea gigas, and can cause strong disorders to consumers at high doses. The impacts of Alexandrium minutum on C. gigas have often been attributed to its production of PST; however some studies revealed that these algae can also produce extracellular substances, some of which have allelopathic or ichthyotoxic properties. These compounds are excreted in the environment thereby impacting phytoplankton, zooplankton but also marine invertebrates and fishes, without implicating any PST. The chemical identity of those extracellular compounds is still unknown. The aim of this work was to compare the effects of three strains of A. minutum producing either only PST, only non-PST extracellular compounds, or both PST and non-PST extracellular compounds, on the oyster C. gigas. Behavioural and physiological responses of oysters exposed during 4 days to these three strains of A. minutum were therefore compared in order to discriminate between the effects attributable to PST and those induced by extracellular compounds. Our results indicate that the three strains of A. minutum induced different behavioural changes and physiological responses of oysters probably depending on bioactive substances produced by each strain. The extracellular-compound-producing strain primarily modifies valve-activity behaviour of C. gigas and induces hemocyte mobilization in the gills whereas the PST-producing strain causes inflammatory responses in the digestive gland. These results suggest that extracellular compounds have a significant harmful effect on the gills, which is the first organ in contact with the extracellular substances released in the water by A. minutum. Conversely, the PST impact the digestive gland, where paralytic toxins are released and mainly accumulated, after degradation of algal cells during digestion process of bivalves. This study provides a better understanding of the toxicity of A. minutum on oyster, and also highlights the significant role of extracellular bioactive compounds in this toxicity and the need to characterize these substances. Keywords: Alexandrium minutum, PST, extracellular bioactive compounds, oyster.

MO204

First insights in the characterization of allelopathic and cytotoxic extracellular compounds from the toxic dinoflagellate Alexandrium minutum

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To explore the chemical nature of putative ETC, we obtained indicated EC50 (48 hrs) values ranging from 0.5 mg/L to close to 100 mg/L for A. preuvianum having a C12 alkyl chain and most of them a 2-pyrrolidone ring, using dihydrotestosterone (DHT), 17β-estradiol (E2) and Triiodothyronine (T3). A. neogracile producing either only PST or PST and non-PST extracellular compounds, on the oyster C. gigas. Behavioural and physiological responses of oysters exposed during 4 days to these three strains of A. minutum were therefore compared in order to discriminate between the effects attributable to PST and those induced by extracellular compounds. Our results indicate that the three strains of A. minutum induced different behavioural changes and physiological responses of oysters probably depending on bioactive substances produced by each strain. The extracellular-compound-producing strain primarily modifies valve-activity behaviour of C. gigas and induces hemocyte mobilization in the gills whereas the PST-producing strain causes inflammatory responses in the digestive gland. These results suggest that extracellular compounds have a significant harmful effect on the gills, which is the first organ in contact with the extracellular substances released in the water by A. minutum. Conversely, the PST impact the digestive gland, where paralytic toxins are released and mainly accumulated, after degradation of algal cells during digestion process of bivalves. This study provides a better understanding of the toxicity of A. minutum on oyster, and also highlights the significant role of extracellular bioactive compounds in this toxicity and the need to characterize these substances. Keywords: Alexandrium minutum, PST, extracellular bioactive compounds, oyster.

MO206

Cytotoxicity to fish cell lines and endocrine effects of the mycoxinos beavericaine, deoxyxinvalenol and ochratoxin-A

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Harmful algal blooms (HABs) are blooms of microalgae able to produce toxic compounds that are accumulated by shellfish and affect human health through its consumption. Monitoring and public health management is therefore important, in order to avoid production of these toxins by forking the harvest of shellfish when a HAB outbreaks and/or toxin levels in shellfish flesh are over a legally regulated level. Research and monitoring of marine toxic microalgae tend to reduce the costs and time between the sampling and the results, in order to support decisions that affect human health, food safety and aquaculture and fisheries economy. Shellfish aquaculture is an important economic activity in some coastal areas of Catalonia and Spain. There have been several closures of Catalan shellfish harvesting areas due to the presence of Paralytic Shellfish Poisoning (PSP) toxins in shellfish flesh since the beginning of the monitoring program in 2002; spirolides were confirmed in 2014. Alexandrium species are among the main producers of PSP toxins and spirolides. There are 6 Alexandrium species in toxicities normally detected in Catalan coastal waters, of which produce PSP toxins (A. minutum, A. cutenella and A. tamarensis), 1 is known to be toxic (A. preuvianum) and 2 are considered non toxic (A. inaequatum and A. margaretae). Methods for microalgae identification (microscopy) and toxin detection (e.g. chemical and immunological) are often laborious and expensive. Recently, several genetic techniques have been developed for the study of mycoligase diversity; among which, NGS, whose throughput exceeds by millions of times the Sanger sequencing, producing high amounts of data cheaply and spreading the use of genetic analysis to a wide spectrum of scientific disciplines. These characteristics make NGS especially attractive for environmental risk monitoring and public health management. The project ALEXNEXT - Alexandrium sp. analysis by Next-Generation Sequencing (TECSR14-2-0012) is a proof of concept of the suitability of a NGS technique for identifying and quantifying marine microalgae. First, specific primers will be developed and tested on single-species Alexandrium samples. Tiered communities based on the DNA or the PCR products of the same Alexandrium samples will be made to investigate the limits of detection and the ability to quantify the content of each individual Alexandrium species DNA in the sample. Finally, the suitability of NGS will be assessed using selected samples collected from the Catalian coast. 
(0.02µM) showed antigestrogens activity, provoking a reduction of the luminiscence induced by E2 EC50 (0.07µM). BEA presented anti-androgenic activity and DON and OTA anti-thyroidal activity, at concentrations 1000 times higher than this of the EC50 of DHT (0.09 nM) and of T3 (0.09 nM). The data obtained in this work showed a high sensitivity of fish cell lines to the three mycotoxins tested and it was evident that these mycotoxins are endocrine disruptors at receptor level. Acknowledgements - Supported by INIA project RTA2012-00053-00-00

MO207 Surfactants from natural products Iaconic and Fumaric Acids: their ecotoxicity and potential for green chemistry M.B. Gösser, University of Bologna / The School of Life Sciences; E. Tagliavini, Università di Bologna / Dipartimento di Chimica G Ciamici / Alma Mater Studiorum; C. Samori, Università di Bologna / Dipartimento di Chimica Giacomo Ciamician; P. Galletti, Università di Bolonia / Dipartimento di Chimica G Ciamici; Z. Gadja-Meissner, T.F. Fernandes, Heriot-Watt University / School of Life Sciences

Interest in surfactants obtained from renewable sources has increased considerably due to their broad spectrum of industrial and everyday applications, the urgent need of decreasing the environmental impact of various chemical products and the general requirement of a more sustainable chemistry that no longer relies on fossil hydrocarbons. One interesting group of surfactants that can be synthesized in a sustainable manner includes products that can be obtained from fatty amines and itaconic and fumaric acids. Iaconic acid can be obtained from the fungal fermentation of waste biomass and is considered to be non-hazardous and biodegradable. Fumaric acid is a biogenic compound, considered to be in general non-hazardous. A series of different compounds have already been synthesized from these acids and have been evaluated focussing on their physicochemical properties and ecological behaviours. In this work a toxicological characterization of the surfactants was conducted in order to evaluate the safety profile of these products. Acute toxicity tests of 6 different surfactants, all of them having a 12 alkyl chain and most of them a 2-pyrrolidone ring, using Daphnia magna were carried out as a first step. Following the OECD guideline 202, young daphnids, aged less than 24 hours at the start of the test, were exposed to the test substance at a range of 7 concentrations for a period of 48 hours. Immobilization was recorded at 24 hours and 48 hours and compared with control values. Results obtained indicated EC50 (48 hrs) values ranging from 0.5 mg/L to close to 100 mg/L. The most toxic chemicals are now the focus of chemical modifications which aim either to lower the toxicity observed, or to increase it in view of possible uses as bacterial biocides.

MO208 Comparative toxicity of Quillaja saponins extracts and fractions X. Jiang; A.M. Hansen, B.W. Strobel, H.C. Hansen, University of Copenhagen / Plant and Environmental Sciences; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences

Saponins are natural surfactants, found abundantly in various plant species. Quillaja saponins (extracted from Quillaja saponaria) have been approved for use as a type of biofungicide. The extract contains around 70 structurally distinct saponin fractions. Among all fractions, QS-18 is the dominant and most toxic one. Another fraction, the QS-21 fraction, is used as an adjuvant. The Quillaja extract contains around 13.5% of QS-18 and 3.7% of QS-21. However, most research so far has been done on the entire plant extract containing all saponins as well as other bioactive compounds. Hence, it is not specifically known where the bioactivity comes from: the saponins or non-saponin content. In addition, as only few of the saponin fractions can be analytically detected, the half-lives in different environmental compartments are rarely known. In this study, the QS-18 and QS-21 fractions were isolated by preparative HPLC. The two isolated fractions will be used in both toxicity and degradation studies. Their toxicities will be compared with the toxicities of the commercially available extracts, Quillaja Saponin (S79000) (>10% saponin content) and Quil-A® (>95% saponin content), with Daphnia pulex and Vibrio Fischeri tests. The relative concentration of the different saponin fractions will be calculated by an internal standard (α-Hederin) using LC/MS. Preliminary results show EC50 of Quillaja Saponin S79000 and Quil-A® extracts towards Daphnia magna equal to 32.4 ± 1.0 and 19.3 ± 0.6 mg/L, respectively. When the relative concentrations of the different saponin fractions are known, the total dose and the dose of the two fractions can be determined using the assumption of concentration addition. Knowledge of the toxicity of the different saponin fractions together with knowledge of their degradation under different conditions, will contribute considerably to environmental risk assessment of saponin applications.

MO209 Exposure and risks of aquatic organisms to genetically modified corn pollen M.H. Wagelmans, Bioclear; E. Dijkhuys, FLORON

In this study the potential exposure of aquatic organisms to Bt-proteins in Corn pollen has been examined. The objective of this study was to gain insight into the potential exposure of aquatic organisms to Bt-proteins in Corn pollen and estimating the possible effects of this exposure in order to assess whether or not the aquatic ecosystem needs to be considered in the assessment of transgenic Bt-corn events in the Netherlands. This research was performed commissioned by COGEM (The Netherlands Commission on Genetic Modification). The research was carried out in phases. In the first phase a literature review to gain insight in the occurrence routes and uptake in ecosystems. The second phase was a field study to determine pollen deposition and dispersion in surface water. For this purpose, samples were taken in waterbodies bordering plots with corn. A total of nine sub locations have been sampled across three areas in the Netherlands. In addition a column test was carried out in order to determine how long pollen of Corn are residing in the water column. In phase 3 the results of the literature study and field study were combined and potential effects on aquatic organisms to Bt-proteins in Corn pollen were estimated. Also, just after the harvest, a visual inspection of the waterbodies was conducted to verify the assumption that crop residues of Corn in the Dutch situation do not end up in waterways. We have identified that Bt-proteins is contained in plant material and pollen of Corn if cultivation takes place adjacent to surface water. In case of cultivation of Bt-Corn potential risks of Bt-proteins for organisms in the aquatic environment are assessed as very low (based on the measurements of pollen deposition, numbers of pollen in surface water and dose-effect relations described literature). At this moment, based on current knowledge, the aquatic ecosystem does not need to be considered at admission of new GM event of Corn.

MO210 The risk mitigation of E. coli O157 in agricultural environment S. Taroni, O. Kaya, Adnan Menderes University /., Kırkhan, Adnan Menderes University / ad. U. Parfüm, Adnan Menderes University /. Escherichia coli O157:H7 causes several outbreaks as foodborne diseases by consuming leafy vegetables as lettuce and this pathogen has emerged as very important public health issues because of the gastrointestinal illness and long-term, chronic sequelae from an infection in many countries including Turkey. Many studies have been conducted that aquatic environments are exposed to plant material and pollen of Corn if cultivation takes place adjacent to surface water. In case of cultivation of Bt-Corn potential risks of Bt-proteins for organisms in the aquatic environment are assessed as very low (based on the measurements of pollen deposition, numbers of pollen in surface water and dose-effect relations described literature). At this moment, based on current knowledge, the aquatic ecosystem does not need to be considered at admission of new GM event of Corn.

Advances in exposure modelling: bridging the gap between research and application (P)

MO211 How to account for the variability in chemical emissions from consumer products? M. Douziech, Radboud University Nijmegen / Institute for Water and Wetland Research; A. Franco, Unilever / Safety and Environmental Assurance Centre SEAC; J. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; M. Huijbregts, Radboud University Nijmegen / Department of Environmental Science; H. King, Unilever RD Colworth; R. Oldenkamp, Radboud University Nijmegen / Department of Environmental Science; R. Van Zelm, Radboud University / Department of Environmental Science

To quantify the environmental risk of the increasing use of personal care products, accurate emission estimates of their components are required. In order to be reliable, these estimates need to consider the variability bound with the use of those products. Variability is induced by economic, demographic and social factors as well as by differences in consumer behaviour. Accounting for this variability is important to identify chemical emission hotspots. Recent (top-down) tonnes approaches estimate chemical emissions through disaggregation at smaller spatial resolution of products sales data using information about population density and GDP. The aim of this work is to explore potential improvements of methods to estimate chemical emissions from consumer products. One possible improvement is the consideration of more variables like age stratification level and ethnic origin when aggregating products sales data at smaller spatial resolution. Another possible approach for estimating the amounts of chemicals released to the environment is to use average product consumption data from consumer surveys thus accounting for differences in consumer behaviour (bottom-up approach). Both approaches define the amount of product used per time which can be expressed in form of a probability density function. In a second step, the inclusion levels of chemicals are derived from the chemical
composition of single consumer products. Finally, these values are used to estimate probability density functions of chemicals emitted to the environment. This work focuses on countries for which data is available for laundry products and shampoo, e.g. the European Union, the U.S., India and China. The chemical emission estimation techniques derived from this work can be used to derive global chemical emission maps. These maps can be used both for risk assessment and when conducting life cycle assessments of consumer products.

MO212

Modelling for engineered nanoparticle emission estimation: York case study

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Urban pollution has become one of the main threats to human health in developed and developing countries. Particulate matter (PM) is already a health concern in many cities, and predictions of urban population growth are estimating 2.5 billion more inhabitants by 2050 (UN, 2014). As populations rise in urban areas there will potentially be an increase in the emissions of contaminants to the environment. These emissions will include novel and non-regulated contaminants such as engineered nanomaterials (ENPs). A wide variety of ENPs are already integrated in many different commercialized products such as cosmetics, fuel additives, paints and coatings and will be released to the aquatic environment following use and disposal (Nowack et al. 2012). These ENPs vary in composition, shape, size and surface coating, which will determine their fate and behaviour after use. At the same time, environmental conditions such as pH, salt concentration and soil moisture of the local environments are good indicators of behaviour and fate. All these factors make the exposure estimations a complex issue. However, the use of models can help us to better understand this complex systems and to fill data gaps where monitoring data is not yet available (Prataortius et al. 2013). In this context the aim of this project was to develop a realistic, small scale model for the prediction of environmental concentrations of several ENPs in urban surface waters. By using local specific data on the use and disposal of commercialised ENP-containing products, jointly with high resolution data of the water chemistry and the physical characteristics of the studied area, we are able to realistically predict the local exposure to these nanomaterials and to estimate their potential impact on urban ecosystems by mapping out exposure hotspots. The high resolution local model for ENP exposure in urban areas was developed by combining two individual but interconnected models: an emissions estimation model and a river model. A multimedia box model approach was used. The emission estimation model includes two main pathways of emission of the ENPs (i.e. via the sewage system and direct run off from field and roads). Emission estimates are obtained at high spatial resolution and are fed into the river model, where different processes, such as hetero-aggregation and sedimentation, are simulated and a final estimate of ENPs exposure is obtained.

MO213

Predicting wastewater treatment plant emissions of pharmaceuticals

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Due to the increased use of human pharmaceuticals, emission of those substances into the environment have become a major concern. After consumption, pharmaceuticals are primarily metabolised in the liver and by the kidneys. After metabolism and excretion, a fraction of the substance is less polar and therefore remains in the body, while a fraction is excreted as metabolites or unchanged. This fraction is then released into wastewater treatment plants (WWTPs). Not surprisingly, concentrations higher than 0.1 μg/L have been detected in WWTPs. Because many compounds are incompletely transformed in WWTPs, they are discharged into the environment via effluent water and sludge, which forms a potential risk for humans and ecosystems. Therefore, understanding and predicting the fate of pharmaceuticals through WWTPs is important. SimpleTreat is a simple box model that estimate the relative emission of a chemical from a WWTP to the environment. The model has become accepted as an evaluation tool for generic exposure assessment of substances emitted via sewage treatment plants in the European Union. Recently, the model was adapted to ionized substances and provided with new equations for a higher variability in operation parameters. In this study, it is tested whether the fate and elimination of pharmaceuticals are well predicted by the improved SimpleTreat 4.0. Variabilities in predictions are explained. Field data obtained from literature, which are measured in activated sludge WWTPs, were used. This study yielded modelling concentrations of 44 different WWTPs. The modelled concentrations of SimpleTreat 4.0 were compared to observed effluent concentrations and chemical emission rates as derived from reported influent concentrations. The model predicts well in a number of cases. However, monitoring data are in some cases contrasting, due to variations in the operational system and environmental conditions, which are not included in SimpleTreat. Next to this, differences in detection techniques and the time between sampling and measuring, can cause variability in data, because biodegradation still takes place, which especially can influence the measured effluent concentrations. Furthermore, the chemical-specific data related to ionizing properties of certain pharmaceuticals are uncertain, leading to divergent model outcomes.

MO214

Model for assessing the fate of contaminants in constructed wetlands

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INTRODUCTION Qatar is an arid country with limited amount of natural freshwater resources. As a result, the country has been involved in the construction of wastewater treatment plants (WTPs) in order to reuse the treated wastewater. However, the economy continues to expand there is growing need for water resources for many domestic, agricultural, green space and industrial uses. There is currently a heavy demand on desalination of waters from the Arabian Gulf, however, there is value in looking at other potential sources of water to help meet the increasing demand. One such source may be the industrial wastewater which could be treated in wastewater treatment plants (WWTPs) and used, particularly for non-potable purposes. Produced water from the oil and gas industry represent large volumes of treatable water. At ExxonMobil Research Qatar (EMRQ), we are exploring the potential to use engineered wetlands, also known as constructed wetlands, to treat produced water. There are multiple examples of these types of engineered wetland being implemented both industrially and commercially. Cooperation Council (GCC) countries. Constructed wetlands typically consist of water flowing through a matrix of soil or sediment substrate consisting of living plants and associated microbes. The systems can be designed in various orientations, such as surface flow or subsurface flow, depending on the desired contaminant treatment and water characteristics. There are a number of biological and chemical processes that result in the sequestration, breakdown, evaporative loss and/or transpiration of contaminants. A significant amount of the activity occurs in the rhizosphere region and depending on the oxic or anoxic state of the system, different biochemical reactions will occur. These systems are designed to maximize removal of contaminants in water flowing out of the system. Consequently, it is important to model the transformation of the contaminants to be treated and how best they can be treated. An effective approach to help understand constructed wetland behavior and the resultant fate of contaminants is to utilize mass balance models specifically built with the various wetland processes in mind. MASS BALANCE MODEL OF CONSTRUCTED WETLANDS (EMRQ, with collaboration of Simon Fraser University (Canada)), has developed a simple model for the environmental fate of contaminants in constructed wetlands, with the ultimate goal of aiding in the design and monitoring of engineered wetlands to support treated wastewater use applications. This is a preliminary model that has not been tested with field-generated data and, thus, is applied in an illustrative fashion. Regardless, the model is useful as an evaluative tool for exploring the potential treatment of different types of contaminants, ranking treatment effectiveness among contaminants groups, and identifying potential parameters or processes that are important to understand in more detailed to build more representativeness into the model. With time and application to field data, the model may be used to help design similar systems, understand their inner workings and also potential mitigations required to ensure the safe and effective use of the wetland across its lifetime of operation. The model has been developed with modules for: (i) environmental distribution of contaminants in aquatic system; (ii) uptake, translocation and biotransformation of contaminants in vegetation; (iii) bioaccumulation in aquatic biota of wetlands; and (iv) risk evaluation of contaminant toxicity to resident biota. The model computes both steady-state and time dynamic responses of the mass and concentrations of contaminants in the wetland. The wetland model is currently able to simulate free surface flow, horizontal subsurface flow and vertical subsurface flow wetland configurations. Model inputs include wetland characteristics (e.g., compartment volumes, dimensions, soil composition, environmental conditions (e.g., water flow and temperature); and contaminant properties (e.g., molecular weight, degradation half-lives). MODEL ASSUMPTIONS This model is based on some key assumptions. First, it is assumed that the chemical is homogeneously distributed within each of the wetland media (e.g., water, sediments, plants) and that the wetland media can be described as single compartments in its exchange with its surrounding environment. It is recognized that this is an over simplification and that in particular there is likely to be a significant concentration gradient within the different media near the water intake location compared to those same media nearest the outfall. Realistic contaminant exchange between media will be further complicated by this effect. However, for our purposes as stated above, this is a reasonable assumption to make for such a preliminary model. A second key assumption is that the rhizomes are part of the vegetated rooting medium and in equilibrium with the rooting medium. This assumption may be reasonable for many organic contaminants and nonessential metal ions such as nitrates. However, for mercury, this model further assumes that vegetation can increase in biomass, which reduces the chemical concentration but causes no net loss of the chemical from the wetland. While these assumptions may be clearly gross simplifications of reality, they are reasonable first approximations for this screening level tool. A model user must first identify the system dimensions, particularly the area foot print (of the various media, water and sediment compartment depths and intake wastewater flow rate. There are a number of other process specific parameters (e.g., media diffusive exchange rates, media biodegradation rates, etc.) that need to be specified. At the moment assumptions have been made about these parameters on the basis of other studies. In some cases parameters associated with constructed wetlands may be quite different from what is seen in the future.
This work focuses on countries for which data is available for laundry products and shampoo, e.g. the European Union, the U.S., India and China. The chemical emissions will include novel and non-regulated contaminants such as nanoparticles: Analysis of TiO2, Ag and C60. The poster will present the model predictions versus observations, and discuss their structural differences in details.

**MO215** Human exposure to household surface cleaning products: Application of a two-field model

G. Wang, University of Michigan / Environmental Health Sciences; L. Huang, University of Michigan / Dept of Environmental Health Sciences; D. Jolliot, University of Michigan / Atmospheric Sciences

Indoor cleaning activity is a major human household activity and cleaning products contain volatile organic compounds (VOCs) that constitute a potential threat to indoor air quality and occupants health [2]. The overall objective of this study is to evaluate the short-term human exposure to household cleaning products. More specifically we aim 1) to establish a dynamic modelling framework which accounts for the near-person inhalation and dermal exposure to indoor cleaning products; 2) to determine the evolution of chemical mass and concentration associated with indoor cleaning activity, and to determine the key factors affecting chemical fate and exposure for indoor environment; and 3) to determine the short-term human intake, intake fraction (IF), and product intake fraction (PIF). We model a two-zone model to describe the near-person and the far-person field identifying four transfer compartments, i.e. near-person surface, near-person air, far-person surface, and far-person air. Transfers between compartments are described by first order transfer rate constants structured in a matrix (K matrix) to describe the mass flows between different. The exposure matrix (P matrix) relates the mass in a given environmental compartment to the intake by human. Eigenvalues and eigenvectors of the K matrix simulate the dynamic behavior of the chemical mass in each compartment and variations in human intake through different exposure pathways. For both chemicals, mass in each compartment reaches its peak at 1.5 hour, at the end of the application phase. Of special interest is the contrast in mass evolutions between high and low volatile chemicals. For benzene the two air compartment tends to have the largest mass of chemical after the products is applied, whereas the mass on the near- and far-person surfaces are also high for 2-butoxyethanol. For both chemicals, inhalation exposure dominates the human intake during application phase. Compared to low volatile chemicals (2-butoxyethanol), dermal uptake is negligible for high volatile chemicals (benzene). The adapted two-zone model and its dynamic version using eigenvalues and eigenvectors enables us to describe well and with parsimony the dynamic of masses and intakes. The presentation further details and contrast the time evolution and the resulting intake fraction and product intake fraction for the 21 considered substances.

**MO216** Evaluation of models for gas-particle partitioning of nitro- and oxy-aromatic hydrocarbons

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Gas-particle partitioning is an important mechanism affecting the transport and fate of semi-volatile organic compounds (SOCs). The preferential partitioning of SOCs in the atmosphere depends on parameters such as the compound’s molecular structure as well as particulate matter physical and chemical properties. This can be explained by various empirical and theoretical models based on single- or poly-parameter linear free energy relationship (pplFER). To explain the SOC partitioning, each model considers one or more of the compound’s functionality to include other lower order organisms (e.g., phytoplankton, zooplankton, benthic invertebrates) and fish to determine how contaminants may have an impact in the wetland system. This bio-uptake / bioaccumulation module draws upon extensive experience in the field of food web dynamics and ecotoxicology and provides another interesting layer of detail to a system that is known to become relatively diverse in nature and also depend on some of these organisms to maintain its health and effectiveness. Finally, the model has been developed to examine a suite of oil and gas related contaminants. Representative constituents of volatile, semi-volatile and polycyclic aromatic hydrocarbons can be modeled. It is possible to examine other ionizable organic compounds such as Naphthenic acids. Metals can also be modelled in the system. In each case the model requires a description of the physical system that will help determine its ability to transport between media and be sequestered or transform in a media. Ecotoxicity data will be necessary to determine at what concentrations of each constituent effects on microbes, plants and other organisms may be seen. REPRESENTATIVE ANALYSES Three representative wetland organic contaminants (freeruns and surface flow) were modelled using a non-ionizable organic, an ionizable organic and a metal constituent to show the functionality of the mass balance modeling tool. Differences in the ability of each wetland to remove each contaminant from the wastewater stream can be expressed. The removal fraction of each constituent is estimated for the various processes, both at steady state and with time using the dynamic analysis. The steady state analysis provides a good indication of the anticipated removal efficiency in the wetland and the ability to quickly compare removal processes across contaminant constituents and across different wetland systems. This is quite helpful for comparison and ranking purposes. The temporal simulation provides the benefit of predicting the time-variable loading of constituents in media which may provide some indication of the timing required for remedial action in the system (e.g., plant harvesting once the holding capacity is met and removal efficient significantly declines; metal toxicity to microbes in the sediment/rhizosphere). This too is beneficial specifying the design of the system and determining the cost-benefit analysis of different approaches and systems. CONCLUSION Because there are currently insufficient data to conduct an actual model performance evaluation, the model has only been applied in an illustrative fashion. This application aims to demonstrate some of the key model features. The model may be most useful in evaluating the relative treatment capacity of wetlands for certain contaminants where certain contaminant removal efficiencies are unaffected by wetland treatment. The model can also illustrate the relative importance of various chemical removal mechanisms for particular contaminants and illustrate the relative capacity of wetland treatment for different contaminants depending on substance and wetland properties. Also, model results provide insights into the temporal response of contaminant concentrations in wetland media and resulting contaminant concentrations in different wetland media. Finally, it may help to assess if the build up of concentrations in the wetland media can pose ecotoxicological concerns for resident macrophytes, micro-organisms, invertebrates and fish that inhabit the wetland. Such evaluations are regularly used in environmental assessments of commercial chemicals and may also be useful in assessing the potential of wetlands to treat contaminated wastewaters. The ability of wetlands to treat contaminated wastewater is highly dependent on the contaminants and wetland characteristics. While some contaminants will be efficiently removed from wastewaters by the wetlands, others will be minimally affected. The models may be of value in predicting contaminant concentrations. The models can make estimates of contaminant removal efficiency of the wetlands throughout their lifetime and predict whether wetland treatment can be expected to produce effluent streams that meet health and environmental standards. They can also be used to investigate key wetland design specifications such as size and vegetation type.

**MO217** SimpleBoxx nano, A Screening level Environmental Exposure Model for Nanoparticles: Analysis of TiO2, Ag and C60

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In order to sustain the development of nanomaterials by industry, a robust risk efforts in model parameterization and performance analysis, the model may become more useful as a predictive tool for assessing the remediation capacity of contaminants using various engineered wetland designs. It is recognized that future work should include the evaluation and testing of the model capability to predict non-target effects and to process and interpret field data. This type of assessment could provide guidance to field experimentation on what testing and refining of the engineered wetland model presented in this study, with future
assessment framework is needed. A screening level environmental exposure model is one of the first steps of such a framework currently in place in Europe and the United States, but this needs adaptation for use with nanomaterials. We incorporated the SimpleBox4nano model definition previously published into the Simplebox model currently implemented in USES as part of REACH. This is a new multi-scale model that can project concentrations (PECs) for 4 different chemical species in the environment. We use this model to show the uncertainty of estimating PECs for TiO2, Ag and C60 nanoparticles (nPs). These nPs represent three common nP types, being a poorly soluble metal oxide, a readily soluble metal and an insoluble carbonaceous nP, respectively. The default scenario of SimpleBox v4 is used, which includes the Rhine catchment, Europe and the whole Northern hemisphere. In order to assess the uncertainty of the steady state PECs we conducted a probabilistic assessment based on the uncertainty in nano specific input parameters. The variability and uncertainty of other parameters was not included due to this being similar for conventional chemicals and is expected to currently be at an acceptable level for screening purposes. The results show that the variation of the obtained PECs can be explained largely by the uncertainty in the emission rates. Other important parameters explaining the variation in PECs are (i) the uncertainty in the attachment efficiency between nPs and natural colloids and other particulate matter. And (ii) the dissolution rate of nPs. It was found that the dissolved species of nPs reached very high concentrations in certain environmental compartments, this is however due to the low removal rates from these compartments. The concentrations were much lower when considering the 1 year PECs calculated using the dynamic module of SimpleBox4nano. This suggests that for regulatory purposes, time dependent PECs might be more relevant than steady state concentrations in exposure assessment of nPs.

MO218 A risk management model for evaluating the impact of sediment resuspension on the distribution, bioavailability and toxicity of harmful contaminants in the Port of Antwerp and beyond. H. Hetiens, S. Spore / SPHERE, J. Teuchies, R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology

The port of Antwerp is Europe’s most central and second largest sea port, ranking second behind Rotterdam by total freight shipped. It is a cluster of industry and marine and road traffic in a densely populated area. Present and historical activities have caused the contamination of sediment and water and led to a moderate to low water quality within the harbour docks. Under undisturbed circumstances the impact on water quality of contaminants fixed in the sediment layer is relatively low, but has been investigated to increase significantly when contaminated sediments are resuspended. In order to maintain or improve the current water quality of the Port of Antwerp as well as the connected Scheldt estuary areas, the aim of the present project, which is part of EcoDocks+, a cooperation between the Antwerp Port Authority and the University of Antwerp, is to develop a dynamic risk management model for contaminants under disturbance in the aquatic environment. With this model the main sources for sediment resuspension within the port area will be determined and the re-accumulation behaviour of contaminants in the water phase investigated. It is expected that sediment resuspension and transport and therefore the distribution, bioavailability and toxicity of harmful contaminants is mainly caused by three major factors: (1) advective sediment flux through the sluices, (2) ship traffic and (3) sediment mining activities. In the coming months, existing literature and data sets as well as the results from own measurements will be used to develop a user friendly interface that can be used to calculate the short and long term changes in concentrations of contaminants in water and sediment layers during and after resuspension and to determine the effect size on the aquatic environment of each of the three factors. In the future, our results and the model itself can be used to identify areas with high risk potential and evaluate possible risks of future maintenance or construction works on harbour sediments on current water quality status.

MO219 Does the inclusion of mass balance and degradation processes to vegetative filter strips impact long-term pesticide environmental exposure assessments? A. Ritter, Waterborne Environmental, Inc.; R. Muñoz-Carpena, University of Florida / Department of Hydrology Water Quality Agricultural Biological Engineering; O. Storløkken, University of Tromsø an Medical University; G. Gjøsteen, Department of Food Technology and Agricultural Engineering; O. Perez-Ovilla, Bayer CropScience LP

Vegetative filter strips (VFS) are widely adopted for reducing pesticide transport from adjacent fields to receiving water bodies. The long-term VFS efficacy is dependent on site-specific factors related to soils, weather, land use, vegetation and maintenance. The previous version of the well-tested process-based model for VFS (VFSMOD) did not incorporate processes that can project multi-scale results for residual US EPA scenarios. Global sensitivity analysis (GSA) was used to assess the relative importance of adding or removing mass balance and degradation processes in the context of other input factors like VFS length (VL), pesticide organic-carbon sorption coefficient (Koc), and half-lives in both water and soil phases. It was concluded that considering degradation in the VFS was not relatively important if single, large events were controlling the transport process, consequently, VFS length and higher inputs than exposure assessments. Degradation processes become more important when considering percent reductions in acute or chronic EECs, especially under scenarios with lower pesticide losses.

MO220 Development of a scoring system for surface soil pollution potential of chemicals from accidental release using a multimedia model SoilICCA K. Kim, J. Jung, Seoul National University; Y. Lee, L. Chang, Seoul National University / Dept of Environmental Studies; D. Lee, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies

Hazardous chemicals accidentally released into air can transfer to surface soil by dry and wet depositions, leading to surface soil pollution which can adversely impact the soil ecosystem. A new scoring system was developed to evaluate the surface soil pollution potential of chemicals accidentally released into air. Scores for surface soil pollution potential of individual chemicals were to be determined by multiplying three indicators i.e., level of pollution, persistence of pollution, and spatial extent of pollution given a unit quantity (1 ton) of release. A new dynamic multimedia model named SoilICCA was developed to calculate the scores. SoilICCA consists of a total of 3100 cells to cover an area of 15 km (L) x 3 km (W) as a model domain. Each cell is of a 150m x 150m x 1000m size with three compartments, i.e., air, surface soil, and vegetation. The temporal and spatial variation of concentrations in surface soil was investigated for five typical types of chemical release. It was found that the concentration profiles with time and space were practically independent of the release type with a difference only in the concentration level and the rate of decrease of the concentration among the chemicals. From the findings, the three equations were derived to calculate the indicators: i) pollution level as represented by the average concentration, ii) persistence of pollution by the slope of linear relationship between logarithm of the surface soil concentration and time, and iii) spatial extent of pollution by logarithm of the maximum concentration in surface soil. About 600 chemicals in the list of “Toxic Chemicals Control Act” of Republic of Korea will be evaluated for their surface soil pollution potential.

MO221 Evaluation of accidentally released chemicals for their surface soil pollution potential by using SoilICCA K. Kim, J. Jung, Seoul National University; Y. Lee, L. Chang, J. Park, Seoul National University / Dept of Environmental Studies; D. Lee, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies

Hazardous chemicals accidentally released into air were evaluated for their surface soil pollution potential. Scores for surface soil pollution potential of individual chemicals were to be determined from three indicators (level of pollution, persistence of pollution, and spatial extent of pollution given a unit quantity (1 ton) of release) by using SoilICCA. About 600 chemicals in the list of “Toxic Chemicals Control Act” of Republic of Korea were evaluated. Watershed and semi-volatile compounds showed higher scores than VOCs, which was qualitatively anticipated prior to the evaluation. Chemicals of the greatest surface soil pollution potential include heavy metals and semi-volatile organic compounds such as benzo(a)pyrene as their wet deposition flux and sorption capacity onto soil particles are high while the loss rates by diffusion to air and surface run-off are low. For the organic compounds evaluated, only the indicator for the spatial extent of pollution showed negative correlations with the Henry’s law constant exceeding a certain value depending on the chemical groups. The low pollution potential of VOCs can be accounted for primarily by their high vapor pressure and Henry’s law constant. Of particular use of SoilICCA was made of evaluating organic compounds that belong to neither semi-volatiles nor VOCs, which would not readily be done without a quantitative tool.

MO222 Monitoring and modeling of POPs in air at Cape Verde, Africa T. Voss, A. Hackl, NIU - Norwegian Institute for Air Research; S. Eckhardt, NILU - Norwegian Institute for Air Research / Atmosphere and Climate Change Department; A. Halse, NILU - Norwegian Institute for Air Research; M. Schlabach, NILU - Norwegian Institute for Air Research; K. Breivik, Norwegian Inst. for Air Research

Ambient air is among the core media selected for the sampling and analysis of Persistent Organic Pollutants (POPs) under the Stockholm Convention. Ambient air also provide useful information for (i) studies of global transports of POPs, and (ii) atmospheric sources and source regions. Yet, existing data on POPs in air based on active air sampling remain scarce and mainly limited to industrialized regions in the northern hemisphere. The primary objectives of this study were to i) evaluate the concentration of selected POPs in air outside West Africa, and (ii) to evaluate sources and source regions affecting measured concentrations. For this
purpose, an active high-volume air sampler was installed on The Cape Verde Atmospheric Observatory (CVAO: 16.848°N, 24.871°W), a World Meteorological Organization (WMO) global station at Cape Verde outside the coast of West Africa. Sampling commenced in May, 2012 and 43 samples were collected until June 2013 (24 h sampling). The samples were analyzed for selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), hexachlorobenzene (HCB) and chlordanes. The source regions of the air masses were evaluated for each sample using the FLEXPART model. FLEXPART is an atmospheric Lagrangian transport model, developed by Andreas Stohl and co-workers, which calculates the trajectories of so-called tracer particles (FLEXPART.eu). It was run in backward mode, in order to identify the source regions of air pollutants at a particular site. A general finding is that the concentrations measured are in the lower range in comparison to data from the literature, which is mainly attributed to the remoteness of the station from major sources and source regions. Factors controlling measured concentrations will be evaluated and discussed.

MO223 Modelling the behaviour of cyclic volatile methyl siliconeoxanes in an Arctic benthopelagic freshwater food web L.S. Kruger, Wellington Institute for Air Research / Environmental Chemistry; E. Underman, Stockholm University / Environmental Science and Analytical Chemistry ACES; A. Evensen, G. Christensen, Akvaplan-niva AS; M. Whelan, University of Leicester / Geography; K. Breivik, Norwegian Inst. for Air Research; N.A. Warner, NILU - Norwegian Institute for Air Research / Environmental Chemistry

Cyclic volatile methyl siliconeoxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. Due to the high hydrophobicity of cVMS, sediments are expected to be an important source for biotic exposure in benthic and benthopelagic food webs. However, details of the exposure pathways have yet to be fully understood. The main objective of this study was to explore the role of the sediment compartment for bioaccumulation of cVMS in an Arctic lake (Lake Storvannet, 70°N 23°E) through a combination of modelling and measurements. Storvannet is an oligotrophic lake with ice-cover for 6 months of the year and a short summer season. Food webs for resident Arctic Char (Salvelinus Alpinus) and Brown Trout (Salmo Trutta) are strongly connected to the benthic community. Sediment, benthos, and fish were collected and analyzed for cVMS concentrations in 2014. Concentrations of decamethyleneoctanoloxiane (DS) were comparable to concentrations found near more highly populated areas, and were significantly higher in muscle tissue of Arctic Char (3.8 ± 2.8 g/g lipid) than Brown Trout (0.8 ± 0.6 g/g lipid). Higher values of δβ-N in Brown Trout (12.4 ± 0.9) than in Arctic Char (10.4 ± 1.0) indicates trophic dilution, but their different cVMS concentrations may also be influenced by species-specific differences in behavior or biotransformation. To rationalize the measured concentrations, a mechanistic pelagic bioaccumulation model (ACC-HUMAN) was modified to incorporate benthic exposure. The model was specifically parameterized for key benthic species (the deposit feeder Chironomidae sp. and the filter feeder Posidum sp.) and fish, including time-variant uptake and elimination processes for ventilation, feeding, growth, egestion and biotransformation. Measured concentrations in sediments and modelled concentrations in water were used as input to the model. Preliminary simulations show that incorporation of a benthic link in ACC-HUMAN can be important to rationalize observed data, but also cVMS oligomer patterns in fish. The model was used to explore the influence of different dietary scenarios, as well as observed concentration differences across Arctic Char and Brown Trout. This model-measurement comparison helps to both evaluate the new benthic ACC-HUMAN model and to contribute new insights into the trophic transfer of cVMS in benthopelagic ecosystems.

MO224 Critical review and interpretation of environmental data for volatile methylsiloxanes: Monitoring cVMS in non-biological media S. Xu, Dow Corning Corporation / Health Sciences; N.A. Warner, NILU - Norwegian Institute for Air Research / Environmental Chemistry; J.A. Durham, Dow Corning Corp. / Health and Environmental Sciences; D.A. McNett, Dow Corning Corporation / Health and Environmental Sciences

Due to their heavy use, cyclic volatile methylsiloxanes (cVMS) are under increasing regulatory scrutiny. In last few years, the transport and distribution of these compounds have been studied both by multimedia environmental fate modeling and also by the environmental monitoring. The objective of this project was to compare the monitoring results of cVMS with their model predictions and to identify the critical data gaps in our understanding of the major environmental processes regulating the transport and distribution of cVMS in the terrestrial environment. The published modeling assessments included both simple steady state simulations and time variable analysis at local, continental and global scales using both hypothetical and realistic release scenarios. The non-biological media studied in the disclosed environmental monitoring programs included air, soil, water and sediment mostly in regions impacted by human activity, especially some with remote Polar Regions. The temporal and spatial patterns of cVMS concentrations in air, soil, and sediment, were generally in a good agreement with the modeling predictions with exception to a recently published findings in Antarctica. The biggest inconsistency with the model predictions was the concentration ratios of various cVMS measured at different locations. This highlights that future work is needed to develop monitoring methods which can prevent samples from contamination and to minimize artifacts and misinterpretation in reporting cVMS environmental behavior including the concentration ratios.


About five years has passed since the accident of the Fukushima Daiichi Nuclear Power Plant (FDNPP). Topic of interest has shifted from analyzing the current situation to radioactive contamination. Therefore, predicting the future situations of radioactive pollution especially of cesium (Cs-137). Previously, we reported simulation results of terrestrial environmental fate of Cs-137 in Japan about 10 years after the accident, using the multimedia environmental fate model (Grid-Catchment Integrated Multimedia Modeling System, G-CIEMS). Based on this simulation result and published researches, it was revealed that major part of Cs-137 had deposited to forest area, most of Cs-137 strongly attached to soil surface after the deposition, and therefore Cs-137 would slowly run off from the forest area. Several researchers reported that outflow of Cs-137 to the ocean predominantly occurred during typhoon Roke September 21, 2011. Therefore, in this study, we improved our model in order to simulate daily change of environmental conditions and temporal fate of Cs-137. In this model, we 1) calculated amounts of direct deposition of Cs-137 to surface waters (rivers and lakes), 2) calculated daily amount of soil runoff from land area using Universal Soil Loss Equation (USLE), 3) set up daily river flow rate, and 4) calculated daily suspended solid (SS) concentration in river. As the first step, we made polygon data of surface water segments which were used in our model, calculated the area of each surface water segment in each 1-km mesh, and then calculated amounts of direct deposition of Cs-137 to each surface water segment. As the second step, we calculated daily “rainfall” factor in USLE using 30 minutes precipitation data for each 1-km mesh, and then calculated daily soil loss from land by erosion. As the third step, we set up daily flow rate of each river segment based on daily precipitation and simple assumptions. As the fourth step, we calculated daily SS concentration in each river segment using daily input amounts of SS (= soil loss from dry land) and mass balance equation of SS. We simulated annual fate of Cs-137 in Abukuma river basin and river basins in Hamadori region, the surrounding area of the FDNPP, and obtained good agreement between simulated flux of Cs-137 and field observations.

MO226 Long-term and large-scale prediction of air radiation dose rate in Fukushima: incorporating vertical migration of radioactive cesium K. Kuroda, Y. Imaizumi, National Institute for Environmental Studies; M. Takagi, National Institute for Environmental Studies / Centre for Environmental Health Sciences; N. Suzuki, S. Hayashi, T. Ohara, National Institute for Environmental Studies

Long-term prediction of air radiation dose rate is important in Fukushima, Japan, where a large amount of radioactive cesium was deposited following the accident of Fukushima Dai-ichi nuclear power plant in March 2011. In general, air dose rate derived from deposited radioactive cesium is known to decline with time, not only because of natural decay, but also because of various processes, including migration of cesium into deeper soil. The rate of decline excluding natural decay would vary depending on locations and other factors. To date, however, there has been no comprehensive study on this. In the present study, we evaluated the effect of radioactive cesium migration on the decline of air dose rate in a large area, using various geo-referenced and time-course data of deposition and soil depth-profile of radioactive cesium and air dose, which have been published since the nuclear accident. The decline of air dose rate was evaluated, after eliminating the effect of natural decay, as the temporal change of conversion coefficients between the depositional radioactive cesium amount and the air dose rate measured at 1 meter above the ground. The soil migration of radioactive cesium was evaluated by the temporal change of weighted center obtained from the depth profiles. The results showed that the conversion coefficient decreased with time and with depth, although a large variation was noted on the temporal change. Finally, we predicted the long-term change of air dose rate in the large area of eastern Japan, considering the effect of migration of radioactive cesium on the conversion coefficient. Our study highlights the importance of vertical migration of radioactive cesium for a better spatiotemporal prediction of air dose rate.

MO227 A Global Historical Inventory of Individual PBDEs: From Production Towards Emissions A. Geelhoed, NILU - Norwegian Institute for Air Research / Geography; K. Breivik, Norwegian Inst. for Air Research

Despite advances in environmental fate modeling, measuring and monitoring of
persistent organic pollutants (POPs), knowledge about their sources and emissions remains a major knowledge gap. This mitigates our potential to understand and predict historical and future POPs concentrations in various environmental media. The first step towards estimating the global emissions of disproportionately produced POPs and their future trends is to obtain an overview of their production and consumption on a global scale. In this study, we compiled data on the global production of three main commercial mixtures of PBDEs (penta, octa and decaBDE) from 1970, along with data on the chemical composition of these mixtures, in order to estimate historical production trends of selected individual PBDE congeners. The global consumption over time was next estimated from available information on PBDEs in the environment, capita consumption and/or surrogate data. These results will be coupled with material flow analysis to estimate the amount of waste PBDE-containing products that are subject to transboundary movement. The results of this study will be used in a global emission model to estimate temporal trends of PBDE emissions at the global scale.

MO228 Environmental exposure assessment of sucralose in receiving waters at differing spatial scales
K.E. Kapo, R. Yamshi, M.L. Sebasky, Waterborne Environmental, Inc.; D.B. Huggett, Waterborne Environmental, Inc. / Department of Biological Sciences; C.M. Holmes, Waterborne Environmental, Inc.

Down-the-drain exposure models provide a valuable screening-level tool for estimating environmental exposure to product ingredients which are treated and discharged at municipal wastewater treatment plants. We present an enhanced down-the-drain exposure model for sucralose, an artificial sweetener which ultimately ends up in the environment via the drain emissions. Exposure modeling was performed using the iSTREEM® model, a publically-available web-based model supported by the American Cleaning Institute (www.istream.org) which estimates spatially-explicit concentrations of chemicals in effluent and receiving waters across the U.S. at mean and low flow conditions. Wastewater treatment facility influent loadings of sucralose were estimated using per-capita usage derived from market sales volume combined with individual facility population served and daily flow estimates within the iSTREEM® model. The screening-level assessment used an assumption of zero removal during treatment and no non-steady decay, resulting in a representation of “worst-case” environmental exposure estimates. Three case studies of modeling at different spatial extents are presented: national scale of the continental U.S., regional scale of the Lake Erie drainage basin, and local scale of the Grand River Watershed in Canada. US-wide predicted environmental concentrations (PECs) estimated by the model at mean annual flow conditions were comparable to sucralose concentrations typically expected to be observed in the field, with a 90th percentile PEC in surface waters of approximately 1 µg/L. Watershed-scale modeling of the Grand River was compared to published data from 23 sites measured in 2007-2009. This local assessment was enhanced with temporally-specific adjustments to flow. Once time-specific gaging data were added, the model predicted a comparable exposure pattern to those measured across the 23 sites. Maps of the estimated geographic distribution of US-wide and Grand River watershed river concentrations are presented using geo-referenced concentration data generated by the iSTREEM® model. This screening-level environmental exposure assessments provide an estimated distribution of PECs in a spatial and potentially temporal context. These can be used to inform risk management and/or subsequent higher-tier assessment.

MO229 FlowEQ - a coupled flow-network and fugacity based fate and transport model for the assessment of Bispheno A in the environment
M.J. Bock, M. Bunge, M. Vosteen, Ramboll Environ; I. Gutiérrez, BiPRO GmbH; H. Huggett, Waterborne Environmental, Inc. / Department of Biological Sciences; C.M. Holmes, Waterborne Environmental, Inc.

The FlowEQ model was populated with watershed information and emissions of Bisphenol A (BPA), a substance that degrades in the environment, to German watersheds. The model considered a wide variety of processes including industrial and per capita emissions, wastewater treatment, landfill leachate discharges, partitioning between environmental media, soil runoff, atmospheric scavenging, degradation, and the advective transport to downstream areas. Consumer emissions were found to dominate and the predicted surface water concentrations were consistent with those measured in surface water downstream indicating the suitability of the FlowEQ model. The model can be further used to assess the relative importance of various substances and emissions pathways and explore how concentrations change as usage patterns and treatment technologies change.

Modelling of pesticides and biocides fate and exposure in a regulatory context (P)

MO230 Modeling honey bee extinction risk posed by neonicotinoid insecticide (imidacloprid) exposure
N. Hsieh, Department of Bioenvironmental Systems Engineering; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering

Colony collapse disorder has become an ecology crisis for honey bee population in recent years. Neonicotinoid insecticides is the suspected risk factor, which may accelerate the bee population decline. The most widely used insecticide in the world is imidacloprid that can harm honey bees through their pollinating of nectar and other consumers. The purpose of this study was to develop a risk-based population dynamic model and a probabilistic risk assessment framework to predict the potential hazards of imidacloprid toxicity toward honey bee population that may further cause colony collapse disorder. Published toxicity bioassay of imidacloprid exposure to honey bee mortality were used as the study data. The nonlinear regression models were used to reconstruct a dose-mortality response profiles in acute and chronic imidacloprid toxicity. We rebuilt the simple differential equation-based model built on previous study that can be used to simulate the seasonal dynamic of honey bee population and food storage based on the seasonality parameters of laying rate, mortality rate, and food consumption rate. This study also parameterized the imidacloprid toxicity to simulate the toxicity effect on honey bee population. Finally, we applied the probabilistic risk assessment framework to assess the potential risk of environmentally relevant imidacloprid for honey bee population. We further built the interactive web application by using Shiny for the R programming language that have the potential to revolutionize the sharing and risk visualization of population dynamic model simulations. This study provides the novel risk assessment concept to characterize the imidacloprid exposure risk to honey bee population. Current result showed that realistic in-field exposure dose distribution of imidacloprid can only effect the honey bee population slightly. Moreover, the developed population dynamic model can be further extended to simulate and realize the other risk factors that may cause colony collapse disorder. The web-based interactive applications of risk visualization can help people understand the quantitative risk of imidacloprid toxicity.

MO231 Modelling of Acetamiprid residues as foliar application in pepper
C. Turgut, ADU; C. Şarkaya, Agricultural Engineering; M. yalçın, Adnan Menderes University / Faculty of Agriculture; A. Rein, Technical University Munich

Pesticides are worldwide used to control pests, diseases and weeds of many crops. Although pesticide residues are regularly monitored and regulated, they are still detected above MRL (maximum residue level) in many countries. Many models have been developed to predict the residues in plants and crops for soil application and thus not consider foliar application for the following pesticide application. Acetamiprid is a systemic insecticide applied to pepper plants twice in ten days intervals and collected pepper fruits 35 days long. Queches method was used for extraction of acetamiprid and analysed by LC/MS/MS. The cascade model was adopted to foliar application to predict the pesticide residue over the period. The concentration of acetamiprid residues declined very sharply until second application. Simulated concentrations of residues were very similar to experimental results so that curve fitted well. The concentration in pepper reaches almost fifty percent of initial concentration after second application and decreased slowly in comparison to first application. The calculated concentrations in fruit after second application were lower than experimental. This model has advantage to predict PHI (Preharvest Intervals) between applications to decrease acetamiprid residues in many crops.

MO232 Computer simulations of plant protection products on leaf surfaces at the nanoscale

180 SETAC Europe 26th Annual Meeting Abstract Book
MO233 Experimentation of foliar wash-off as factors in input for FOCS exposure modelling
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The foliar wash-off factor is a compound and product (formulation) specific modelling input parameter relevant for the aquatic exposure assessment after foliar application. To ensure a realistic worse case for soil residues, the foliar wash-off coefficient was originally set to default value of 0.5 cm⁻¹ for FOCSow crops. Revisions. EFSA revised later on the default value to 1 cm⁻¹ if no experimental data are available. The use of measured refinement values for the wash-off factor reflects specific compound behaviour and is part of FOCUS guidance and accepted by EFSA. Recently EFSA published a guidance document in which the foliar wash-off factor also needs to be considered for the soil exposure assessment. Thus, the foliar wash-off factor may be experimentally determined if a refinement is needed for the aquatic or soil exposure assessment of a specific compound. A generic frame work of an experimental study design has been derived in a workshop organised by the European Crop Protection Association (ECPA). This frame work was used for the experimental determination of wash-off factors for modelling purposes. The wash-off studies were conducted under GLP for different compounds of foliar applied products applying different rainfall scenarios. Four main conclusions could be derived from the presented measurements: The wash-off factor → → → → → is independent from the investigated crop and BBCH stage, if leaf surface properties are comparable regarding their respective wetting regimes. → → → → → can be influenced by both the formulation composition and the physico-chemical characteristics of the active ingredient, in some cases strongly. → → → → → is comparable for standard, non-optimized formulations where the nature of the deposits is similar (e.g. SC, WP and WG formulations). → → → → → of optimized formulations (resistance to wash-off by rain) can be significantly reduced compared to standard formulations.

MO234 Exposure and Risk Assessment for operator exposure to Insecticide thiamethoxam during grape cultivation in vineyard using Whole Body Dosimetry
J. Lee, E. Kim, Seoul National University / Department of Agricultural Biotechnology; Y. Shin, Seoul National University; J. Lee, J. Lee, M. Jung, J. Sung, Seoul National University / Department of Agricultural Biotechnology; J. Kim, Seoul National University

Thiamethoxam belongs to the neonicotinoid class of chemicals. It is broad-spectrum insecticides that are used to control a wide range of diseases in fruit and vegetables. Assessment for operator’s dermal and inhalation exposure to thiamethoxam during cultivation of grape in vineyard was carried out. For dermal exposure measurement, whole body dosimetry (WBD) was performed in Korea. WBD consists of cotton/polyester outer clothes and cotton inner clothes. Hand exposure was measured by washing of nitrile gloves and hands while hand exposure was monitored by face/neck wipe technique. Inhalation exposure was monitored with personal air sampling pumps and IOM sampler (glass fiber filter). Analytical limit of quantitation was 5.0 ng/mL. Good reproducibility (C.V < 7.6%), linearity (R² > 0.99) and recovery (75–113%) were obtained. Field recovery of thiamethoxam was 88–106%. Field experiments were carried out 11 replicates, and data was processed with 75 percent statistical method. The result shows that, during application, total dermal exposure was 32277.0 μg, and that of mixing/loading case was 194.5 μg. During mixing/loading, hand exposure of thiamethoxam (156.4 μg) was slightly less than that of application case (519.3 μg). But considering the ratio of hand exposure to the total dermal exposure, mixing/loading was lower (80.4%) than that of hand exposure (1.6%). During mixing/loading, all parts were similar density, but application case, exposure of thigh and shin was high density (47.0%). Penetration rate between outer and inner dosimeter was about 10%. Inhalation exposure during application (10.8 μg) was about 30 times more than that of mixing/loading case (0.3 μg). Margin of safety (MOS) was calculated for risk assessment using male Korean average body weight (70 kg) and acceptable operator exposure level (PEL) (2500 μg/kg/day) to 7.3, suggesting that health risk of operator during treatment of thiamethoxam for grape in vineyard could be safe.

MO235 World Exposure Assessment Tools and Scenarios
A. Ritter, Waterborne Environmental, Inc.; M. Cheplick, Waterborne Environment; G. Hoogeweg, C.M. Holmes, Waterborne Environmental, Inc.

Modeling platforms have been developed to evaluate the potential impact of crop protection chemicals on the environment throughout the world. The tools currently have been configured with scenarios containing crop, soil, and weather conditions for agricultural areas across the United States, Canada, Central America, and Europe. Furthermore, agricultural scenarios have been developed for countries such as Peru, Ecuador, Taiwan, Philippines, and Korea. All these scenarios are simulated using fate and transport models that have been accepted for regulatory assessment in the U.S. and the European Union, including the Pesticide Root Zone Model (PRZM), Exposure Analysis Modeling System (EXAMS), Rice Water Quality Model (RICEWQ), and Toxic Substances in Surface Waters (TOXWA). Development of country specific scenarios and tools will be described. A key strength of the tools are that scenarios can be added for additional geographical areas with relative ease and the appropriate regulatory endpoints.

MO236 First industry experiences with the new exposure assessment guidance documents and models in China
H. Shihai

Since almost a decade ago, China has started to develop their own framework for the registration of plant protection products. A major building block of this framework is the guidance documents on environmental risk assessments. In total there are 6 (draft) guidance documents, aquatic (rice), groundwater, birds, bees, NTA and silkworms. These include two models for groundwater calculation (ChinaPEARL) and one for surface water paddy rice (TopRice). After the first commenting round in beginning of this year, in which CLC (CropLife China), the CERA team (Chinese Environmental Risk assessment) and CLI (CropLife International) provided more than 400 comments, the guidance documents have been updated and are now in the process of finalization (most probably, by the time the SETAC conference takes place, these guidance documents will be already published and came into force). ICAMA has given training using the final draft guidance documents to different stakeholder. This gives the ideal time point to report on the first experiences the industry has made using these "new" exposure assessment models and guidance documents. This includes comparison between EU and China PEARL, and also model testing for the TopRice model.

MO237 Modelling and monitoring travel distance of pendimethalin in air
B. Jense, J. Hassink, BASF SE / Environmental Fate

Based on the physical-chemical properties of pendimethalin volatilization can be a relevant dissipation pathway. Laboratory studies with pendimethalin SC and EC formulations showed significant volatilization from plant and soil surfaces. The travel distance in air is mainly influenced by the aerial half-life of pendimethalin which is estimated to be 4.2 hours (Atkinson calculations) and confirmed by experimental studies. Multimedia models were used to estimate the distribution and expected concentrations in environmental compartments at different distances from the source. Aerial transport parameters such as characteristic travel distances (CTD) were also derived. Existing monitoring data show that pendimethalin findings are highly correlated with time and location of application. The modelling results confirm that aerial transport of pendimethalin is possible over a few tens of kilometres whereas transport over longer distances to remote areas is unlikely. Mitigation of aerial transport is achieved with a new encapsulated CS formulation. A significant reduction of the volatilization rate of encapsulated pendimethalin compared with EC and SC formulations was shown in laboratory and wind tunnel (semi field) experiments where also the transport and deposition of pendimethalin was significantly reduced. Trace detections of pendimethalin in air can be further minimised by use of alternative application techniques and avoiding application at unfavourable climatic conditions. Adjuvants that reduce the volatility of pendimethalin containing products are under evaluation.

MO238 A mono-modelling approach for risk management of pesticide at territorial level
A. Di Guardo, Universita degli Studi di Milano-Bicocca / Department of Earth and Environmental Sciences; A. Finizio, University Milano - Bicocca / Department of Environmental Science and Sustainability; A. Miron, Technical University of Milan / Department of Chemistry

Decision-making for the risk management of pesticides is a challenge that requires the use of appropriate assessment tools. A mono-modelling approach was developed and applied to pesticide risk management at territorial level. The approach is based on the World Exposure Assessment Tools and Scenarios (WEXAS) platform, which includes models for different environmental compartments and a variety of processes including industrial and per capita emissions, wastewater discharges at municipal wastewater treatment plants. We present an experimental study on the determination of wash-off factors for modelling purposes. The wash-off studies were conducted under GLP for different compounds of foliar applied products applying different rainfall scenarios. Four main conclusions could be derived from the presented measurements: The wash-off factor → → → → → is independent from the investigated crop and BBCH stage, if leaf surface properties are comparable regarding their respective wetting regimes. → → → → → can be influenced by both the formulation composition and the physico-chemical characteristics of the active ingredient, in some cases strongly. → → → → → is comparable for standard, non-optimized formulations where the nature of the deposits is similar (e.g. SC, WP and WG formulations). → → → → → of optimized formulations (resistance to wash-off by rain) can be significantly reduced compared to standard formulations.

MO239 Monitoring and evaluating the effectiveness of management measures for the contamination of surface waters by pesticides: A multi-scale approach
A. M. Jochum; L. Garcia, Crop Protection; B. Gottesbueren, BASF SE / Crop Protection

Foliar applied plant protection products (PPPs) may be washed-off the leaf surface during rainfall events, leading to varying temporal exposure patterns of the pesticides on soil. Good descriptors of this process are, however, still missing due to a lack of understanding of the key factors involved. Thus, current environmental fate regulatory assessments for PPPs rely on worst case descriptors to account for the wash-off process. The objective of this work is to use molecular modeling to fill in the gap and allow for the establishment of higher tier refinements for environmental fate models. Using molecular dynamics simulations, we can elucidate the relevant interactions involved at the nano/micro-scale using coarse-grained models of the PPP’s formulation components (active ingredients, dispersing agents, emulsifiers, solvents, etc.) and of the plant leaf surface. The leaf surface is simulated by a thick hydrophobic layer covered by a mixed polymer matrix of the main epicuticular waxes. The composition of this matrix is obtained by ATR-FTIR spectroscopy of cuticles measurements for the specific plant of interest and thus characterizes the modeled interactions for the various types of crops under investigation. All coarse-grained models are based on the individual molecules’ octanol-water partitioning. By examining how the individual formulation components are distributed on the leaf surface with respect to the aqueous phase of the PPP, we aim to estimate its wash-off potential.
from the risks associated to the use of Plant Protection Products (PPPs) fall within two categories: monitoring campaigns or predictive models. Both approaches show pro and cons. Monitoring programs are required in current EU regulations, as they are very useful to verify whether the concentration of chemicals exceed predetermined trigger values (i.e. for PPPs it is 0.1 µg/L in groundwater). On the other hand, they represent a single point in space and time (static), in a situation in which different dynamic processes act at the same time; consequently, they cannot be used to forecast the future state of the environment. On the other hand, predictive models are very useful to spatially represent the forecasted contamination starting from several sources on the territory. However, they rely on assumptions, requiring the parameterisation of the model inputs which could introduce biases and uncertainties in the spatial estimation of pesticide transport toward water resources. In our opinion, a new methodology that provides the integration of both approaches (what we here call mono-modelling approach) could provide invaluable help for risk managers. In fact, this approach would be very useful, particularly on a territorial scale, for a proper risk management of PPPs. In fact, it would be helpful to: a) identify sensible areas where implement mitigation measures or limitation of use, b) re-design future monitoring plans, c) better calibration of the pedo-climatic input data for the environmental fate models. As a case study, the proposed approach has been applied to Lombardy region (a NUTS 2 zone in North of Italy) using six active ingredients with different leaching behaviours.

MO239
The Wash-off Model - A new approach to estimate wash-off of biocides from different surfaces

B. End, WSC Scientific GmbH

Biocidal products can be applied outdoors on various surfaces like terraces, balconies, walls, fences, pavements etc. as a protection against insects or different herbs. These products can then be washed off by precipitation and reach soils and drainpipes. This route of entrance needs to be considered in the Environmental Risk Assessment of Biocidal Products. Unfortunately, the proportion of the active substance washed off is rarely known so that complete wash-off immediately after application is often assumed as a worst-case. Since this is rather unrealistic, a wash-off model was developed for different surfaces, which calculates the amount washed off due to a known wash-off factor which has been the proposed procedure so far. Additionally, the amount can be estimated using the substance water solubility and the mean precipitation. The amount being washed-off may further be reduced by losses due to photodegradation and volatilization between the application and the first rainfall event. Relevant input parameters are the amount of active substance applied (mg/m² or mg/m), the area (m²) or perimeter (m) of the outdoor surface based on the Emission Scenario Documents (ESDs) and, if possible, the volatilization rate (d⁻¹) and photodegradation half life (d) along with the time (d) between application and the first rainfall. If the last three parameters are not known, default values of 1000 days for photodegradation, 0 of volatilization rate and 0 days between application and rain are assumed as worst-case. For the established approach only the wash-off factor has to be entered in addition to the inputs mentioned above. For the alternative approach, the user has to enter the water solubility (mg/L) along with a mean rainfall amount (mm).

Data on precipitation can be estimated using e.g. FOCUS scenarios from the current FOCUS models (e.g. FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3). The two approaches are described in a short example. A substance is applied with the amount of 10 mg/m² on a 30 m² area, resulting in 300 mg a.s. applied and washed off when using a worst-case wash-off factor of 1. But due to its low solubility of 1 mg/L and assuming a mean rainfall of 4 mm, the actual amount washed off during the next rain event is reduced to 120 mg a.s. In conclusion, the wash-off model can help to estimate realistic wash-off amounts of biocidal products from outdoor surfaces, either based on a given wash-off factor or on the water solubility of the active substance.

MO240
Outdoor use of Biocidal products: considering contaminated rainwater pathways in the risk assessment of biocides

F. Eicher, C.E.A.; S. Taylor, Cambridge Environmental Assessments

Contaminated rainwater and, in particular, pathways of exposure to surface water via sewage treatment plant bypass and separated rainwater systems, are increasingly considered within Biocidal risk assessments. A first tier approach was described for the evaluation of a number of preservative products (UBA, 2014) and there is indication that other product types may be considered in the future. In addition, an existing higher tier approach involving the HardSpec model is commonly used for plant protection products applied in urban areas and it is proposed that the data generated for the design of this model could be the basis for a second tier approach for biocides. Particular consideration is given to those substances exhibiting potential for dissipation and to those uses where infiltration and retention may be significant.

MO241
In silico Prediction of the Formation of Non-Extractable Residues (NER) in Soil: a New Tool to their Environmental Hazard

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Organochlorine complexes are the major sink for xenobiotics in soils and sediments. Products can be degraded and transformed by biotic and abiotic mechanisms. Additionally, the parent xenobiotics and their transformation products are immobilized as non-extractable residues (NER). All chemicals are assumed to form NER to different extents, but the actual amount of NER formation depends not only on the chemical structure but also on the environmental conditions. Three types of NER have recently been classified: Type I are xenobiotics sorbed or entrapped, type II are xenobiotics covalently bound, and type III are biogenic residues. The possible remobilisation of incorporated xenobiotics is of environmental concern and refers particularly to type I NER. Type II or type III NER formation is considered not to cause harm. We therefore launched a project aiming at predicting the potential for formation of different types of both xenobiotic and biogenic NER formation from xenobiotics, in particular pesticides. To this end, derivation of respective structural alerts had been envisaged originally. However, we found that the available experimental data are not sufficient to derive general structural alerts. Structural alerts thus can only address a subset of potential NER forming chemicals. Acknowledging this restriction, the structure has been altered based on insights on identified mechanisms of physicochemical and molecular properties and their control on NER formation. The paper provides an overview of the original and adapted approach and reports modelling results. The financial support of this study by CEFI-LRI (ECO24-UFZ) is gratefully acknowledged.

MO242
KINETIC EVALUATION OF FIELD DISSIPATION STUDIES: APPLYING MCMC SIMULATION DREAM FOR PARAMETER INFERENCE AND UNCERTAINTY ANALYSIS

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In legacy field dissipation studies, plant protection products (PPP) were applied to soil surface or to cropped plots. Consequently, surface processes other than (microbial) degradation might contribute to the overall dissipation of PPP mass in a soil profile. The properties of the compound as well as agro-climatic conditions will influence degradation, plant uptake and leaching below the lowest sampling depth, particularly if the depth of sampling is limited. A recent EFSA guidance document (1) provides criteria to be followed to determine a true DegT50 from those field trials. The guidance is lacking some information about the detailed procedure to derive DegT50 of metabolites, particularly if global solvers or inverse modelling approaches are to be considered. This presentation describes a novel methodology to derive the true DegT50 of a primary metabolism from legacy field studies employing a global solver. The evaluation considers requirements of latest regulations, i.e. ignoring masses below 10 mm of rainfall, and using state of the art field weather data. For an improved understanding of the results of field dissipation studies the leading model PEARL 4.4.4 is coupled with a Markov Chain Monte Carlo sampler (Differential Evolution Adaptive Metropolis algorithm, DREAM_ZS)(2) to efficiently search the parameter space of the advective-dispersion model. Standard statistical measures according to FOCUS are given, e.g. Chi-square test and an analogon of t-test statistics. This overall procedure allowed for a representation of the distinct processes occurring under field conditions, i.e. degradation, leaching, plant uptake (in cases of cropped trials). Consequently, in legacy studies a true DegT50 can be estimated other than an overall DT50, employing a standard time-step normalisation procedure. We will present the results of field trial experiments along with an analysis of parameter uncertainty. (1) EFSA (2014): EFSA Guidance Document for evaluating labels of new and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil.European Food Safety Authority. EFSA Journal 2014:12(5):3662. (2) Vrugt (2009): J. Vrugt, C. ter Braak, C. Diks, B. Robinson, J. Hyman, and D. Higdon. Accelerating markov chain monte carlo simulation by differential evolution with self-adaptive randomization subspace sampling. Int. J. Nonlinear Sci., 10:273–290, 2009.

MO243
Estimation of degradation rates of pesticides in surface water from higher-tier effect coom studies by inverse modelling with TOXSWA

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In water bodies with low flow velocities or with multiple spray drift deposition events, measure concentrations of pesticides are influenced by their degradation rate in water. DegT50water derived from water-sediment studies do often not

182 SETAC Europe 26th Annual Meeting Abstract Book
represent well degradation rates in water in realistic, outdoor cosom systems. Therefore, we developed a method to estimate degradation rates in water from available higher-tier effect cosom studies (which have often limited fate data). Results obtained with this method can be useful in higher tier exposure assessment for authorization purposes of pesticides on a national and zonal level. We derived DegT50,water values from outdoor cosom studies for three compounds with relatively low Koc values (metribuzin, linuron and imidacloprid). In total 18 outdoor studies were analysed. Studies were only included if aqueous concentrations were available for at least five sampling times and if the water depth was reported. Concentrations in the sediment were not used for the fit (although for some compounds). We observed a significant difference between simulated and measured aqueous concentrations using PEST vs 13.0 and running FOCUS_TOXSWA 4.4.2 many times. The quality of the fits was assessed visually (considering also trends in residuals) and by calculating the error level of the χ^2 test as recommended by FOCUS Degradation Kinetics. All fits were found to be visually acceptable (for χ^2 without a reason to reject the fitters and a common 11%). The error values were between 20% and on average 11%. The 95% confidence interval of the DegT50,water values ranged typically from 75 to 125% of the fitted DegT50,water value. Thus, the estimation procedure resulted in sufficiently accurate DegT50,water values for all eight outdoor higher-tier effect cosom studies. For all eight studies, the DegT50,water values obtained appeared to be shorter than those derived from hydrolysis and sediment-water studies (the difference was typically an order of magnitude and at least a factor of two). 

MO244

VFSmod - A comparison with field derived values of pesticide removal efficiency under controlled conditions

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Consideration of the pesticide removal efficiency of vegetative filter strips (VFS) within the regulatory process has been through the use of standardised values for buffer strips of sets of classes of width (10-12m and 18-20m) and adorption (aqueous and sediment associated ). These are applied to all compounds for all FOCUS scenarios, all crops and all usage periods. The adoption and usage of more dynamic models, such as VFSmod, by Member State regulatory authorities has been relatively slow, despite efforts to underpin this model with a wide database of field data, improve model parametrisation, and further align scenario development with existing FOCUS frameworks. As a complimentary approach to the use of VFSmod, and in support of a number of regulatory submissions, a series of replicated field studies have been carried out as part of the data package where the efficiency of VFS for specific compounds and specific VFS widths has been tested under controlled conditions representative of a selected worst case FOCUS scenario covering the intended use profile. This study explores these FOCUSsw scenario related experimental VFS data with a view to examining VFSmod performance further and potentially expanding the validation dataset that underpins it.

MO245

Spray Drift: Representation, Mitigation and Future Directions

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Spray drift measurement studies have been carried out to assess the drift downwind from application and deposited on adjacent surfaces. Evaluation reference conditions are detailed in ISO 22866 but allow some variation in environmental characteristics at point of application (wind speed, temperature, humidity), application settings (nozzle height, travel speed), crop and landscape influences (bare ground / grass versus crop, choice of crop) and measurement standards (choice of collector, relationship of measurement to edge of field / end nozzle location). As a consequence, drift trials may provide variable drift representations. SETAC MAGPIE WG concluded that consideration of harmonization of testing standards beyond ISO 22866 would be better addressed independently via spray physics expert working groups. To improve understanding of spray drift and the regulatory basis for risk assessment, a SETAC workshop (DRAW) is underway to facilitate efforts in a number of areas. - Assemble a database of existing spray drift trials for boom sprayers - Analyse and interpret available data; highlight areas where protocol-derived differences would hinder the comprehensibility of trials - Identify and explain differences emerging from measurement methodology and choices - Develop proposals for standardized measurements to investigate key drivers or influences on drift - Support further interpretation of existing spray drift trials - Determine which collector type best represents the actual deposition capture by the relevant risk assessment. Developing a more robust basis for risk assessment refinement The valuable heritage of research data on spray drift must be harnessed for the longer-term goal of facilitating greater flexibility with drift representation through mathematical modelling of drift arising from boom applications. As representation of spray drift affects a range of risk assessments (aquatic, non-target arthropods/terrestrial plants etc.), the workshop is considering collaboration with other working groups to develop an expanded, secure toolbox of risk mitigation measures. This action will enable efficient transition to a new framework of drift representation coupled with options to accommodate change.

MO246

Research of boscalid and markers of exposure to boscalid in honey bees

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The intensification of agriculture has been characterized in last decades by a significant use of pesticides. To protect bees against their effects, it is necessary to develop analytical tools for the detection of these compounds in bee products and in bees. The search for pesticides in bee generally involves native target substances. However they may have been degraded or metabolized by bees or due to delayed mortality findings; thus the parent compound may no longer be detectable in the bee. Even for the contact of contact bee and pesticide may, nevertheless, be obtained by detecting metabolites/markers of exposure. However, the study of metabolites and of their toxicity in the bee is still undeveloped. The reasons are firstly, the lack of information on the nature of the metabolites, and secondly the lack of analytical standards, which are essential for conducting the tests. Our work is part of this context and aims firstly at characterizing metabolites of boscalid in bees, and next to search them into bees from symptomatic colonies. Boscalid was chosen for this study as it is a new-generation fungicide which use is increasing, it has a broad spectrum of activity, and it has been detected in bee matrices. Honeybees were sprayed with boscalid in a Potter spray tower and left in crates 24 h at 28°C before being frozen to stop metabolism. Boscalid and its metabolites were extracted from bees by QuEChERS method prior to analysis by liquid chromatography coupled to high resolution mass spectrometry (LC-Q-TOF). Metabolites research was performed by comparing the retention time and mass/charge ratio between sprayed bees and control ones. Discriminant masses (exact mass, isotopic profile, mass fragments) and empirical formula next obtained were compared with databases and/or in silico fragmentation. Thus 5 markers of exposure to boscalid were determined. Then they were synthesized and quantified in bee samples collected from various beehives located in France. The analysis of boscalid and the 5 identified markers of exposure was performed on 42 bee samples from symptomatic colonies. Among these markers, 4 were present in the samples (levels comprised between 0.2 and 36.3 ng/g). About half samples were positive for at least one of the 6 target molecules whereas boscalid was detected in 11 samples but quantified in only 4 with levels up to 330 ng/g. These results highlight the importance of not limiting the analysis of the parent drug in sentinel organisms such as bees.

Prospective and retrospective soil risk assessment of chemical stressors (P)

MO247

How to deal with different soil organic matter contents in ecotoxicological studies for the risk assessment of soil organisms?

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The risk assessment for non-target soil meso- and macroorganisms is based on ecotoxicological tests performed in standardized test soils as a first step. Depending on the test organisms, the guideline recommendations regarding the soil organic matter content (OM) of the test soil vary. For earthworms, the OECD Guidelines 207 and 222 recommend 10% OM (as peat) for the composition of the artificial test soil. The OECD Guidelines 226 and 232, targeting chronic effects on mites and collembolans, propose a soil OM (peat) content of 5%. The quantity and quality of OM in soils might exert a strong effect on the sorption and bioavailability of tested substances and therefore alter the determined toxicity of the tested compound. Amount and quality of OM used in tests with artificial soil might strongly diverge from the humic materials in natural agricultural soils. EFSA (2012) reports e.g. for the Central Zone of Europe OM contents between 1.8 and 8.6% (10th and 90th percentiles of the distribution), respectively. This adds to the uncertainties accompanying the extrapolation of toxicity endpoints measured under standard laboratory test conditions to the situation in the field. To account for the effects of OM content on sorption and thereby bioavailability of tested compounds, the current Guidance document (GD) on Terrestrial Ecotoxicology SANCO/10329/2002 (European Commission 2002) recommends a reduction factor of two for material of agricultural origin (soils with lipophilic substances (defined by a logKoc > 2) in artificial soils with 10% OM. At the time the current GD was established, tests with soil organisms were conducted with 10% OM as peat in the soils exclusively. The OECD guidelines 226 and 232 were adopted later, and therefore no proposal exists in the current GD, how to deal with toxicity endpoints for soil organisms derived from tests with 5% OM. Currently, the risk assessment scheme for soil organisms deals similarly with
endpoints conducted in test soils with different peat contents. This contribution presents the outcome of a comprehensive analysis of all available test data with soil organisms in artificial soils of different OM contents that were submitted to authorities in the framework of PPF registration or active substance evaluation. Moreover, it reviews available literature on compound toxicity for soil organisms in soils of different properties, with a focus on the effects of different soil OM contents.

**MO248**

**Soil enzyme activities reveal re-functionalization of trace metal agricultural contaminated soils after Miscanthus giganteus plantation**

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**Context** The use of contaminated soils for non-alimentary cultures is gaining importance in view of the scarcity of agricultural surfaces and the presence of some potential harmful pollutants in surface horizons that could impact the food chain. But in the case of trace metal contaminated soils few information are available on the effects of land use and soil management changes on soil quality parameters and/or soil health. Suggestion was made in literature that enzymatic activities could be good indicators of soil functioning, providing special attention to confounding effects. In this work the objective was to assess the impact of change of cultural practices on the soil quality, as seen by soil enzyme activities.

**Materials and methods** We used trial essays were contaminated parcels were divided in two, one part was left under annual culture and the other was planted with the high biomass plant Miscanthus giganteus. The enzymatic activities of the C and N cycles were measured (soil mites and collembolans). In addition, we determined the activities of some enzymes (soil mites and collembolans). In a diachronic study – including higher tested insecticidal soil concentrations – with focus on the structure of Collembola populations showed some initial transient effects, but also no longer term impact. On the basis of these results it can be concluded that the functional field test on organic matter breakdown is sensitive and covers the effects on soil mites and Collembola. Sensitivity and initial effects in field tests on organic matter breakdown were also reported in a previous industry-wide ECPA review of such data (Dinter et al 2008). It is concluded that functional test system on organic matter breakdown is sensitive and covering potential effects on soil non-target meso- and macrofauna and considered – due to the direct link to protection goals derived from the EsS concept (plus the robustness of the test conduct itself) a superior testing tool for soil assessment of plant protection products.

**MO249**

**Gaining a better understanding of toxic effects on soil communities, using the example of AgNP**

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Resource limitations and ethical concerns often hinder field studies in ecotoxicology. One way to meet the frequent calls within the scientific community for the rapid answer of the indicators, three years were necessary to significantly quantify an evolution. N cycle was more sensitive to point out the re-functionalization of the contaminated soil. In the soil profile results of the enzymatic activities under miscanthus highlighted the evolutionary structuration of microorganisms in contaminated soil. This evolution was faster near the soil surface than in subsurface horizons and better assessed for N cycle.

**MO250**

**A comparison of functional and structural soil testing for risk assessment of plant protection products**

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In the development process of a new active substance of a plant protection product (PPP), an insecticidal, functional tests and structural tests were performed, which allowed a comprehensive understanding of the effects on soil organisms and soil properties. In the functional field study – a test on the breakdown of organic matter following OECD GD 56 (2006) and EPFES WS recommendations (2002) – slight transient effects due to the insecticide were found on mass loss of organic matter at 1 month after treatment, while at 3 and 6 months after treatment no significant impact on organic matter decomposition was found. The potential effects of the test substance on structural parameter (soil mites, and collembolans for which a potential risk was identified based on worst-case laboratory testing) were addressed via additional soil samplings and organisms extraction on the same plots of the organic matter breakdown study. Samplings performed at 3 and 5 months after treatment indicated no significant effects on the structural parameters. In a diachronic follow up in surface horizons showed that soil mites activities under miscanthus differ clearly from those under annual culture with higher values under perennial culture. This was particularly observed as the setting-up of miscanthus was former. β-Glucosidase activities, while less indicative, also suggested an enhancement of microbial activities under miscanthus. When normalizing with the enzymatic activities of the parcel under annual culture, results clearly show the effect of the plantation duration on the re-functionalization of the soil. But despite the rapid answer of the indicators, three years were necessary to significantly quantify an evolution. N cycle was more sensitive to point out the re-functionalization of the contaminated soil. In the soil profile results of the enzymatic activities under miscanthus highlighted the evolutionary structuration of microorganisms in contaminated soil. This evolution was faster near the soil surface than in subsurface horizons and better assessed for N cycle.

**MO251**

**Making soil protection goals based on the ecosystem services concept operational in ecotoxicological risk assessments**

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What, where, and when to protect are fundamental questions that need to be addressed before designing environmental risk assessment (ERA) schemes. The ecosystem services (EsS) concept can give guidance in what to protect for ERA schemes. Here we use the EsS concept to propose a novel and science based soil risk assessment for PPPs. As a first step we define registration principles followed by identification of soil relevant EsS as a second step. Thereafter, we formulate relevant protection goals (PGs) which are transferred into test systems. Finally our goal will be to define a tiered ERA based on acceptability criteria. The registration principles we identified were that ERA should be workable, scientifically sound and locally acceptable. In the context of the ERA approach, reduce uncertainties and data gaps concerning organism potentially at risk. A ERA scheme should use validated test systems only, needs to have conservative trigger values, and needs clear acceptability criteria. Soil relevant EsS (e.g. nutrient cycling) can be affected by PPPs. While it is relatively easy to define general PGs, it can be quite challenging to define specific PGs. In the soil area the current focus is on structural PGs (testing of surrogate species) and lacks a clear link to PGs derived from EsS. On the other hand functional PGs directly correlate to EsS. Thus suitable tests based on functional parameters will reduce uncertainties with regard to EsS. Although several functional tests are already available, a critical evaluation of their effectiveness on functional sensitive test systems. Additionally, these novel functional tests need to be linked to information obtained in structural tests for the final risk assessment. The EsS and related PGs might be weighted differently on a spatial scale in agricultural landscapes. For in-crop areas functional aspects are in focus (e.g. food production & nutrient cycling) whereas other crop areas structural aspects are additionally of interest. Ecosystem services and the PGs derived from them are suitable to design a new soil ERA scheme. Several EsS are relevant to soil and can be affected by PPPs. Ongoing research will help to define a workable ERA scheme for soil that combines structural and functional endpoints. PGs based on EsS should be addressed by validated and suitable functional tests as these in contrast to structural tests directly link to PGs and thus fulfil registration principles.

**MO252**

**Statistical Power and MDDs in Earthworm field testing**

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Earthworm field tests are carried out as part of the risk assessment of plant protection
products according to the ISO guideline 11268-3 (ISO 2014). This
document provides information on experimental design, test site requirements,
data assessment and validity criteria of the test, but does not give indications about the
required statistical power of the test or about the effect sizes that should be
detectable in such a field study. In contrast to that, the guidance for summarizing
earthworm field studies published by the National Institute for Public Health and
the Environment of the Netherlands (De Jong et al., 2006) demands that the
minimum effect level that could be detected as statistically significant in a study
should be reported. For aquatic test systems, it has been suggested (EESA, 2013
and Brock et al., 2015) to use the minimum detectable difference (MDD) to
evaluate the statistical power of aquatic test systems. In order to provide a better
understanding of the earthworm field test we carried out a retrospective power
analysis of a series of earthworm field studies covering a range of land uses and
soil types. The objective of the MDD calculation was to estimate the MDD with
regard to the overall earthworm population as well as for individual taxa and for ecological
groups. The impact of site related (size of the earthworm population, homogeneity of
earthworm distribution across the test site) and study design related criteria (e.g. number of replicates) on the statistical power in terms of MDD will be
discussed.

Brock TCM, Hammel-Wirtz M, Homen U, et al., 2015. The
minimum detectable difference (MDD) and the interpretation of
treatment-related effects of pesticides in experimental ecosystems.
Environmental Science and Pollution Research International 22(2):
1160–1174. doi:10.1007/s11356-014-3398-2. De Jong, FMW., Van Beelen, P.,
Smid, CE. & Montforts, HHMM., 2006: Guidance for summarising
each earthworm field study. Guidance Document for the
determination of the MDD for the assessment of higher tier studies. RIVM, The Netherlands, 47 pp. EFSA
[European Food Safety Authority] Guidance on tiered risk assessment for
plant protection products for aquatic organisms in edge-of-field surface

Statistical Power and MDDs in Earthworm field testing
addressed by validated and suitable functional tests as these in contrast to
parameters and/or soil health. Suggestion was made in literature that enzymatic
parameters (soil mites and collembolans). In another field study
implications (i.e. organic matter breakdown). In this study the suitability of the
minicontainer test did not show a clear effect of insecticides on the mesofauna
while no lasting effects were observed in the community. This shows that either the extraction procedure or
stressor on the fertility of soils. Furthermore, functional test systems can help to
evaluate the ecological relevance of a density change of a soil organism
population affected by a certain stressor. In order to improve the current
toolbox for the soil risk assessment, a project on soil functional test systems was initiated by
the European Crop Protection Association in 2014. In a first step a literature
search identified several promising functional test systems which could provide
valuable and ecologically relevant information. In a second step a field study was
set up in 2015 which measures the impact of two insecticides (Methidathion,
Lindane) on organic matter degradation (minicontainer, bait lamina, litterbag test).

Soil micro-arthropod abundances are monitored in parallel to determine the link
between effects on the structure of soil micro-arthropods and their soil functional
implications (i.e. organic matter breakdown). In this study the suitability of the
different functional test systems related to organic matter degradation is evaluated
with regard to their potential use in soil risk assessment of plant protection
products. The functional test systems (i.e. the minicontainer test) showed overall
consistent and reproducible results in the field trial. The results indicate that the
process of organic matter degradation is dominated by soil microbes. Soil
mesofauna contributed only to minor extend to organic matter degradation. Thus,
the minicontainer test did not show a clear effect of insecticides on the mesofauna
development of the test organisms. Although a high degree of SCAR material
were heavily reduced by the insecticide applications. Thus, the relevance of
structural endpoints on soil micro-arthropods (population/community level) within
an in-field soil risk assessment for plant protection products which focus on
maintenance of soil fertility (protection of soil functions) is questionable.

Evaluation of epoxiconazole bioavailability in soil to the earthworm
Aporrectodea icterica
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In soil, the determination of total concentration using an exhaustive extraction
method was then applied to explore the relationship between total and
bioavailable concentrations in soil and in A. icterica, over 7 or 28 days exposure
times. This demonstrated the proportionality between epoxiconazole concentration in earthworm and available in soil (up to 96%, with regression coefficient R²
0.98). Sublethal effects on earthworm remained not significant. References 1. C.
quality – effects of pollutants on earthworms. Guidance on the determination of
effects in field situations.

Bioassay (Eisenia andrei) and soil microbial communities
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Fumigation is an important crop protection practice to control agricultural crop
pathogens and diseases. Methyli bromide (MeBr) and it success as nematocide
since the 1940’s is well documented. It has, however, been identified as ozone
depollutant and it was to be phased out by 2005 under the Montreal Protocol
(2002). The more common alternatives to MeBr include metam sodium (MNa)
and cadusafos but very little information is available about their effects on
non-target and beneficial soil organisms. The aim of the study was to determine
the ecotoxicity of MNa and cadusafos using a standardised earthworm (Eisenia
andrei) ecotoxicity test (OECD 2004). In addition to this the genotoxicity of MNa
and cadusafos was assessed by investigating DNA damage (comet assay) of
individual earthworm cells. Further, to assess changes in the microbial community
function and structure by means of Biolog™ Ecoplates and phospholipid fatty
acid (PLFA) analyses. The short-term exposure of earthworm and PLFA analysis
of soil with chemical fumigants. The
chemical fumigants had a marked negative impact on the survival, growth,
reproduction and the genotoxicity of the earthworms with MNa causing
greater harm than cadusafos. The effects on the microbial community varied. Both
chemicals had an inhibitory effect on the microbial growth in terms of the viable
biomass determined by PLFA and the average well colour development in the
Biolog™ Ecoplates. No lasting effects were observed in the community structure. Overall, cadusafos has a more pronounced effect on the microbial community
functional diversity than MNa.

Application of Equilibrium partitioning-based model framework for
evaluating soil and sediment hazards of lipophilic non-polar organic
substances
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The target lipid model (TLM) is a QSAR framework used to predict acute and
carcinogenic toxicity of substances based on structure. This framework has been
extended for calculation of predicted no effect concentrations (PNEC) of highly
lipophilic hydrocarbon substances to support risk assessment activities of single
chemicals as well as complex substances such as fuels, solvents, and lubricants.
Recently, this framework was extended into soils and sediments using equilibrium
partition coefficients (n-octanol/water, n-octanol/soil) as input and evaluated for
read across activities thereby maximizing use of available data. The TLM-EqP

SETAC Europe 26th Annual Meeting Abstract Book 185
framework established the range of sensitivities for common test species (invertebrates, microbial endpoints, plants), which supports read across from the results of one assay to another. This framework was applied to recent dossier updates to address existing data gaps. The approach consisted of a weight of evidence built using TLM-EqP predictions and read across to available data, which allowed an improved experimental design for additional testing. This framework was used to establish the upper limit of the predicted porewater solubility (~100 mg/kg in bulk soil) to avoid potential formation of oily residues, which introduce the potential for physical oiling. While the mechanism of physical oiling could be a true hazard, predicted environmental concentrations in soil for most substances under typical use patterns are very low (<0.01 mg/kg). Confirmatory testing in soil and sediment showed lack of toxicity consistent with model predictions and consistent with existing test data in water and soil. This framework promotes realism in chemical risk assessments by designing tests based on physicochemical properties and likely hazards of the test substance. This promotes the technical basis for the framework and illustrate the application of the methodology to available case studies, i.e. dossier updates to insoluble, nonpolar organic substances.

MO257

Effects of wheat seed dressings with neonicotinoid insecticides and fungicides on soil organisms

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Seed dressing with pesticides is widely used to protect crop seeds from pest insecticides and fungicides. The technical basis for the framework is that neonicotinoid seed dressings detrimentally affect pollinators, surprisingly little is known on potential side effects on soil biota. We hypothesized that soil organisms would be particularly susceptible to pesticide seed dressings as they get in direct contact with these chemicals. Using mesocosms with field soil we investigated whether seeds treated either with neonicotinoid insecticides or fungicides influence the activity of earthworms, collembolan, protozoa and microorganisms. Additionally, we also examined interactions between soil organisms and seed dressings. The full-factorial design consisted of the factor seed dressing (control vs. insecticide vs. fungicide), earthworm (no earthworms vs. addition of Lumbricus terrestris L.) and Collembola (no Collembola vs. addition of Sinella curviseta Brook). We used wheat seed material (Triticum aestivum L. cf. Lukullus) available for farmers at a recommended seeding density of 367 m² (18 seeds per mesocosm). Seed dressings (particularly fungicides) increased Collembola surface activity, increased the number of protozoa and reduced soil decomposition rate but did not appear to affect earthworm activity. Earthworms decreased wheat growth, reduced soil basal respiration and microbial biomass but increased soil water content and electrical conductivity. Collembola increased earthworm surface activity but reduced soil basal respiration. Earthworms interactively affected effects of seed dressings on Collembola activity. We find it remarkable that the reported non-target effects of seed dressings on soil organisms were found after 14 days. However, we showed that earthworms are more susceptible than collembolan. Because of the fundamental role of soil organisms in agroecosystems, investigations on their interactions with pesticides should receive more attention.

MO258

Sublethal effects of exoperoxidase on the earthworm Aporrectodea ictericia

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Earthworms play a key role in agroecosystem soil processes. This study aims to assess the effects of different doses of a commercial formulation of exoperoxidase (Opus®), a persistent and widely used fungicide, on the earthworm Aporrectodea ictericia. A laboratory study was conducted in a natural soil in order to measure effects of Opus® on earthworm mortality, uptake and weight gain, energy metabolism (catalase and glutathione-S-transferase), and energy resources (lipsids and glycogens). The estimated LC50 was 45.5 mg kg⁻¹, or 268 times the recommended dose. Weight gains were 28, 19, and 13 % of the initial weight after 28 days of exposure in the control and D1 and D10 (1 and 10 times the recommended dose) treatments, respectively. No difference was observed for catalase activity between the three treatments, at 7, 14, or 28 days. The glutathione-S-transferase (GST) activity was two times as high in D1 as in D0 at 14 days. At 28 days, glycosen concentration was lower in D10 than in the D1 treatment. This study highlighted moderate sublethal effects of the commercial formulation Opus® for earthworms. Considering that these effects were observed on earthworms found in cultivated fields, even at recommended rates, much more attention should be paid to this pesticide. Keywords: Ecotoxicology, Fungicide,

Biochemical biomarkers, Mortality

MO259

Poplar-assisted bioremediation of a PCB historically contaminated soil in Southern Italy


The plantation of selected plants in contaminated sites can support organic contaminant degradation. Plant roots can release exudates and supply nutrients useful for autochthonous microorganisms, which in turn enhance their biological activity and can degrade contaminants. A synergistic action between biohetero microorganisms and plants can lead to an increase in bioavailability of hydrophobic compounds, making them more susceptible to degradation. The results of the first 30 months of a poplar-assisted bioremediation application to a multi-contaminated (PCBs and heavy metals) soil in Southern Italy are here reported. The investigated site has been used for several decades as an unsupervised waste disposal. A specific poplar clone (Monviso), was applied in an area of 785 m² of the contaminated site where six hundred poplar cuttings were planted. Sampling were performed 14 and 30 months after the poplar-plantation. At each sampling, soils at different depths and distance from the tree trunk, tree roots and leaves were collected for chemical and microbiological analysis. PCB and heavy metal (HM)s analyses were performed using a GC-MS and an ICP-MS, respectively. Microbial analysis were carried out to assess total microbial abundance, cell viability and dehydrogenase activity (DHA) of the autochthonous microbial community. The comparison of the chemical and microbiological analyses performed at day 0 and 14 and 30 months after the poplar plantation, showed a decrease in soil of PCBs under the Italian legislation limits and also a significant decrease in soil of all HMs. The poplar plantation showed its effectiveness both in promoting the persistent organic contaminant degradation and in the phyto-containment of the inorganic ones. In line with these results microbiological activity increased significantly in the rhizosphere soil.

MO260

Re-calibration of the earthworm tier 1 risk assessment of plant protection products

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To address potential risks of plant protection products to earthworms, a risk assessment is required. This risk assessment comprises two steps 1) deriving no-observed-effect levels (NOEL) from laboratory reproduction tests and applying a trigger value, to cover uncertainties, and if this indicates a potential risk, 2) conducting field studies. In this review paper the tier 1 earthworm risk assessment for plant protection products is calibrated by comparing the NOEL in laboratory earthworm reproduction tests with effect levels on earthworm populations under real world field conditions. A dataset of 54 pairs of studies conducted in the laboratory and in the field with the same plant protection product was compiled, allowing a direct comparison of relevant endpoints. The results indicate that a tier 1 assessment factor (AF) of 5 combined with a regulatory relevant soil layer of 0.5 cm provides a conservative tier 1 risk assessment. A risk was identified by the tier 1 assessment in the majority of the cases at application rates which were of low risk for natural earthworm populations under field conditions. Increasing the conservatism in the tier 1 risk assessment by reducing the depth of the regulatory relevant soil layer or by increasing the tier 1 AF would increase the number of false positives and trigger a large number of additional field studies. This would however not increase the margin of safety for earthworm populations. The analysis revealed that the risk assessment is conservative if an AF of 5 and a regulatory relevant soil layer of 0.5 cm are used.

MO261

Determination of Soil Adsorption Coefficient (Koc) Values for a Series of Aliphatic Alcohols using the OECD 121 HPLC Method

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A study has been performed to generate experimental data for the organic carbon-water adsorption coefficient (Koc) of a range of alcohols (C6-C16) using an accepted OECD method (OECD 121). This Test Guideline describes an experimental method using High Performance Liquid Chromatography for the estimation of the adsorption coefficient Koc. The HPLC method is an estimation method based on correlation of log Koc values with true Koc values determined in soil/sediment/sludge-water systems. A series of reference substances, as
recommended in the OECD 121 guideline, were assessed using a HPLC system under the specific conditions outlined in the method. The retention data were used to generate a correlation plot of literature adsorption coefficient (log Koc) values and the log capacity factor (k) for the reference substances. A range of linear alcohols, ranging from C6 (1-hexanol) to C16 (1-hexadecanol), was subsequently run under the same conditions and calculated. The calibration line (log k vs log Koc) for the reference substances was then used to estimate the log Koc values for all the individual linear alcohols. Applying the OECD 121 guideline method, the HPLC-derived log Koc values for these alcohols ranged from 1.25 (1-hexanol) to 5.40 (1-hexadecanol). When using this method to calculate log Koc for the pesticides in the studies (n = 4) and a recent OECD Guideline 106 test for 1-decanol, very good agreement with HPLC screening data was obtained. The HPLC log Koc value for 1-decanol was within a factor of 2 compared to the value obtained from the OECD 106 study which demonstrates the accuracy of the technique. However, QSAR predictions of log Koc for atrazine are in agreement with its highest measured concentration data (dependent on which QSAR method (regression-based log Kow or fragment-based Molecular Connectivity Index) is selected. This study demonstrates the good performance of the HPLC screening method for determination of log Koc of aliphatic alcohols without the complications associated with definitive experimental determination using the OECD 106 method due to their rapid biodegradability. An evaluation of the various QSAR-based methods for prediction of log Koc for aliphatic alcohols is also discussed.

MO262 How representative is Eisenia andrei as a standard species for soil ecotoxicological testing? S. Reinecke, Stellenbosch University / Dept Botany and Zoology; A. Reinecke, Stellenbosch University / Botany and Zoology The widely distributed composting earthworm species Eisenia andrei is recommended and used for ecotoxicological testing. However, the scientific literature about its sensitivity about its suitability to HPLC’s representativeness because it is a litter-dwelling species representing a totally different ecotype than most other soil dwelling oligochaetes. To address the question whether toxicity data obtained with E. andrei could be extrapolated on reasonable grounds to other earthworm species, we compared the sensitivity of E. andrei and four other species representing different ecotypes: E. andrei, Aporrectodea trospezoides, Amyntas diffirigens, Paxonioux excavatus and an indigenous Chilota species were exposed under similar conditions to a concentration series of copperoxychloride as prescribed by OECD guidelines for toxicity testing. We monitored mass change and cocoon production over time and compared body copper accumulation at the end of the exposure. No worms in the uncontaminated control soil or in the 20 mg/kg exposure lost significant body mass. With exception of A. trapezoides no other species lost significant mass at 80 and 160 mg/kg exposures. At 320 mg/kg exposure significant differences occurred between species with E. andrei and P. excavatus (the two litter-dwelling species) seemingly more robust. Cocoon production differed between the composting and soil dwelling species with the latter species being more severely affected. Body concentrations of Cu increased similarly with increasing exposure concentrations in all species. At the lower, environmentally more relevant concentrations mass change and body load of Cu were not strong discriminating factors. However at the higher concentrations it clearly affected the soil dwelling species more detrimentally. Copperoxychloride is known to cause mortality and a distinct between soil dwelling and the litter dwelling species was obvious at lower concentrations. The simultaneous onset of the detrimental effect of copperoxychloride on cocoon production (albeit at different levels of severity) in all species provided evidence that extrapolation to other species may be warranted, provided that the results are interpreted with the necessary caveat that the intensity of effects may differ as a result of differences in habitat type and lifestyle.

MO263 Monitoring the residues of currently used pesticides in agricultural soils - the first step of the post-registration control J. Hofman, Masaryk University, RECETOX / Faculty of Science RECETOX; M. Hvezdova, Masaryk University / Faculty of Science RECETOX; P. Kosuoba, Central Institute for Supervising and Testing in Agriculture; Z. Simek, Masaryk University / Central Institute for Supervising and Testing in Agriculture; M. Sokolov, Masaryk University / Central Institute for Supervising and Testing in Agriculture; J. Vasiczka, Masaryk University / Faculty of Science RECETOX; L. Bielska, RECETOX, Faculty of Science, Masaryk University / Faculty of Science RECETOX; M. Sudoma, M. Svozilova, L. Skulcova, Masaryk University / Faculty of Science RECETOX Data on the current use of pesticides in soils (CUPs) in surface and ground water are relatively abundant and also to ongoing water monitoring in many EU countries. Several CUPs or/and their degradation products have been repeatedly found at high frequency and also at considerable concentrations. Water monitoring scientists have hypothesized that the secondary source of CUPs in water are soils in which are used as disposal site (for long persistence pesticides degradation products) to ground/surface water. However, there are surprisingly no data available for CUPs in soils in EU to work on this hypothesis. Not only for the purpose above, but also for the post-registration control of approved plant protection products, the monitoring of CUPs in Czech agricultural soils was performed last year. Fluvisols (alluvial soils) were selected for the study as they are intensively used for agriculture and actively communicating with surface-ground water. Soil sampling was collated in February to March to address the “long-term” (oldest possible) residues before new pre-emergence applications and to avoid the peaks caused by recent applications. In 75 soils, 65 active ingredients were determined by multiple residue method using QuEChERS extraction and UPLC-MS/MS detection. The results identified several CUPs frequently found above LOQ. The most frequently detected compounds were azole fungicides epoxiconazole (36% of samples), tebuconazole (27%), flusilazole (17%), prociloraz (16%), and propiconazole (10%). Their mean concentrations in positive samples were above 0.01 mg/kg. The second most frequently detected compound was 2-hydroxy atrazine (29%) with mean concentration in positive samples above 0.03 mg/kg. The highest measured concentration was 0.135 mg/kg. This finding is surprising as atrazine was banned in CR in 2006. It can also explain still frequent detections of atrazine degradation products in water monitoring. Other CUPs with frequent findings were fenpropimorph (15%), diflufenican (13%), terbufthaline (13%) and others. The results also show how complex is the mixture of CUPs in current agricultural soils - half of the soils had 5 or more CUPs detected. The soil residues of CUPs should be definitely considered and soil monitoring programs for CUPs should be initiated in EU countries along with water monitoring. Financial support was obtained from GACR project 15-20065S.

MO264 PERSAM PESoil calculation and pesticides risk assessment: support or burden for regulators? S. Ullucci, L. Menabulli, M. Luini, International Centre for Pesticides and Health Risk Prevention; F. Marchetto, ICP; A. Riva, C. Riva, F. Galimberti, International Centre for Pesticides and Health Risk Prevention; A. Moretto, Università degli Studi di Milano Pesticide risk assessment for soil organisms is currently performed by calculating the toxicity/exposure ratio, that is the comparison of the toxicity that a specific pesticide has for a soil not-target organism and the pesticide exposure predicted for that same organism. The substance toxicity is assessed with targeted ecotoxicological studies on certain representative species, while exposure is assessed by calculating a Predicted Environmental Concentration (PEC) with models. According to the new EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil (EFSA Journal 2015:13(4):4093) the new model PERSAM has to be used for the calculation of pesticides PECsoil. This model is structured in five Tiers with different levels of complexity. Tier 1 is a very conservative one while the higher Tiers, even if closer to realistic conditions, are quite complex and time-consuming since the entire calculation process is quite slow and long-lasting. This work addresses such a complex exposure assessment in the context of the standard evaluation procedure that regulators have to deal with in their activity of registration of both active substances and PPPs. In particular, attention will be focused on how often a higher tier results necessary and how often Tier 1 may be used to exclude pesticide of low risk for soil non-target organisms. A subset of Plant Protection Products (PPPs) with Tier 1 (may have some risk to soil non-target organisms) authorized at National level has been considered. Ecotoxicological endpoints on soil arthropods and earthworms (NOEC and EC50) have been taken mostly from EFSA Conclusions and Review Reports and recorded in an MS Excel spreadsheet. RAC (Regulatory Acceptable Concentrations) have been derived from these endpoints. PECsoil for the selected active substances have been calculated with PERSAM and with the simple Tier 1 presently used in registration and then compared with the RAC. The percentage of active substances which showed an unacceptable risk for each Tier has been calculated, and comparison between the old system and the new one has been presented together with some considerations on the efficacy, reliability and usefulness of all the PERSAM tiers.

MO265 From bioavailability science to regulation of organic chemicals: a communication perspective J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo; J. Harmens, Alterta, Wageningen-UR / CALM; J. Parsons, University of Amsterdam / IBED; J. Roembeck, ECT Oekotoxikologie GmbH; K. T. Semple, Lancaster University / Lancaster Environment Centre The bioavailability of organic chemicals (i.e., mainly industrial chemicals, pesticides, biocides and fungicides) in soil and water is an important area of scientific research. However, this area remains only partially recognized by regulators and industries working in the environmental sector. Based on the positive experiences already gained with metals, regulators have recently started to consider bioavailability within retrospective risk assessment (RA) frameworks for these contaminated sites and chemicals. By doing so, realistic decision-making in terms of hazard definition and priority considerations finally
MO266
General overview of experimental terrestrial toxicity studies submitted in the framework of the REACH Regulation


The REACH Regulation entered into force in June 2007. Since then European Chemicals Agency (ECHA) has received a large amount of scientific and administrative information related to chemical substances submitted in the registration dossiers. To avoid unnecessary testing, the REACH Regulation provides registrants with the possibility to build testing strategies and to adapt the standard information requirements based on the specific conditions listed in the Regulation. In order to understand what type of data on terrestrial ecotoxicity were submitted in REACH registration dossiers, a detailed analysis of the availability and content of relevant information was performed. The similar analysis was performed after the first registration deadline (Versonnen et al., 2013). The current analysis covers the data submitted for the two registration deadlines (1 December 2010 and 1 June 2013) and new experimental data produced as a consequence of the evaluation of testing proposals and the compliance checks. As in the previous analysis, both the most used test guidelines and test species were investigated. As the outcome of the conducted analysis, it can be concluded that for soil invertebrate testing mostly standard guidelines were used and consequently, a clear prevalence has been detected for testing on the species recommended by the standard test guidelines. Nevertheless, the reporting included a large variety of species from very different families. For terrestrial plants, the most extensively reported test guidelines were OECD 208, ISO 11269-1 and ISO 11269-1. It is important to stress that for many cases, the information on the test guideline according to which the study was conducted, was not reported or was not reported correctly. Moreover various adaptations and waiver justifications were used to omit the experimental testing for terrestrial toxicity. When interpreting the findings of the current analysis, it should be noted that in principle the current results may be affected by the outcome of the dossier evaluation works performed by ECHA. By November 2015, ECHA had issued more than 100 final decisions, which requested terrestrial toxicity studies to be performed according to standard test guidelines. The analysis of the terrestrial ecotoxicity data submitted by registrants to fulfil REACH data requirements will be presented.

MO267
Natural transformation of chlordecone into 5b-hydrochlordecone in French West Indies soil: a statistical evidence for investigating long-term persistence of organic pollutants

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Chlordecone (CLD) was an organochlorine insecticide whose previous use resulted in an extensive pollution of the environment with severe health effects and social consequences. A closely related compound, 5b-hydrochlordecone (5b-hydroCLD), has been searched for and often detected in environmental samples, from the areas of contamination where this compound was not used. A recent consensus considered that its presence was not the result of a biotic or abiotic dechlorination of CLD in these matrices but rather the consequence of its presence as impurity (synthesis by-product) in the CLD released into the environment. The aim of the present study was to determine if and to what extent degradation of CLD into 5b-hydroCLD occurred in the field. To test this hypothesis, the ratios of 5b-hydroCLD and CLD concentrations in a dataset of 810 soils collected between 2009 and 2012 in Martinique were compared to those of negative soil reference samples (commercial formulations). The statistical analysis highlighted the unexplained high amount of 5b-hydroCLD in soil associated with the CLD spread. The hypothesis proposed for explaining why 5b-hydroCLD/CLD ratios exceed the corresponding ratio in commercial formulation leads to the hypothesis of natural transformation of CHL to 5b-hydroCLD. The significance of the 5b-hydroCLD/CLD ratio in the soils—25 times greater in soil than in commercial formulations—when the investigations dealing with the differential transfer of 5b-hydroCLD compared to CLD lead to promote a decreasing mass ratio in situ compared to the formulation, the opposite of the trend observed. This suggested that natural CLD transformation into 5b-hydroCLD over the long term occurred in these soils. Previously, considered as a by-product due to CLD synthesis, 5b-hydroCLD turns to be a highly probable dechlorination product of CLD. However, the details on 5b-hydroCLD formation are still to be determined although some potential pathways for this formation could be advanced. Results from this study may impact future decisions for the remediation of polluted areas.

MO268
Toxicity depends on the life stage: proposal for a new testing method using Folsomia candida

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Toxicity of pollutants depends on the life stage of the test organisms. Different sensitivities between juveniles and adults on earthworms have been reported, meaning that often juveniles are more sensitive than older stages. Current test methods seldomly cover this aspect and may underestimate toxicity. Hence the development of new test methods to assess the environmental risk of contaminants is of high interest. The aim of this study was to develop and assess a novel test method using the standard test species Folsomia candida. The current OECD test is standardised for the exposure of juvenile organisms being 10-12 days old. In the present study the toxicity of Cd was compared between 3 different developmental stages: eggs, juveniles (10-12 days) and adults (21 and 28 days old). Results showed that Cd was not affecting significantly the hatching success of F. candida at 60mg/kg (the reproduction EC50), but survival of juveniles was affected as well as reproduction. These effects were stronger compared to the results of 10-12 days old organisms. Exposure of adults to Cd showed less effect in terms of reproduction compared to those observed after exposure of juveniles. The present assessment of effects at different life stages allows to identify the stage which the effects occurred. Moreover, exposure of eggs enhanced the effects in terms of reproduction compared to exposure of juveniles and adults.

MO269
Effects assessment on terrestrial organisms: Integrated testing strategy under the REACH Regulation


The scope of the terrestrial effect assessment under REACH is restricted to soil non-vertebrate organisms living the majority of their lifetime in the soil and being exposed to substances through the soil pathway. Under REACH framework risks to predators from the secondary poisoning due to chronic exposure to a substance through the terrestrial food chain should also be assessed by registrants. The REACH "standard information requirements" aim to minimise the ratios VILL to X to the REACH Regulation. The minimum dataset depends on the annual tonnage (tpa), i.e. quantity manufactured or imported by a registrant per year. This principle also applies to the standard terrestrial toxicity data necessary to be provided in the REACH registration dossier. For registrations from 100-1000 tpa, results of short-term toxicity studies on soil invertebrates and plants should be provided together with the results of toxicity study for soil microorganisms. When the registration tonnage is above 1000 tpa, long-term toxicity studies with soil invertebrates and plants should be provided. For highly adsorptive (log Kow>5) and very persistent substances (half-life in soil>180 days), long-term toxicity data instead of short-term data should be considered even at registration tonnage from 100-1000 tpa. Column 2 tests (OECD TEEA, Part IX) were considered to gather all necessary information for running preliminary risk assessment: a further step is to develop exposure models that are appropriate at the scale of the garden, rather than the treated soil, is not generally considered. The exposure models integration test method (EPM) may be applied to assess the hazard to soil organisms. The model accounts for the potential for recovery. In addition, consideration should be given to the non-chemical alternatives. There seem to be sufficient alternatives to replace formaldehyde with less hazardous substances and to stimulate the use of non-chemical alternatives.

Result of the present study is to establish compliance of registration dossier with REACH Regulation and to set the next steps of testing strategy to be performed -Quantitative biological tests and/or sub-lethal tests for different life stages should be considered -Microorganisms and plants should be included in the tests -Quantitative and qualitative recovery of the terrestrial effect of the registered substance should be considered -Biocides: risk assessment of formaldehyde should be considered -Biocides: risk assessment of formaldehyde should be considered with respect to the eventual use in biocidal products and to the absence of non-chemical alternatives.
MO270 Example of a conceptual garden scenario and potential for ecosystem services and ecological modelling to support the regulatory process
F. Eriech, CEA; S. Taylor, Cambridge Environmental Assessments
At the present time, outdoor risk assessments of biocidal consumer products are typically based on the impact they have on the immediate treated area. Recovery at the scale of the garden, rather than the treated soil, is not generally considered when reviewing the risk and benefit of the product. This poster aims to demonstrate how treated area and the garden itself could be considered as a part of a larger urban environment, where the fraction of impacted soil can be balanced with the potential for recovery. In addition, consideration should be given to the role of the product and the ecological relevance of the treated area with respect to the wider environment. Finally, the importance of a consensus on the ecological relevance of urban area is highlighted, in order to provide effective guidance to risk assessors.

MO271 Aspects of scoping and running environmental risk assessment for a Biocidal Product Family
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The Biocidal regulation requires an environmental risk assessment (ERA) to be performed on the active substance (AS), for the precursor(s) of in-situ generated AS and for any substance of concern (SoC) present in any biocidal product (BP) to be marketed in the EU market. In the meantime, the regulation allows the creation a biocidal product family (BPF) in order to facilitate a group of products to access the market. Within a BPF, all BP have the same AS and have similar uses. Variations in the composition, replacement of non-active substances is allowed, providing that change do not affect the level of risk or reduce the efficacy. The number of BP and AS in a BPF is not defined. A simple and straightforward BPF includes a single AS and omits any SoC. However, more complex BPF may be built and include more than one AS and SoC for environmental purposes. Since the assessment of the BPF shall consider the maximum risks to the environment over the whole potential range of products within the BPF, preliminary risk assessments is needed to define the scope of ERA (i.e. compartment at risks, composition leading to maximum risks). The initial step is to gather all necessary information for running preliminary risk assessment: -Product Type, detailed information on use claimed -Identification of relevant emission scenario document. However, for many biocide uses, no ESD have been developed and will require tailor-made scenarios to be performed -Quantitative composition and variation of components within the BPF -Collecting hazard data for all components including PNEC values. Which methodology to be applied for running the risk assessment? Various methods have been proposed to assess the risk of mixture for the environment. This includes e.g. component-based approach. Among the modelling tools, EUSES has been extensively used for the assessment of risks posed by AS under the review program of existing AS. The methodology is quite clear for assessment of BPF containing a single AS, the picture much more complicated with combination of AS and SoC. A transitional guidance has been made available by ECHA. This poster will address a hypothetical case study with BPF containing more than one AS and relevant substance to assess together with a combination of wide dispersive use. The purpose of this work is to initially review the issues with ERA for BPF, the need for an adaptable methodology able to cover complexities associated with variation of composition and uses.

MO272 Testing antifouling sealers efficiency and toxicity
J. Wezenbeek, E. Smitt, RIVM / Centre for Safety of Substances and Products
The Dutch Ministry of Infrastructure and the Environment will use the inventory to review – in collaboration with industry branches – whether there will be bottlenecks when the use of formaldehyde is further restricted. Also, the study will be used to inform users about the need to restrict exposure to formaldehyde and formaldehyde emissions as much as possible. This is in line with the current policy of the Ministry of Infrastructure and the Environment for priority substances of concern (ZSV substances). The Ministry aims to substitute the use of formaldehyde with less hazardous substances and to stimulate the use of non-commercial alternatives.

MO273 Harmonizing the environmental risk assessment for antifouling paints
J. Wezenbeek, E. Smitt, RIVM / Centre for Safety of Substances and Products
Replacement possible for biocides with carcinogenic formaldehyde. However, the suitability of these alternatives needs to be reviewed per sector and application. Formaldehyde is the active substance in many disinfectants and preservatives. It is expected to be classified as carcinogenic by 1 January 2016 at European level. This can have implications for the use of biocides containing formaldehyde that are currently on the market. In the Netherlands, the national risk management of banned paint components is made available on the market are assessed by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) and authorised as safe when used according to the product label. These products are used by professionals only. There are alternatives for the following applications: cleaning and disinfecting of surfaces and instruments in the food sector; cleaning and disinfecting of public areas; disinfection of stables and animal housing; preservation of detergents and cleaning products; preservation of paints, varnishes, inks and other industrial liquids, preservation of liquids in cooling systems and for slime control in the paper and pulp industry. For some applications, very few alternatives were found. This lack of concerns, for example, the disinfection of mushroom-growing rooms, litter bins for sanitary towels, footwear and cattle hooves. This also applies to some preservation products, such as in lubricants, metalworking fluids, and the preservation of human and animal corpses and biological tissues. The Dutch Ministry of Infrastructure and the Environment will use the inventory to review – in collaboration with industry branches – whether there will be bottlenecks when the use of formaldehyde is further restricted. Also, the study will be used to inform users about the need to restrict exposure to formaldehyde and formaldehyde emissions as much as possible. This is in line with the current policy of the Ministry of Infrastructure and the Environment for priority substances of concern (ZSV substances). The Ministry aims to substitute the use of formaldehyde with less hazardous substances and to stimulate the use of non-commercial alternatives.
normally preceded by the use of sealer coats (SC), which are developed to prevent product incompatibilities and leaching from older paint layers. Therefore, the efficiency of such SC is of importance if leaching from older paint products to the environment is to be avoided, especially nowadays with the increase usage of mechanical brush-based devices for removal of biofouling from boat hulls. In this study, we manipulated share-based exposure treatment of five SC from leading paint producers. Sealer coats efficiency in locking underlying AP, its resistance to brushing action and toxicity to aquatic organisms were evaluated. The toxicity of SC leachedates produced in 7%K natural seawater was tested on four organisms, the bacterium Vibrio fluvialis, the alga Ceratium tenuissimum, the crustacean Niscaea spinipes and the gastropod Theodoxus fluviatilis. All SC showed to be toxic, causing increased mortality, inhibition of growth or bioluminescence inhibition, to at least one of the tested species. In terms of locking efficiency, none of the SC showed to be able to successfully lock biocide release from all the AP tested. Locking capacity of SC was dependent on the underlying layer of AP being tested and identity of the metal being released, with brushing action generally reducing locking efficiency of SC. Our results emphasise the need to improve the locking efficiency of SC in order to prevent release from underlying AP. Moreover, we showed that the use of mechanical brushing devices on SC is also not recommended as it reduces SC locking capacity and consequently increases leaching rates from underlying paints. As SC leachedates were toxic to tested organisms, the use of biological tests in the regulatory process of such products is recommended.

MO275

Emission sources and photo-transformation of biocides on building facades using the example of terbutryn
U. Bollmann, Aarhus University / Environmental Science; G. Minelgaita, Aalborg University / Civil Engineering; M. Schlesinger, German Federal Institute of Hydrology; T. Termes, German Federal Institute of Hydrology BfG; J. Vollertsen, Aalborg University / Department of Civil Engineering; K. Bester, Aarhus University / Environmental Science. Biocides as triazines or isothiazoliones are added to render and paint in order to protect building facades from microbial deterioration. Previous studies have shown that biocides leach out of the material during rain events and can be detected in the urban environment. In the present study the leaching of terbutryn from artificial walls equipped with two types of render (acrylic and silicone resin render) was observed for 19 months. On the one hand, the emissions of terbutryn (concentration and mass load) were determined; on the other hand, photodegradation products were identified and studied in the leachate as well as in the render. It could be shown that biocides leach predominantly within the first months of the facade lifetime. While the leaching was determined by driving rain within this period the leaching was reduced afterwards and determined by other factors. Several photo-transformation products could be detected in the facade runoff. However the major fraction of the transformation products was still remaining in the facade material. Based on the amount of terbutryn and its transformation products in the leachate as well as remaining in the material, the overall mass balance can be closed. This study showed, that the focus on transformation products during the assessment of biocides in building material is of high importance: not only concerning the emissions during life-time, but also when it comes to its disposal of the coating material as waste.

MO276

Non-monotonic dose-response relationships for toxicity of the biocide DiPel ES on Daphnia magna
A. de Souza Machado, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; C. Zarfl, University of Tuebingen / Center for Applied Geosciences; S. Rehse, Leibniz-Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; W. Klaos, Liebniz Inst. of Freshwater Ecology & Inland Fish. Currently, there is a trend to increase the use of supposed environmentally friendly biopesticides. In this context, Bacillus thuringiensis (BT) pesticdes, such as DiPel ES used in Germany for about 40 years, had been most used to control caterpillars. BT produces endotoxins that cause effects described as highly specific to some insects. These biopesticides are used worldwide without environmental limits because they are considered to present negligible toxicity to non-target organisms. This study investigated the toxic responses of the non-target organism Daphnia magna to waterborne DiPel ES based on results tested for EC50 and LC50 and EC50 estimation. D. magna 17-21 days old were similarly exposed for EC50 and biomarkers. The biomarkers evaluated (whole body) were body weight, global protein, chitin, casein, glutathione reductase, glutathione S-transferase, and acetylatedalaminesterase. Particle size distribution in the exposure media was considered to present negligible toxicity to non-target organisms. This study shows that the focus on transformation products during the assessment of biocides in building material is of high importance: not only concerning the emissions during life-time, but also when it comes to its disposal of the coating material as waste.

MO227

Secondary poisoning risk assessment for insectivorous species under Biodical Products Regulation: case of insecticides used against Ants
V. Lamz, A. Scole, ANSES
The risk of secondary poisoning is a part of the environmental risk that has to be assessed under the Biodical Products Regulation 528/2012. It relates to toxic effects occurring in higher levels of food chains which result from ingestion of contaminated organisms from lower trophic levels. Insectivorous animals have potentially a risk of secondary poisoning through eating treated insects, especially during the outdoor use of insecticidal products, like those against ants. A harmonized approach to assess the secondary poisoning risk for insectivorous birds or mammals is actually available in the Exposure Scenario Document for household and professional insecticides (ENV/JM/MONO(2008)114). However, this approach based on the SANCO/4145 (2002) EU guidance for Plant Protection Products is relevant only for spray products and not for other type of application such as gel bait often used against ants. This poster describes an example of biopesticide use for which an exposure scenario is currently not available. It presents first thoughts on the parameters which could be considered for the construction of a new model of exposure more specific to gel bait product against ants. Considerations related to the type of product, its mode of action, specificities of target (here ants) and predator species (behavior, biology) are exposed and discussed. This can be useful for a future scenario that could better reflect the real worst case of secondary contamination of insectivorous species eating ants treated with a gel. Due to the variety of uses, target organisms and application type, the biocidal environmental risk assessment regularly requires the adaptation or development of new exposure scenarios which complete existing European guidance documents and enhance the environmental risk assessment.

MO278

Exposure of non-target prey and predators to anticoagulant rodenticides
A. Gedehn, Julius Kuehn Institute, Federal Research Centre for Cultivated Plants; A. Esther, IJK / Vertebrate Research; D. Schenke, Julius Kuhn-Institute, Federal Research Centre for Cultivated Plants / Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; J. Jacob, Anticoagulant rodenticides (ARs) are the main management tool for the reduction of commensal rodents worldwide. However, ARs are toxic to all warm blooded organisms and can lead to wildlife exposure and poisoning. The details of the connection between primary exposure of non-target small mammals by direct AR bait consumption and secondary exposure of predators through uptake of contaminated prey are often unknown. In field studies we assessed the exposure of small mammals and estimated AR exposure risk for barn owls (Tyto alba) based on AR residues in small mammals during baiting campaigns on farms and the diet of barn owls in the farm environment. In addition, we screened barn owl spit pellets, liver samples of prey caught by barn owls, and carcasses of predators for AR residues to clarify the exposure pathway from bait to predator. Furthermore, we analyzed local parameters affecting AR exposure in red foxes on regional scale to identify spatial drivers of AR exposure in top predators. AR residue concentration of non-target small mammals was highest within 15 m of bait points and low concentrations were detected in animals caught at a distance of up to 87 m. Apodemus species and the shrew Crocidura russula regularly carried residues (sometimes at high concentration), whereas exposure in Microtus species was low. The exposure risk of barn owls was highest in autumn when owls increasingly preyed on Apodemus species and low in summer when owls mainly preyed on Microtus species. ARs were rarely found in spit pellets but were present in 13% of prey items collected from nest boxes. About 36% of predatory birds and owls and 60% of red foxes (Vulpes vulpes) that were sampled across Germany carried AR residues. In foxes, the presence of AR residues was correlated to the percentage of urban areas and livestock density. The results demonstrate that AR exposure of non-targets occur more or less often depending on species and several environmental correlates incl. distance to bait points (small mammals), seasonal prey composition (barn owls), and livestock density (foxes). Knowledge about environmental drivers of non-target exposure to ARs can be used to adjust risk mitigation strategies. (this study was funded by German Federal Environmental Agency grant #371063401)

MO279

Resistance of Norway rats (Rattus norvegicus) to anticoagulant rodenticides: Distribution in Germany and factors determining the spread
Life Cycle Data and Modeling Developments - From Data Collection to Usage (P)

MO280

Life Cycle Assessment of bio-based solvents for paint and coating industries

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Keywords: Solvents, Bio-based products, Environmental impacts, Sustainability

The global consumption of solvents is significant, with almost 28 million metric tons consumed in 2012 (Linak and Bizzari, 2013). The paints and coatings sectors are the main solvent consumers. This demand is predicted to increase by 2.9 % per year until 2019 (Ceresana, 2014). Nowadays, the solvents used in the paint and coating industries are currently produced from petrochemical sources. In the context of sustainable development, the replacement of traditional solvents by new green alternatives has to be studied. The ECOBIOFOR project, developed by a consortium of 14 European partners from 6 different countries, will help the transition of European paints and coatings industry from petrochemicals to bio-based products. The main objective of the ECOBIOFOR project is to develop novel bio-based solvents from renewable building blocks thanks to easier chemical or biotech transformations processes. In parallel to the syntheses development, a Life Cycle Assessment (LCA) of these new bio-based transformation processes is considered, using the ILCD method with some improvements, in order to assess their potential environmental impacts and so select the best-developed options. These new pathways are also compared to traditional petrochemical processes. After we defined the goal and scope of this LCA study, difficulties have been encountered on the data collection due to confidentiality issues and lack of data, especially for the chemicals in the Ecoinvent database. Methods to overcome these difficulties have thus to be developed from the point of view of the foreground and background data. Therefore, data could be obtained either from specific collection (directly from the manufacturers or in the R&D laboratory), by using proxy to model the considered molecule by a similar one already available in the databases or finally by semi-specific modelling, i.e. reconstructing the synthesis pathway through retrosynthesis to obtain building blocks available in Ecoinvent v3. References: Ceresana (2014). Market Study: Solvents. Linak, E., and Bizzari, S. (2013). Global Solvents: Opportunities for Greener Solvents (IHS Chemical)

MO281

Conversion of international environmental reporting data to LCA data for SCP

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Conversion patterns have shown to increase the use of natural resources for the production of goods and services. A national project has been set up to provide support to Swedish authorities (notably the Swedish Environmental Protection Agency and the Swedish Agency for Marine and Water Management) to gain a better understanding of the reasons for why some environmental problems are difficult to forecast - thereby making them difficult to mitigate by conventional means - with a special focus on the environmental impacts of production (SCP). The theoretical parts, which cover a major portion of the overall work, include a methodological development on a scientific basis where long-term national monitoring data provided by the Consortium for Environmental Data reporting in Sweden (SMED), in various contexts such as the Industry Emissions Directive (IED), the UNEP guidelines, and so forth, are summarized. The main findings are that data can be converted into LCA-based information. Such an assessment will primarily include two essential aspects - one dealing with a number of technical considerations for allocation and other LCA aspects, the other with non-technical aspects, e.g. the proper use of confidential data. This part of the project will also examine and assess potential for trend analysis and support the identification of key pollutant emission sources as an input to a deeper understanding of why some environmental quality objectives, specifically those related to SCP, are hard to fulfil. The practical and operational parts will focus on synthesis and implementation of results, in which conclusions are drawn on how the SCP work can be better linked to the work related to the Swedish environmental production goals, as well as providing some perspectives for suggesting additional needs of legislative and other means for better control of SCP. This part will also focus on international outreach, in which the key focus will be to provide a future input to the UN work on establishing a “Global Network for Interoperable LCA Data Bases (GlobalLCAccess)”, and link these activities to European and international standardisation work.

MO282

LCI data sets for up-stream processes of fossil energy for Germany today and 2030


The German “Energiewende” (Eng. energy transition) aims at enhancing the share of renewable energy carriers in order to mitigate Green House Gas emissions. Mitigation is calculated against the reference of fossil energy sources such as crude oil and natural gas. However, environmental impacts of extraction of fossil energy carriers also change in the course of time due to technological performance but also due to an increasing use of unconventional sources. For a realistic estimate of national GHG balances in different energy scenarios, data for up-stream-processes are most crucial: Whereas the environmental impacts of the combustion technologies mostly are well-known, the extraction of fossil fuels receives little attention in life cycle assessment (LCA) studies (Bouman et al., 2015). This translates into the lack of data availability showing changes of conventional and unconventional extraction technologies in LCA databases. The performance of high quality life cycle assessments requires life cycle inventory data representing the current state of technology. To this end, this study analyses environmental impacts of current production technologies of petroleum and natural gas. Life cycle inventory datasets describing the current production of crude oil and natural gas in various regions are provided and compared with older datasets produced in 2000. The production regions examined are those which provide Germany with crude oil or natural gas. Furthermore, in order to analyse the future global warming potential (GWP) regarding the production of 1 kg crude oil and 1m3 natural gas for usage in Germany, scenarios for 2030 are developed. The LCAs are generated according to ISO 14040 and ISO 14044. For the bulk of oil producing regions, a decrease of GWP compared to 2000 is identified. Simultaneously, the GWP of natural gas production has increased in all examined regions of production. Based on the scenarios, future GWP of natural gas production for Germany will be higher than today. The results of the scenarios for the crude oil production are less distinct. The results range from nearly constant GWP to increased GWP. Taking into account the current trend of decreasing GHG emissions during oil production, even a sinking GWP might be possible.

MO283

Assessing the environmental performance of new technologies in the drinking water industry

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Life Cycle Assessment can be applied to a wide range of case studies considering that it can help develop a philosophy for undertaking the countable procedure of all pollutant exchanges between the system analyzed and the environment. This system can take the form of a variety of activities, services and products, from an entire industrial sector of a country to any particular action that a person can perform. The LCA practitioner must face the challenges posed by the environment systems and the demands on data availability, uncertainty of the input data, resolution...
of the model, necessary assumptions, sensitivity to unsettled parameters, etc. In the presented study we analyze the consequences of adopting decisions on the mentioned features in the design of the LCA models used. The systems to be evaluated are the novel platforms developed under the project AQUAVALENS for the detection of pathogens in drinking water. The aim of the project is to enhance the water systems' adaptability to manage new paths and manufacturers to better control the safety of their water supplies by using newfangled technologies that integrate sample preparation and detection into a single platform. The Carbon Footprint calculation of these platforms will be used to determine their sustainability as part of final objectives of the project, which includes, among other problems and benefits, assessment of the improvement of Water Safety Plans, adaptation to climate change and control of outbreaks of infectious diseases. The challenges of developing a LCA of the systems under study rely on the difficulty for setting a high number of parameters related, on the one hand, with the platform manufacturing and, on the other hand, with the protocols and procedures to use them. The complexities have their origin in the diversity of platforms, manufacturers and potential users, which make valid the saying that there is more than one way to skin a cat. This variety have forced us to perform the study in a iterative way, where questionnaires and model drafts have been made in line with the input data from the most general to the smallest detail so that each model was refined to obtain a degree of uncertainty consistent with the objectives of the study. Particularly, the decisions and assumptions made were based on robust uncertainty and sensitivity analyses.

MO284 Prospective performance indicators of electricity production in Spain D. García-Gusano, Instituto IMDEA Energía / Systems Analysis Unit; D. Garrain, CIEMAT / Energy Systems Analysis Unit; D. Iribarren, Instituto IMDEA Energía / Systems Analysis Unit; H. Cabal, CIEMAT / Energy Department Energy Systems Analysis Unit; J. Dufour, Instituto IMDEA Energía / Systems Analysis Unit Although environmental modelling methodologies such as Life Cycle Assessment (LCA) are well-known tools for the analysis of sustainability issues, there is a gap in managing the time dimension. Many studies carry out assessments assuming predefined systems concerning the future or use roadmaps from the literature. This work involves a detailed LCA study from a consequential perspective by using a national energy systems optimisation model, TIMES-Spain. This methodological linkage strengthens the discussion on how the electricity production mixes should be built in the future according to key prospective performance indicators. TIMES-Spain, built with the TIMES energy optimisation model generator developed by ETSAP Implementing Agreement of the International Energy Agency, provides energy-related technology mixes for satisfying the demand of energy services in all economic sectors (industry, transport, etc.) under different assumptions. A couple of scenarios were implemented: a Business as Usual scenario “BaU” with restrictions from European Directives, and an exploratory scenario “80%” enforcing an 80% reduction in the total Spanish CO2 emissions by 2050 with respect to 2005 levels. The evolution of the electricity production mix from the optimisation process shows that the scenario “BaU” results in a major presence of natural gas cogeneration plants in the long term, whereas the scenario “80%” prioritises wind and biomass options. LCA computation was performed for the set of existing and new technologies. Besides, a LCA/TIMES harmonisation process was established to enable a potential extension of TIMES-Spain. In this regard, existing processes in TIMES-Spain were adapted to include a fraction of the burdens linked to infrastructure by assuming a residual lifetime according to the year of installation. The evolution of ten life-cycle indicators (from ILC 2011 and ReciPe methodologies) shows global reductions in the long term ranging from 21% for Ozone Depletion to 85% for Acidification in the scenario “BaU”, and reaching up to 87% for Acidification in the scenario “80%”. Nonetheless, Abiotic Depletion grows significantly due to the rare metals required in the production of solar cells, highly present in the future mix. Energy systems modelling is found to be a valuable tool to perform prospective LCA studies in the field of energy, adding the time dimension to the sustainability assessment.

MO285 Lessons Learned in Developing an Environmental Product Declaration Program for the Asphalt Industry in North America A. Burschke, Michigan Technological University / Civil Engineering; H. Dylla, National Asphalt Pavement Association The objective of this presentation is to report the technical and organizational challenges involved in the development of the North American Environmental Product Declaration program for asphalt mixtures. It explores how differences in stakeholder priorities and perspectives, in the pavement construction industry, directly shape the design of the Category Rules (CRs). Health, the primary challenge identified is how to ensure technical rigour of the underlying LCA, while recognizing the interests of the stakeholders and ensuring the delivery of a program that is effective. The presentation discusses how technical issues regarding system boundary choice, data use and allocation presented challenges for the Expert Group. The paper focuses on how the Expert Group addressed these different stakeholder interests. Insights into the discussions that informed the PCR development are provided.

MO286 LACAnet.de - A new network for LCA practitioners in Germany B. M. Zimmermann, LACAnet.de; P. Peters, KIT Karlsruhe Institute of Technology / Helmholtz Institute Ulm HH; W. D. Baluch, Öko-Institut Darmstadt; S. Weyand, Technische Universität Darmstadt / IWAR Chair of Material Flow Management and Resource Economy LCA networks provide contacts and allow for exchange of knowledge and questions within the LCA community. They can be an extremely helpful address for LCA practitioners on emerging information and development of new publications and upcoming workshops, especially for new practitioners. While there are several important LCA networks on international level or in other regions, there is little local activity in Germany. LACAnet.de is a recently founded non-profit organization dedicated to the exchange of knowledge among – especially young – LCA practitioners. It aims at building up a decentralized structure with several regional nodes in Germany. This should keep distances between the actors short, allowing for direct communication and shorter, cheaper and environmental friendly travels to workshops. LACAnet.de’s first node is in the city of Karlsruhe reaching from Freiburg in the south to Darmstadt in the north. LACAnet.de is an integrative network, open for everyone who is working in a LCA context within the network’s regions. Therefore the language of communication is English. By developing organizational tasks among the network’s members, LACAnet.de can run on a small budget. Consequently member fees are very low, and participation in workshops is either free or inexpensive. This eases the access for people who are not able to pay considerable member fees such as students. Professionals or companies, who are hardly connected to the academic LCA community, LACAnet.de provides a web page with relevant news and a forum for addressing specific questions to the community. The core activity of LACAnet.de is the organization of regional workshops at least twice per year. Each workshop addresses a single, LCA-related topic, aiming at learning, discussing, problem solving, method development and networking. This poster presents the non-profit organization LACAnet.de, provides information about its principal goals, how to become member and about the services and networking activities provided by the network. It invites especially young, but also experienced LCA practitioners to join and to actively contribute to the development of this initiative.

MO287 Ecospold 02 - ILCD: mapping and format converter S. Fazio, EC-JRC / Institute for Environment and Sustainability; F. Mathieuex, EC JRC; IES; G. Biengini, European Commission DG Joint Research Centre; C. Servaes, GreenDelta@ECC, European Environment Agency / Institute for Environment and Sustainability / Sustainability Assessment unit; C. Rodrguez, GreenDelta GmbH In a recently completed project carried out for the Joint Research Centre (JRC) of the European Commission, GreenDelta updated the International Reference Life Cycle Data system (ILCD) reference elementary flow list. Basing on the existing mapping file available in GreenDelta’s open LCA format converter, the ILCD flowlist has been revised and updated, in collaboration with JRC, in order to cover all the elementary flows available in EcoSpold02 nomenclature. For the EcoSpold2 flows not mappable in the ILCD reference flow list, new flows have been added to the ILCD list. Almost 400 new flows have been created, and more than 400 have been mapped, and the nomenclature has been further investigated also on the new formats of the upcoming Environmental Footprint (PEF and OEF) scheme, which is now in the pilot phase, under the supervision of DG Environment. The project has been funded by JRC within the institutional activities, therefore the converter was developed only for the conversion from EcoSpold2 to ILCD and not vice-versa, since the other formats and nomenclature are not officially adopted or endorsed by EU Policy. However, the results of the project are now available also in the framework of the international cooperation for the Global Network of interoperable LCA databases, led by UNEP, where the aim is to expand the mapping also to other format and nomenclature systems, and to enhance the converter in order to allow multi-directional convertibility.

Improving the usability of ecotoxicology in regulatory decision-making: findings from a SETAC Pellston® Workshop (P)

MO288 Updating Environmental Quality Standards using REACH disseminated data: an illustration of transfer of knowledge between policies G. Desviller, CIEexpertPOPs Article 16 of the Water Framework Directive (WFD, EC 2000) sets out the strategy against chemical pollution of surface waterbodies. The chemical status assessment is used alongside the biological status assessment to determine the overall quality of a waterbody. The Environmental Quality Standards (EQSs) Directive (EC 2008a) establishes concentration limits for 33 Priority Substances and 8 other pollutants. EQSs were derived for the Priority Substances at a European level and are used to assess the chemical status of water bodies in all Member States. In addition, the WFD establishes the principles to be applied by
The member States to develop EQSs for Specific Pollutants that are ‘discharged in significant quantities’. Compliance with EQSs for Specific Pollutants forms part of the assessment of ecological status. Derivation of EQSs is based on (eco)toxicological data on organisms that are relevant for the identified protection goal: the pelagic organisms for waters (fresh and marine), the benthic organisms for sediments (fresh and marine) and mammals/birds for secondary poisoning. Many of the EQS that have been derived up to now have used peer-reviewed data and in many cases data gaps were observed for one or more compartments. However, it is recognised that the risk assessment paradigm on which the EQS derivation is based could lead to unworkable and/or unrealistically low EQS values in the case of deriving an ETP Technical Guidance (2011). With REACH entering into force, many studies have been performed by industry in order to comply with their regulatory obligations. The dissemination of data of registered dossier on ECHA’s publicly available website has given considerable opportunities to improve our knowledge of the substances properties and to reassess previous work like EQS derivation. This presentation will show how REACH disseminated data have been assessed for EQS derivation purposes (relevance and reliability) and if these new data could decrease the uncertainties attached to the existing EQS and finally improve the global assessment of the chemical status of European waters.

Innovative techniques for monitoring chemicals in the environment (P)

TU001 THE IMPACT OF β-BLOCKERS ON THE SOIL MICROBIAL PHYSIOLOGICAL DIVERSITY

M. Pino, San Jorge University / Facultad ciencias de la salud; S. Muñiz, Pyrenean Institute of Ecology (CSIC); J. Val, San Jorge University; E. Navarro, CSIC - Spanish National Research Council / Biodiversity conservation Ecosystems Restoration

Pharmaceuticals and their metabolites reach the terrestrial ecosystems through the application of wastewater and contaminated biosolids to agricultural soils. These products may impact the microbial community. We have evaluated the impact of six widely consumed β-blockers on soil microbial communities from an ecological farming crop field using the community-level physiological profile –CLPP-. This method is based on the rate of carbon substrate degradation by microplankton (Biolog Ecoplates®). The impact of the pharmaceuticals has been assessed by comparing the Averaged Well Color Development –AWCD- of each treatment, as an integrative indicator of the physiological ability of the microbial community for degrading all carbon sources. Nadolol provoked a slight decrease in the AWCD compared to control. On the other hand, a stimulatory effect was observed for the other β-blockers. The Shannon diversity index (H) was used to evaluate the physiological diversity of the microbial communities. The soils treated with propranolol (pc.0.05) and metoprolol (pc.0.01) presented a significantly lower physiological diversity. The β-blockers also significantly decreased the degradation of polymer carbon and carboxylic and ketonic acids sources and significantly increased the amines/amides. No differences were detected in the use of carbohydrates and amino acids, except for the atenolol that showed a significant increase (pc.0.05) in the use of amino acids. Results show that pharmaceuticals on soil microbial communities, at environmentally relevant concentrations (around 100 mg/kg), altered key ecosystem functions as that of the carbon cycle.

TU002 Eco-toxicity and human toxicity impact in urban area: case of accumulated trace metal air pollution analysis on cemetery mosses in Paris city

A. Yalaltdinova, Tomsk Polytechnic University / Geoecology and Geochemistry; J. Kim, University of Technology of Troyes / Department of Humanities Environment Information Technology CREIDD; M. Natali, IEN-CNRS; A. Zanella, University of Padua / TESAF; A. Rankovic, J. Lata, University Pierre et Marie Curie / Institute of Ecology and Environmental Sciences

The monitoring of atmospheric pollution is a matter of great importance to the environmental protection of peri-urban areas in a crucial phase where expectations of society in terms of environmental issues often exceed the capacity of integrative measures to track pollutants in the long term. Mosses are useful ubiquitous accumulation bio-monitors and as such were used for European surveys. Furthermore, anthropogenic emissions (e.g., heavy metals) being closely related to ecosystem and human health, integrative risk assessment is also of a major interest in citizens' expectations. In this study, we tested the hypothesis that cemeteries are appropriate sampling sites to evaluate moss accumulation of air pollutants in urban areas, and that the USEtox impact assessment model is useful to provide relevant eco-toxicity and human toxicity impact assessment. For this purpose, we sampled mosses growing in 21 urban and peri-urban cemeteries in the Parisian metropolitan area. We focused on Grimmia pulvinata, a species abundantly found in all studied cemeteries. We quantified 19 elements with TXRF-SSM method. For eco-toxicity and human toxicity impact mapping, the use of USEtox method of life cycle impact assessment and its characterization factors allowed to characterize factors and categories (human toxicity and ecotoxicity) related to trace metals in air emissions. We show that enrichment factors for some markers of road traffic (e.g. Pb, V, Zn, Cu) indicate that the highest polluted cemeteries were located near the highly frequented ring road of Paris and under the influence of prevailing winds coming from the Southwest. The sites with the lowest pollution appeared in peri-urban cemeteries, adjoining forest or farming landscapes, but also in large and relatively wooded cemeteries in the center of Paris. The calculation and mapping of the accumulated eco-toxicity and human toxicity impacts allowed showing the distribution of highest and lowest polluted areas in complex urban fabric as Paris area. Observed differences between environmental and human toxicity distributions show that we can expect more spatial variability of toxicity impact even in densely urbanized areas that usually thought.

TU003 E-Board - a new in situ method for assessing the reproduction-related traits of chironomids: proof of concept and potential perspectives of use

B. Ferrari, Ecotax Centre CH; J. Fabian; AgroParisTech / UMR ECOSYS INRA Université ParisSaclay

Further progress in the development of reliable biomonitoring strategies requires better link effects in aquatic ecological systems to ambient contaminations. Among existing tools, in situ biomass tests using caged method represent an interesting way to achieve this challenge. However, elaboration of adapted exposure chambers and suitable operating procedures is still required, particularly to assess ecological relevant traits such as those related to the reproduction. In such context, we developed a new device (Emergence bubble - E-Board) which allows assessing in rivers the development of the Chironomus riparius species from the early fourth instar larvae to the adult stage. The system is based on the hypothesis that chironomids mainly feel oxygen deficiency (Deposition of oxygenated matters that represent the major exposure route to contaminants for them. The system acts as a suspended matter trap floating in the subfascate of the water equipped of an emergence trap for catching chironomid adults. The system was tested in actual field conditions. Its easy handling allowed obtaining data which demonstrated its applicability for assessing the development of the chironomids. Moreover, by adapting energy-based models specifically developed in the laboratory for the species C. riparius, we were able to predict the growth pattern and the emergence of chironomids in real environmental conditions. The method offer the possibility to follow ecologically relevant responses on sensitive life stages or periods for individuals which are difficult to take into consideration in conventional community surveys and which are crucial to infer impacts at population level. The E-Board represents thus a promising new in situ tool in perspective of evaluation of the quality of the ecosystems.

TU004 Development of sensor based on optical fibre for identification of chemical parameters in surface water

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Permanent monitoring of contaminants that can disturb the quality of surface water is required, due to increased environmental pollution from anthropogenic sources. Standard laboratory methods revealed certain disadvantages relating to the high cost, complexity of analysis and the possibility of losing the desired analyte caused by human error in sampling, storage and transport of samples. Fiber optic sensors (FOS) are convenient technology for monitoring of surface and ground water and to provide data for assessing the development of the current state of water bodies. FOS have certain advantages over conventional methods such as: simple design, small size, possible use in hazardous chemical environments that can damage the measuring equipment, can be used for remote sensing and in distant areas, safety because of the low optical power and the absence of electric current in the sensing point and resistance to electromagnetic fields. This project presents a prototype of colour FOS for detection of different inorganic parameters in surface water. Implemented sensor converts the RGB color model to HSV color model and based on the color intensity of the sample determines the concentration for parameters of interest. The concentration of the chemical parameters is calculated by introducing the obtained values of H (Hue), V (Value), S (Saturation) in transfer function. Colour FOS based on HSV model was designed for the determination of orthophosphate, nitrite, sulfate, Cr(VI), and total chlorine in surface water samples. Results obtained with the applied sensor are compared to the results obtained with laboratory analysis to prove the efficiency of the device. FOS has proven to be very suitable for use in the laboratory under controlled conditions as low-cost solution to replace expensive standard equipment. Future research should be focused on performance improvement and on increasing the
sensitivity of the device, which will be achieved by choosing better quality LEDs with a wider wavelength range. Keywords: fiber optic sensors, low-cost measurements, surface water Acknowledgement: The research has been supported by Ministry of Education, Science and Technological Development of Republic of Serbia under project Development of methods, sensors and systems for monitoring quality of water, air and soil, IIIA08.

**TU005**

**FROM TOXICOLOGICAL BIOASSAY AND CHEMOMETRICS ANALYSIS TO A RAMAN SPECTROSCOPY MULTIPARAMETRIC BIOMONITORING**

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In the field of toxicological bioassays, the toxicity assessment of a pollutant or a mixture on living organisms is often based on the observation of a single physiological parameter (e.g.: bioluminescence inhibition, cellular death, enzymatic activity). The monoparametric approach of such bioassays limits their performance in terms of specificity and sensitivity. They need to be associated in order to provide a global toxicity measurement, and lead to the multiplication of the implemented tests. In this context, the latest progresses in the Raman spectroscopy open new research perspectives on fast method of observing metabolic responses against toxic agents. Indeed, each spectrum is made of more than 2700 data points which represent a molecular fingerprint of the observed samples. This approach permits to highlight the physiological changes caused by the pollutants. Thus, the comparison of spectra from bacteria exposed or not, is used to define a “signature” for a given toxic effect and overcome the limitations of other biosensor techniques. Moreover, the physiological spectral fingerprint is both rich and complex. Consequently, to properly analyze it needs complex chemometrics methods. In this study, statistical multivariate analyses have been implemented to highlight the differences between spectra of the bacteria *Escherichia coli* exposed to varying increasing arsenic concentrations. The results show both a dose-response and an exposure time effect of the arsenic on *E. coli* cells. Furthermore, the fine analysis of the Raman spectroscopy fingerprints permits to identify the cellular macromolecules impacted by this chemical. The highlighted changes are in accordance with the expected toxic effects. In the future, a toxic signature of pollutants and a more precise overview of their metabolic target might be identified by the Raman multiparametric observations of toxic effects on microorganisms’ metabolism coupled to chemometrics tools.

**TU006**

**Application of LC-APCI-qTOF-MS for simultaneous analysis of short-, medium- and long-chain chlorinated paraffins in technical and environmental samples**

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Chlorinated paraffins (CP) are high production volume chemicals (1 Mio t/a in 2009) used as extreme pressure additives, plasticizers and flame retardants. They are of concern due to their persistence, bioaccumulation potential and the limited information on their environmental fate and toxicity. Analysis of CPs in environmental samples is a nightmare due to the large number of congeners and isomers (chain length: C5-C45; Cl-degree: 30-70%) and the lack of suitable standards. Recent analytical methods mainly focus on short-chain CPs (SCCPs). SCCPs are currently discussed to be classified as persistent organic pollutant by the Stockholm Convention Review Committee. Thus, a shift in production and emission to medium- and long-chain CPs (MCCPs and LCCPs) is expected, requiring respective analytical methods. Most studies on CPs apply gas chromatography (GC) with mass spectrometry (MS). However, GC is not suitable for analysing CPs with increased chain length due to their low volatility. Recently, Bogdely et al. 2015 (Anal. Chem. 87, 2852-2860) developed a method overcoming the need for separation using direct liquid injection and focussing on accurate mass determination. They apply atmospheric pressure chemical ionisation (APCI) under chlorine-enhanced conditions in negative ion mode followed by quadrupole time-of-flight (qTOF) MS. Their method allows rapid (< 1 min) and simultaneous analysis of SCCPs, MCCPs and LCCPs, featuring the high sensitivity of increasing chain length. The present study investigates how different CP congeners compete for the available chloride. Liquid chromatography (LC) was applied to partially separate CPs. The developed LC-method was able to overcome drawbacks of the direct injection such as matrix interferences and co-elution of analytes with similar masses. The optimised method has been applied to technical and environmental samples and compared to direct injection.

Homologue-specific patterns were validated using a carbon skeleton method which relies on the complete hydrodechlorination of CPs to alkane/pentanes. Corresponding results will be presented. This LC method is the first chromatographic method for simultaneous analysis of SCCPs, MCCP and LCCPs since 2004 and it is the first LC method based on high-resolution MS for CP analysis applied to environmental sample.

**TU007**

**Monitoring of pesticides in air - sampling technique implications**

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Pesticides applied on agricultural fields can enter the atmospheric compartment due to volatilization. Depending on e.g. compound intrinsic properties and climatic conditions pesticides can thus be subjected to short- or long-range atmospheric transport and following application. Monitoring studies frequently demonstrate occurrence of currently used pesticides in the atmosphere, including trans-boundary transport. However, less is known on the importance of the collection procedure of air-born pesticides in order to enhance interpretation of the transport processes involved. Air samples were collected during 2010 – 2012 in a forested area in the south of Sweden, with little agricultural activities in the vicinity. Using high-volume sampling air was pumped, during 5-7 days, through a glass-fiber filter followed by polyurethane foam (PUF) and a hydrophobic crosslinked polystyrene copolymer resin (XAD), in a PUF-XAD-PUF-sandwich for simultaneous sampling of both particle bound and gas phase pesticides. The filter and the three adsorbents were Soxtec extracted separately to investigate the distribution of pesticides. The use of XAD revealed a low number of pesticides on GC-MS. Overall, a total of 43 pesticides were detected in the air samples. The most frequently detected (100% of the samples) were lindane, α-HCH, HCB, α-chlordane and γ-chlordane, i.e. pesticides banned since more than a decade within the EU. The pesticide found in the highest concentration was prosulfocarb at 13 ng m⁻³. On the filter 25 pesticides were identified, most of them currently being used in Sweden. Some of these were only detected on the filter. The first PUF contained 81% of the total pesticide concentration and out of the 33 pesticides detected 19 of them were not approved for use in EU. The XAD and the second PUF generally contained low amounts of a few substances also found in the first PUF. Of all 43 detected pesticides only dichlobenil, α-HCH, HCB and trifluralin gave a breakthrough of more than 30% from the first PUF to the XAD and second PUF. The XAD was essential for capturing a few very volatile, obsolete pesticides, but generally the first PUF was efficient enough to adsorb pesticides in gas phase at environmental relevant concentration levels. For an accurate estimate of the pesticide concentrations in air, especially for currently used pesticides, it is vital to include the particulate phase by adding a filter material in the collection procedure.

**TU008**

**Species - temporal quantification of gadolinium and iodide complex uptake in zebrafish embryo**

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Key parameters for adverse effects of chemicals to organisms are chemical uptake by and distribution within the organism leading to the “internal exposure” as pivotal factor for biological effects of chemicals. Recently the zebrafish embryo (ZFE) has been suggested as a model organism to observe potential toxicological effects of anthropogenic compounds. In order to correlate toxicological effects in the ZFE with uptake and distribution data analytical methods have to be provided. In future such correlations are required as input for toxicokinetic/toxicodynamic models which will be able to predict effects of chemicals on ZFE. Thus, predictions are an important component of risk assessment approaches. Quantification of uptake based on internal concentration analysis can be regarded as state-of-the-art. Compounds such as gadolinium and iodide complexes used as contrast agents were selected as examples to study the uptake and the distribution in the ZFE because of their increasing application. This work aims at developing analytical methods to provide internal concentration data as well as compound distribution data in ZFE by means of laser ablation hyphenated to inductively coupled plasma mass spectrometry (LA-ICP-MS). Exposure studies with ZFE and gadolinium and iodide complexes are presented. Internal concentration and compound distribution analysis was determined and the mutual relationships between determined concentrations. Only external element concentrations were quantified by ICP-MS. LA hyphenated to ICP-MS was applied to map gadolinium and iodide distribution in cross-section of ZFE. Calibration of LA-ICPMS instrumentation was done by agarose standards with known gadolinium and iodide concentration. Exposure studies with iodide compounds (iohexol, iomeprol, iopromide, iopamidol, amidotrizoic acid) revealed a considerable uptake between 10% to 30% of the environmental concentration. Uptake of the investigated gadolinium complexes (Gd-Dota, Gd-Dtba, and Gd-EOB-Dtba) is acknowledged for financial support.
was in the range between 1 and 3% of the external concentration. Qualitative spatial resolved analysis indicated elevated iodine concentrations in the ZFE cross-sections. For both, iodide and gadolinium complexes quantitative distribution data will be discussed. The presented method provides a significant progress towards quantitative data which is required for process understanding and risk assessment. Additionally the analytical methodologies presented here can be transferred to other elements which are accessible to ICP-MS detection.

TU009 Determination of short-chain and medium-chain chlorinated paraffins in fish tissues by gas chromatography coupled to high-resolution time-of-flight mass spectrometry

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Short-chain chlorinated paraffins (SCCPs) and medium-chain chlorinated paraffins (MCCPs) are alkylated alkanes with a chlorine content ranging from 1 to 10%. They constitute a complex mixture of straight and branched compounds (>10,000), used in a wide range of applications as lubricants in metal working or in the leather industry, plasticizers or flame retardants as well as in sealants, paints and coatings. Both SCCPs and MCCPs are considered to fulfill the PBT criteria (persistent, bioaccumulative and toxic chemicals) since they exhibit high bioaccumulation factors in fish as well as elevated acute toxicity towards aquatic biota. As a matter of fact, SCCPs have been proposed for listing under the Stockholm Convention on Persistent Organic Pollutants. Although Low Resolution Mass Spectrometry (LRMS) operated in Electron Capture Negative Chemical Ionization (ECNI) is frequently used for the determination of SCCPs and MCCPs, the methodological and methodological limits of the LRMS are well known. In consequence, High Resolution Mass Spectrometry (HRMS), usually performed with a magnetic sector analyzer. This technique minimizes interferences from other contaminants and between SCCPs and MCCPs; it also enables the quantification of specific homologue groups (i.e. identical chain length and number of chlorine atoms). Over the years, High Resolution Time Of Flight (ToF) and Orbitrap MS technologies have become available in numerous laboratories; these techniques, however, have not been fully explored for the determination of SCCPs and MCCPs in environmental samples. Thus, the aim of the present work was to develop a simple method for the determination of SCCPs and MCCPs in fish tissues, based on a single-step adsorption chromatography clean-up followed by analysis using gas chromatography coupled to ToF MS. The validated and optimized method was applied to the determination of SCCPs and MCCPs in the fillets of selected fishes from the Rhône River basin (South Eastern France). The target species was the Common barbel (Barbus barbus), a typical benthic feeder widely used for biomonitoring purposes in the European Union. Acknowledgements - This study was undertaken with the financial support of ONEMA, in the frame of the Investments for the future Programme (Cluster of Excellence COTE, ANR-10-LABX-45). CPER A2E (Aquitaine region and FEDER (Europe is moving in Aquitaine with the European Regional Development Fund (FEDER)) is acknowledged for financial support.

TU010 Performance of polystyrene passive samplers to assess DDX bioaccumulation in freshwater mussels

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Contaminants in the aquatic environment are present as dissolved, colloidal-bound, and sediment-associated forms. The distribution of contaminants between these phases is a central issue as it strongly influences the sediment and water quality. In the past, sediment quality criteria were set using a simple equilibrium partitioning (EqP) approach seeking to relate dissolved and sorbed contaminants. However, this approach does not account for different aspects such as colloid speciation and specific sorption effects, that may dramatically change environmental impacts. However, until now, there has been no new method that can allow us to account for these issues when defining the quality of a sediment. Among these, the use of passive sampling is becoming an attractive option, as this method allows direct estimation of the pore water concentration of a chemical, i.e. a metric that reveals the bio-available fraction. In this work, we used passive sampling to assess the bioavailable forms of DDX in sediments collected from a lake located in northern Italy; the results were compared with those obtained by exposing the freshwater mussels, Unio pictorum, to the same sediments. Low density polystyrene devices (PEDs) and organisms were exposed in glass jars containing the sediment collected from different sampling stations of the lake for either 10 or 28 days. At the end of each test, the PEDs and mussels were analyzed for target concentrations. Only the concentrations in the PEDs were used to estimate the tissue concentrations in the corresponding exposed mussels. The results showed that the PED-deduced values were consistent with the measured levels for the 28-days tests, whereas the tissue concentrations predicted from passive samplers data were in some cases higher than the ones measured in the organism tissue after 10 days of exposure. This suggests that approach to equilibration of the sediment with a PED was faster than uptake by the benthic organism. These results show that PE samplers data may be quite effectively used to predict the concentration of a contaminant expected in the tissue of freshwater mussels, such as U. pictorum, thus allowing investigators to predict the bioavailable fraction of organic contaminants in an easier and reliable way.

TU011 Perfluoroalkyl substances in aquatic environment - comparison of fish and passive sampling approaches

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The concentrations of seven perfluoroalkyl substances (PFASs) were investigated in 36 European chub (Squalius cephalus) individuals from six localities in the Czech Republic. Chub was selected as working or in the leather industry, plasticizers or flame retardants as well as in sealants, paints and coatings. Both SCCPs and MCCPs are considered to fulfill the PBT criteria (persistent, bioaccumulative and toxic chemicals) since they exhibit high bioaccumulation factors in fish as well as elevated acute toxicity towards aquatic biota. As a matter of fact, SCCPs have been proposed for listing under the Stockholm Convention on Persistent Organic Pollutants. Although Low Resolution Mass Spectrometry (LRMS) operated in Electron Capture Negative Chemical Ionization (ECNI) is frequently used for the determination of SCCPs and MCCPs, the methodological and methodological limits of the LRMS are well known. In consequence, High Resolution Mass Spectrometry (HRMS), usually performed with a magnetic sector analyzer. This technique minimizes interferences from other contaminants and between SCCPs and MCCPs; it also enables the quantification of specific homologue groups (i.e. identical chain length and number of chlorine atoms). Over the years, High Resolution Time Of Flight (ToF) and Orbitrap MS technologies have become available in numerous laboratories; these techniques, however, have not been fully explored for the determination of SCCPs and MCCPs in environmental samples. Thus, the aim of the present work was to develop a simple method for the determination of SCCPs and MCCPs in fish tissues, based on a single-step adsorption chromatography clean-up followed by analysis using gas chromatography coupled to ToF MS. The validated and optimized method was applied to the determination of SCCPs and MCCPs in the fillets of selected fishes from the Rhône River basin (South Eastern France). The target species was the Common barbel (Barbus barbus), a typical benthic feeder widely used for biomonitoring purposes in the European Union. Acknowledgements - This study was undertaken with the financial support of ONEMA, in the frame of the Investments for the future Programme (Cluster of Excellence COTE, ANR-10-LABX-45). CPER A2E (Aquitaine region and FEDER (Europe is moving in Aquitaine with the European Regional Development Fund (FEDER)) is acknowledged for financial support.

Biological effects of emerging micro pollutants at realistic environmental concentrations (P)


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The concentrations of seven perfluoroalkyl substances (PFASs) were investigated in 36 European chub (Squalius cephalus) individuals from six localities in the Czech Republic. Chub was selected as working or in the leather industry, plasticizers or flame retardants as well as in sealants, paints and coatings. Both SCCPs and MCCPs are considered to fulfill the PBT criteria (persistent, bioaccumulative and toxic chemicals) since they exhibit high bioaccumulation factors in fish as well as elevated acute toxicity towards aquatic biota. As a matter of fact, SCCPs have been proposed for listing under the Stockholm Convention on Persistent Organic Pollutants. Although Low Resolution Mass Spectrometry (LRMS) operated in Electron Capture Negative Chemical Ionization (ECNI) is frequently used for the determination of SCCPs and MCCPs, the methodological and methodological limits of the LRMS are well known. In consequence, High Resolution Mass Spectrometry (HRMS), usually performed with a magnetic sector analyzer. This technique minimizes interferences from other contaminants and between SCCPs and MCCPs; it also enables the quantification of specific homologue groups (i.e. identical chain length and number of chlorine atoms). Over the years, High Resolution Time Of Flight (ToF) and Orbitrap MS technologies have become available in numerous laboratories; these techniques, however, have not been fully explored for the determination of SCCPs and MCCPs in environmental samples. Thus, the aim of the present work was to develop a simple method for the determination of SCCPs and MCCPs in fish tissues, based on a single-step adsorption chromatography clean-up followed by analysis using gas chromatography coupled to ToF MS. The validated and optimized method was applied to the determination of SCCPs and MCCPs in the fillets of selected fishes from the Rhône River basin (South Eastern France). The target species was the Common barbel (Barbus barbus), a typical benthic feeder widely used for biomonitoring purposes in the European Union. Acknowledgements - This study was undertaken with the financial support of ONEMA, in the frame of the Investments for the future Programme (Cluster of Excellence COTE, ANR-10-LABX-45). CPER A2E (Aquitaine region and FEDER (Europe is moving in Aquitaine with the European Regional Development Fund (FEDER)) is acknowledged for financial support.

Biological effects of emerging micro pollutants at realistic environmental concentrations (P)
of 1990 - 2015, Web of Science was used as the main database. The focus was set on selected micropollutants (poly cyclic aromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB), and bisphenol A (BPA)) and environmental media (water, waste water, and sea water). The results of the study would provide an information on the current status of the micropollutant species largely found in industrial, agricultural, natural or everyday life, which would supply input for both national and international obligations, and would provide contribution to the development of our country-specific quality standards on many micropollutants in the near future. The authors would like to acknowledge the financial support from TUBITAK (Project No: 115Y309).

TU013
Ecotoxicological monitoring of systems II inhibitors in 5 small streams in Switzerland
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Small streams represent a major part of the Swiss river network, but so far information about pesticide pollution and pollution dynamics is limited. To investigate the situation in such small rivers, a special monitoring program (SPEZ) was conducted on five streams as part of the National Surface Water Quality Monitoring Network (NAWA) in spring and summer 2015. The aim of this project was to obtain knowledge about peak and baseline pesticide concentrations of over 250 different pesticides as well as their ecotoxicological effects. Selected sampling sites were located in areas intensively used for agriculture with different types of crops (grain, vegetables, vineyards and orchards). 12 composite water samples were taken continuously from April to August 2015 using automated sampling devices. Samples were extracted using solid phase extraction and subsequently examined for 250 pesticides and metabolites using HPLC-HRMS. To complement the chemical analysis with ecotoxicological effect data, one week composite samples were analyzed for effects on algal photosynthesis and growth using the combined alga assay. This assay is especially sensitive for systems II-inhibiting substances. Toxic effects were expressed as diuron equivalent concentrations (DEQ) for photosystem II inhibition and as baseline toxic equivalent concentrations (baseline-TEQ) for growth inhibition. Photosynthesis inhibition was observed in 100% of the water samples, but at different levels. Calculated DEQs ranged between ca. 5 and 300 ng/L. Highest DEQs were found whilst DEQs remained elevated. Consequently herbicides were introduced in samples and their fortifications obtained by adding a mixture of methylaralen (9 µg/L), PFOS (60 µg/L) and fluoxetine (400 µg/L). Three different exposure times were used: 10-day tests (modified OECD 210 method), 4-day tests (modified ASTM test, 2004) and 21-day tests (nonstandard method) for Oryzias latipes, Xenopus laevis and Physa acuta, respectively. Capability of methods used to predict ecotoxic and risk assessment, speciation of electrophilic correlations between embryotoxicity and analytical measures are discussed. This work has been funded by the Spanish Ministry of Economy and Competitiveness through CTM2013-44966-R and CTM2014-52888-R projects.

TU015
Effects of the cytotoxic drug cyclophosphamide exposure in the polychaete Neris diversicolor
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Cyclophosphamide (CP) is a worldwide applied cytotoxic drug used in cancer therapy and even less to sediment quality assessment. Hence, there is a lack of knowledge about the bioavailability and possible adverse effects over the benthic biota of coastal waters regarding chronic exposure to low levels of such cytotoxic drugs. Cyclophosphamide (CP) is a worldwide applied cytotoxic drug and one of the most potently alkylating activity in the DNA, able to interact to double strands of prokaryotes and eukaryotes. In this sense, the present study applies a multimbiomarker approach including behavior impairments (burrowing kinetic), biochemical biomarkers and genotoxicity, assessed by the Comet assay, in the sediment-dwelling polychaete Neris diversicolor exposed to the alkylating agent CP in water-body systems, over 14 days. A range of concentrations that cover environmental relevant levels and worst-case scenarios were used (10 ng L⁻¹; 100 ng L⁻¹; 500 ng L⁻¹; 1000 ng L⁻¹). Results showed impairments of burrowing at the lowest concentration of the drug (i.e. 10 ng L⁻¹) and at 500 ng L⁻¹. Antioxidant enzymes activity and DNA damage were altered at higher concentrations.

TU016
Genotoxicity assessment of environmental contamination of veterinary antibiotics in fish (Prochilodus lineatus) using the micromucous test and others nuclear abnormalities
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Veterinary antibiotics are one of the most used pharmaceuticals class in Brazilian fish farming for the treatment of bacterial diseases, and because of their intensive use, contamination of the aquatic environment may occur with effects to the native biota and to the fish farming industry. The goal of this study was to investigate the genotoxic effects of environmental concentration of oxytetracycline and florfenicol on juveniles of native fish from Brazil (Prochilodus lineatus) through the occurrence of micromucous (MN) and other nuclear abnormalities (NA) after 96 hours exposure. Environmental concentration of oxytetracycline (4000 ng L⁻¹) and florfenicol (425 ng L⁻¹) were determined in surface water of a fish farm located at Ilha Solteira Reserve (Santa Fé do Sul, São Paulo, Brazil) through Online-Solid-Phase Extraction coupled to Liquid Chromatography-Tandem Mass Spectrometry (Online-SPE-LC/MS-MS) in a previous study of our laboratory and exposed along with its double (16000 ng L⁻¹ and 125.25 ng L⁻¹) and its half (400 ng L⁻¹ and 125 ng L⁻¹) on P. lineatus during 96 hours in a static system. Although MN was observed after exposure to the concentrations 4000 and 8000 ng L⁻¹ of oxytetracycline no statistical differences were observed when compared to the negative control (p< 0.05). No MN was observed after exposure to florfenicol concentrations. Four kinds of others NA were observed after exposure to oxytetracycline, which are: notched nuclei, lobed nuclei, and bisected nuclei and five kinds after exposure to florfenicol with: notched, blebbed, lobed, broken eggs and binuclei, but in both cases no statistical differences were observed as well as for the sum of abnormalities when compared with the negative control (p< 0.05). Environmental concentrations of oxytetracycline and florfenicol are not genotoxic to P. lineatus according to the MN test.
Effects of exposure to carbasazone and ibuprofen on the mussel Mytilus trossulus from the Baltic Sea

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Pharmaceuticals in aquatic ecosystems are an emerging issue in environmental research and there is a lack of knowledge in literature on their possible effects on non-target organisms. In order to examine biological effects induced by the common drugs carbasazone and ibuprofen in the mussel Mytilus trossulus from the Baltic Sea, a semi-static laboratory exposure experiment was conducted. Consistent with available data on the residual concentrations of the compounds recorded in the Baltic Sea the mussels were exposed for 7 days to nominal concentrations of 4.0 mg L^{-1} and 2.0 mg L^{-1} of carbasazone and 8.0 mg L^{-1} and 4.0 mg L^{-1} of ibuprofen, and to their respective low and high concentration mixtures. Measured concentrations of the test compounds in water samples taken prior to each renewal of the test media at 48 hrs intervals were ca. 70-85% of the nominal ones. As consolidated biomarkers of oxidative stress the enzymatic activities of catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) as well as lipid peroxidation (LPO) were measured from the digestive gland tissue. The phase II detoxifying enzyme glutathione transferase (GST) was determined from the same tissue while the activity of acetylcysteinesterase (AChE), an indicator of neurotoxic effects, was measured in the gill tissue. Apparent effects on the oxidative defence system with marked changes in CAT, GR and SOD activities could be recorded for both the higher concentrations of carbasazone and the mixture whereas no significant effects could be shown for ibuprofen. At the lower concentration range the mixture seemed to be more harmful than the individually introduced compounds, suggesting a possible additive or synergistic effect. No significant changes in the AChE activity and LPO could be detected.

Detoxification of azoxystrobin via biotransformation in Gammarus pulex is influenced by co-occurring substances

A. Rösch, J. Hollender, Eawag / Environmental Chemistry Xenobiotics, such as pesticides and pharmaceuticals are distributed ubiquitously in the aquatic environment and their co-occurrence is often reported. Combined effects may arise that can influence the detoxification of substances via biotransformation and as a result toxic parent substances accumulate in the organism. In the present study the strobilurin fungicide azoxystrobin (AZ) was chosen to study the influence of co-occurring substances on its biotransformation in the aquatic invertebrate Gammarus pulex (G. pulex). AZ exhibits a generally high toxicity towards aquatic organisms by inhibiting mitochondrial respiration. The effect of an enzyme inductor and an inducer on the oxidative drug metabolism was investigated using two binary mixtures. The mixtures were composed of AZ and prochloraz as well as of AZ and carbamazepine. The imidazole fungicide prochloraz is known to be a strong inhibitor of cytochrome P450 (CYP450) whereas the antiepileptic carbamazepine acts as inducer of CYP450. For the identification of biotransformation products (BTPs) and to investigate temporal trends G. pulex were exposed solely to AZ (40 µg L^{-1}) & 80 µg L^{-1}) and to the two mixtures (concentration of inducer and inhibitor: similar molar concentrations: 1:1) AZ and prochloraz (AZ:PC) and AZ and carbamazepine (AZ:CZ). By using high-resolution liquid chromatography tandem mass spectrometry combined with suspect and non-target screening approaches 17 BTPs of AZ were identified. The major biotransformation pathway was an ester hydrolysis to its acid combined with a hydrogenation. Further reactions detected included hydroxylations and diverse conjugation reactions with glucose, sulfate or the combination of both as well as with glutathione resulting in cysteine products. Temporal trends of the internal concentrations of the substrate AZ and its BTPs were compared between the sole exposure to AZ and the mixtures. Carbamazepine did not show any inducing effect in G. pulex i.e. similar internal concentrations for AZ and its BTPs were observed in the substrate control and in the mixture. For the mixture, where prochloraz was used as inhibitor, a strong inhibitory effect was observed. Maximum internal AZ concentrations were about twice as high in the mixture compared to the substrate control as a result of biotransformation inhibition also reflected in the estimated biotransformation rate constants. As a consequently, mortality of G. pulex increased in the mixture compared to the single exposure to AZ, indicating raised toxicity.

Dietary exposure to PVP/PEI coated Ag nanoparticles in adult mussels causes abnormal embryo development in offspring

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In the last years, silver nanoparticles (Ag NPs) have gained high commercial interest basically due to their antimicrobial properties. Applications for Ag NPs are increasing and thus, concerns about their potential input into aquatic ecosystems and their environmental hazards are also growing. Toxicity of Ag NPs to different freshwater and seawater organisms is being widely studied; however, there is a gap of knowledge on the potential toxic effects of Ag NPs ingested through the food web, especially at environmentally relevant concentrations. Further, most investigations are focused on understanding the effects of Ag NPs on exposed organisms, but potential effects on offspring have not been explored yet. The aim of this work is therefore to study the effects of Ag NPs (PVP/PEI coated Ag NPs on mussel immune status, reproductive success and offspring embryo development. Mussels Mytilus galloprovincialis were fed daily with microalgae hochastris galbana previously exposed for 24 hours to two different doses of PVP/PEI coated 5 nm Ag NPs: an environmentally relevant dose of 1 µg L^{-1} of Ag NPs and a high dose of 10 µg L^{-1} Ag NPs. After 21 days of exposure Ag bioaccumulation was determined in different tissues and immunotoxicity was assessed in hemocytes. In addition, mussels were induced to spawn and sperm motility, spawning success, fertilization success and development of abnormal larvae were checked. Mussels fed with microalgae exposed to both doses of Ag NPs showed significantly lower percentages of transformation of incorporated Ag NPs from microalgae to mussels. Phagocytic activity of hemocytes was slightly reduced, contrary to the immunostimulatory effect reported earlier for Ag NPs and other NPs. No significant differences were observed for sperm motility, number of spawned eggs and fertilization success. However, a significantly higher percentage of abnormal embryos were observed after parental exposure to both doses, even for the dose close to environmentally relevant concentrations. Future studies are needed to address mechanisms and consequences of NP exposure for the early life stages of mussels and other organisms. Funded by: Spanish Ministry of Economy and Competitiveness (NanoSilverOnics MAT2012-39372), Basque Government (SAOTEK S-PEZ14/12 and Concerted Group CT1810-13), UPV/EHU (UF11/37 and PhD fellowship to N.D.) and French Ministry of Higher Education and Research (PhD fellowship to M.M.)

Including different biological and space complexity scales in hazard assessment: a case study with diclofenac sodium PNEC derivation

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Westfalia (Germany) more than 90% of the freshwater ecosystems failed reaching the good ecological status, a target set by the EU’s Water Framework Directive (WFD). Many micropollutants like pharmaceuticals, contrast agents, industrial chemicals, personal care products and some pesticides and biocides enter the aquatic environment by wastewater discharge, because these micropollutants are not or only partially treated and has become a matter of scientific and public concern. Hence, wastewater is considered as one of the major sources of micropollutants in the aquatic environment. Bioassays conducted in the lab with representative species help to investigate the potential effects of complex mixtures of micropollutants on single species. In fact short-term effects of wastewater treatment effluents on different test organisms could be demonstrated by a set of bioassays in several research projects. Additionally, some bioassays conducted as by-pass test systems showed chronic effects of micropollutants on single species, too. Furthermore, monitoring results of freshwater communities in receiving water bodies before and after improvement of a wastewater treatment plant indicated that the same quality of wastewater treatment results in different ecological effects in the communities. However, monitoring freshwater communities always includes many other influencing factors (e.g. temperature, velocity, habitat structure) which makes it impossible to analyse the effects of micropollutants alone. Therefore, it is still unclear how strong the multitude of micropollutants discharged by wastewater treatment plants will actually affect freshwater communities. We will present a conceptual approach of artificial stream mesocosms allowing to evaluate the impact of micropollutants alone without other influencing factors present in natural flowing water bodies. This approach allows us to investigate whether advanced wastewater treatment technologies like ozonation and activated carbon filtration will potentially improve the ecological status of macroinvertebrate communities.

**TU022**

**Biological effects of anthropogenic pollutants present in pond receiving treated municipal sewage water**

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In this study, we aim to assess the biological effects of mixture of emerging pollutants, present in recipients of treated sewage water in common carp (Cyprinus carpio). Four samplings (after 0, 30, 90, 180 days) have been done in April-November, 2015. Cezarka pond, a biological pond which receives water from sewage treatment plant in Vodnany, Czech Republic (approx. 70000 inhabitants) has been studied. Anthropogenic pollutants such as persistent organic pollutants, pharmaceuticals and personal care products have been detected and identified in water and sediments. Fish were exposed in real condition at size of 17.05 ± 1.25 cm total length and 65.80 ± 15.12 g total weight. The effect of complex pollution on fish was identified. The oxidative stress and antioxidant enzymes activities were measured in liver, gill, muscle, and intestine fish tissues. At 180 days of experiment the weight of exposed fish was remarkably higher when compared to control group. Nutrient load in biological pond resulted in high pollutants, present in recipients of treated sewage water in common carp. 

**TU023**

**Effects of environmental realistic concentrations of carbamazepine in a freshwater model ecosystem**

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Pharmaceuticals are widely used in human and veterinary health care products. Their occurrence in the aquatic environment is nowadays a well established issue and has become a matter of both scientific and public concern. Hence, wastewater is considered as one of the major sources of micropollutants in the aquatic environment. Bioassays conducted in the lab with representative species help to investigate the potential effects of complex mixtures of micropollutants on single species. In fact short-term effects of wastewater treatment effluents on different test organisms could be demonstrated by a set of bioassays in several research projects. Additionally, some bioassays conducted as by-pass test systems showed chronic effects of micropollutants on single species, too. Furthermore, monitoring results of freshwater communities in receiving water bodies before and after improvement of a wastewater treatment plant indicated that the same quality of wastewater treatment results in different ecological effects in the communities. However, monitoring freshwater communities always includes many other influencing factors (e.g. temperature, velocity, habitat structure) which makes it impossible to analyse the effects of micropollutants alone. Therefore, it is still unclear how strong the multitude of micropollutants discharged by wastewater treatment plants will actually affect freshwater communities. We will present a conceptual approach of artificial stream mesocosms allowing to evaluate the impact of micropollutants alone without other influencing factors present in natural flowing water bodies. This approach allows us to investigate whether advanced wastewater treatment technologies like ozonation and activated carbon filtration will potentially improve the ecological status of macroinvertebrate communities.

**TU024**

**In silico models for nanoparticles: are we ready for regulatory application?**

E. Paps, Department of Theoretical and Applied Sciences; P. Gramatica, University of Insubria / DiSTA

The use of alternatives to animal testing such as in silico models based on Quantitative Structure-Activity Relationships, grouping and read across methods to reduce experimental costs and speed up risk assessment procedures, is a main innovation introduced officially by the REACH regulation in 2007. However, the need for identification of harmonized criteria for the evaluation of the reliability and applicability of QSAR models represented a real priority since the white paper on REACH, published in 2001. The approval and publication in 2004 of the OECD Principles for the regulatory use of QSAR, and the following OECD (2007) and ECHA guidelines (2008), represented milestones in the process of application of in silico strategies for the modelling and the prediction of properties and activities for traditional chemicals. These documents are based on experiences gained during decades of development, validation and application of QSARs to thousands of organic substances. Currently, the large development of nanotechnologies and the uncertainties related to possible effects and risks posed by these new materials to humans and the environment, have increased the interest of scientist and regulators in computational nanotoxicology, which is expected to provide key elements in the determination of missing data by in silico predictions. However, traditional schemes for models development and validation such as those described in the aforementioned OECD Principles, find difficult application to nanomaterials due to lacking input data, consequent limitations in the quality and dimension of the modelled datasets, and to a variety of factors, which strongly influence the behavior of nanomaterials. In this paper the analysis and present different issues related to the development and validation of computational Qsar-type models generated for nanoparticles. The analysis is performed on real datasets and models, following the OECD Principles for the regulatory use of QSARs and highlighting criticalities associated to the application of these five principles. The aim of this poster is to generate discussion and reflection on a sensitive topic such as the possible regulatory validity and use of predictions generated by in silico models developed for nanoparticles.
needed to leverage existing information in risk assessments. These read-across frameworks (or approaches), which allow users to address gaps in data-poor substances with information from other nanomaterials, have become a priority for most jurisdictions. This need is driven by a combination of: short timelines to make regulatory decisions, conducting assessments which span different physical forms like nanoparticles, and the move towards reducing animal testing. However, many of the published read-across frameworks for nanomaterials are underpinned by principles of conventional chemicals. As such, these frameworks cannot be used for nanomaterials without first identifying the specific nano-properties of interest and second by conducting case studies to validate these approaches for nanomaterials. This presentation will provide an update on Canada’s domestic regulatory program and activities under the Organization for Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterials (WPfNM) on the use of read-across in support of regulatory decision making. The intention of this presentation is to inform on the ongoing need to address association frameworks for nanomaterials highlighting gaps which need to be addressed to improve the uptake of these approaches for regulatory decision making in the future.

TU026

Role of laboratory results the fate and effect of nanomaterials in the environment and represent appropriate data for grouping? K. Schlich, M. Kraas, Fraunhofer IME Institute for Molecular Biology and Applied Ecology; K. Hund-Rinke, Fraunhofer IME The grouping of nanomaterials (NM) is based primarily on standardized, ecotoxicological laboratory experiments. However, whether data of laboratory experiments are suitable for data from field studies is still a matter of debate. The main goal of this study was to show if the data on fate and effects of a silver NM (AgNM; NM: 300K) and a CeO2 NM (CeO2; NM: 212) from the OECD Sponsorship Programme in field lysimeters is comparable with laboratory results. Experiments with AgNM started in May 2014 and with CeO2NM in May 2015. Both will end in 2017. Eight field lysimeters (0.9 m x 0.9 m x 0.9 m) are used and various crops are planted. The NM were applied on the field lysimeters via sewage sludge (Ag, CeO2) and simulated rain (CeO2). For the application of NM via sewage sludge, sludge was removed from a municipal sewage treatment plant and mixed for 24 h with the NM. The sludge was dewatered and introduced into soil in accordance with the German Sewage Sludge Ordinance (5t per ha in 3 years, applied at once). For the application via simulated rain periodically CeO2 NM dispersion was prepared over four weeks, which was applied in 2.5 liters of deionized water on the soil surface. The top 20 cm of soil were circulated to simulate the agricultural use of a plow. After the NM had been incorporated into the soil, wheat was sown at first and subsequently canola and barley. For the AgNM lysimeter, via sludge concentrations of 1.8 and 70 mg/kg dry soil were measured. On the CeO2 lysimeters with the sludge application concentrations of 9 and 40 mg per kg soil were reached and as applied simulated rain 9 mg/kg soil were measured. Directly after the application and at the end of each growing period, soil samples were taken and the influence of the NM on the soil microflora was determined using the potential ammonium oxidation (ISO 15685:2012). In addition the influence on the growth of crops and the fate of the NM in soil and the plants was determined. The first results show that AgNM remain in the top 20 cm of soil and are not present in low concentrations in the roots of wheat. The soil microflora is inhibited by the AgNM. There is a good comparability of the laboratory and the field experiments during the first 140 days. First results regarding the toxicity show, that the CeO2 NM did not affect the soil microflora at test start (lab and field tests).

TU027

Grouping of nanomaterial and hazard assessment: requirements for additional criteria to assess the quality of studies L. geooffy, S. ANDRES, INERIS / Toxicological Ecotoxicological Assessment of chemical Substances

In in silico approaches, scientists can predict the effects of nanomaterials that are in the same category. When possible, grouping is done to simplify analysis, decisions, and to help to select relevant assays. Fate categories could possibly be used to dictate the need for hazard tests or hazard profile to dictate environmental behavior. Nevertheless, to determine hazard in a regulatory framework, assays have to be conducted under defined guidelines. These tests are assessed under a score system (i.e. the Klimisch score) to determine if information quoted are sufficient and incertitude acceptable. With this respect, as work is currently done to improve and adapt existing guidelines for nanomaterials, the need for additional quality criteria is emerging. In particular, nanomaterial identification is crucial in hazard assessment and grouping. A list of 10 parameters considered as necessary to identify nanomaterials. The aim of this study is a tentative to identify parameters needed for nanomaterials to validate a study, in order to improve the score system with adaptation for nanomaterials. Data are collected from literature for different nanomaterial tested (TiO2 and Ce for example) and analyzed. For each study, parameters assigned and results of test toxicity are reported. Parameters identified as potential are used to estimate the quality of the study. Based on these results, a prioritization of additional parameter to be included when assessing the quality of a study using nanomaterials is proposed.

TU028

Ecotoxicological impacts of engineered nanoparticles in aquatic model systems M. Lukas, Federal Environment Agency (UBA) / Ecotoxicological Laboratory; C. Polleichter, German Environment Agency (UBA) / Ecotoxicological Laboratory; C. Kussatz, Federal Environmental Agency UBA / Ecotoxicological Laboratory The ecotoxicological effects of engineered nanoparticles (ENPs) are mostly unknown. However, their industrial use is continuously rising. Particularly, studies on prevalent and commercial relevant ENPs with innovative functionalized variations (e.g., coating or doping) are lacking. Therefore, the German Federal Ministry of Education and Research (BMBF) has launched the programme “NanoNature” in 2008. This project is supported by 24 partners and the initiative projects aiming to understand the impacts of ENPs on the environment in general and on organisms in particular. As a part of NanoNature “DENANA (“Design criteria for sustainable nanomaterials”) is a joint research project of 10 academic, industrial and regulatory partners that currently tries to conflate the conflict of immediate marketing of innovative ENP-products and the precautionary principle regarding the potential environmental risks. Within this framework the Ecotoxicological Laboratory of the German Federal Environment Agency (UBA) is investigating the biological effects of differently functionalized ENPs (silicon dioxide, cerium dioxide and silver nanoparticles) on various aquatic organisms. Main objective is to provide a solid long-term data set on ENPs in aquatic ecosystems which can be used for hazard estimation and risk assessment in chemicals policy. Therefore, ecotoxicological tests with several species representing different trophic levels in aquatic ecosystems, ranging from green algae (Desmodesmus subspicatus) and higher macrophytes (Lemma minor) to invertebrates (Daphnia magna) and vertebrates (Danio rerio) started. Moreover, test designs were proposed for multi-generational studies with daphnids were developed to estimate the long-term impact of ENPs. Additionally, several analytical methods such as dynamic light scattering (DLS) to describe the particle size distribution of ENPs, the determination of zeta potentials and chemical analysis of ENPs in aqueous solutions began. Results will show if the currently used classification of the investigated ENPs as less toxic (e.g., SiO2 nanoparticles) holds also for functionalized variations and will offer criteria for designing sustainable nanomaterials. Furthermore, experiments will add up of long-term data on the environmental exposure which provides a basis for regulatory concerns.

TU029

SOS-Nano: the Structure-Oxidative Stress relationship of metal oxide nanoparticles in the aquatic environment S. Novena, University of Exeter / Biosciences College of Life and Environmental Sciences; T. S. Galloway, University of Exeter / Biosciences Department The Marie Skłodowska-Curie Action funded SOS-Nano project addresses one of the pressing issues of ecotoxicology: to investigate how the structural properties of nanoparticles (NPs) can be used to predict their potential toxicity in aquatic environments, the final sink of released NPs. The project is using the oyster (Crassostrea gigas) as a model organism to investigate the toxicity of a range of metal oxide (MOx) NPs exhibiting different physico-chemical properties. The experimental design has been designed to highlight the relationships between the physico-chemical properties of the model NPs (MnO2, CeO2 and ZnO) and the toxic activity under the influence of natural water properties, and to rank their toxic potential through a multi-tier system combining genomics and functional measurements. The NOx NPs exposure settlement mimics different conditions of salinity and natural organic matter concentration, expected to influence NPs aggregation-stability-reactivity in marine environments; the characterization of the behavior of model-NPs in the exposure settlements is complemented by the assessment of bandgap energies, dissolution and NP biological fate within target-organisms. The induction of oxidative stress in the oyster (Crassostrea gigas) is being examined in accordance with the Hierarchy of Oxidative Stress Paradigm, which describes the progressive induction of cellular responses classified as antioxidant defence, pro-inflammatory effects and cytotoxicity. The toxic responses are being ranked through a multi-tier system combining genomics and functional measurements. The poster will present preliminary data on the physico-chemical properties of our chosen model NPs under different exposure scenarios and the extent to which they induce oxidative stress in the test organisms. This project has received funding from the European Union’s Horizon 2020 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 655134.

TU030

Categorisation approaches and grouping for read-across of manufactured nanomaterials L. Lamon, Euro Mediterranean Centre for Climate Change / Institute for Health and Consumer Protection BICP; D. Asturiol, K. Aschberger, European Commission Joint Research Centre / Institute for Health and Consumer Protection BICP; A. Worth, Joint Research Centre / Institute for Health and
The Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH, EC No 1907/2006) is safeguarding the safety of manufactured nanomaterials (NMs) while at the same time encouraging the reduction of in vivo tests promoting in vitro and in silico approaches. Annex XI of the regulation prioritises testing in vivo when testing is not considered scientifically necessary and categories can be properly defined in terms of similarity. The need to apply grouping approaches to NMs testing has been highlighted by several guidance documents from regulatory bodies given that assessing risks posed by all NMs on a case-by-case basis is challenging in terms of financial resources, ethical considerations and time demand. However, a reference guidance framework for MN read-across is not available yet. The ECHA Group Assessing Already Registered Nanomaterials (GAARN) has identified key issues and concepts to be explored in NMs grouping and read-across, including the need to consider properties beyond chemical composition (e.g., particle size, shape, solubility, etc.), the identification of similarity rules in support of the application of REACH Annex XI to NMs, the relevance of toxicokinetic studies in grouping and read-across and in extrapolation from in vitro to in vivo tests. We have analysed the published literature on categorisation schemes and read-across for NMs. The proposed categorisation approaches cover a variety of assessment goals, including priority setting of NMs for further evaluation (including ranking based on level of concern) and guiding the choice of relevant endpoints and methods. In this paper we report what is available in the literature and identify research gaps, with a special attention to the provisions of REACH Annex XI in order to support further activities in this area.

**TU031**

**Agglomeration behaviour of nanomaterials in environmentally relevant aquatic media - towards a nano-specific OECD test guideline**

P.A. Kozin, Vienna University / Environmental Geosciences; F. Von der Kempow, Vienna University / Department of Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences

Agglomeration behaviour is an important parameter affecting the environmental behaviour of nanomaterials. It depends on physicochemical characteristics of the dispersion media and agglomerating nanomaterial, its concentration and concentration of other substances in the dispersion. This study suggests an experimental method (proposed as an OECD test guideline) and provides international regulatory guidance for testing nanomaterial agglomeration behaviour in environmentally relevant aquatic media. Proposed method determines nanomaterial agglomeration behaviour by analysing remaining particle concentration in the top 0.5 cm volume of the dispersion over a 6 hour time period (0-6 hours). For a fully stable dispersion, this concentration did not change over time, while agglomeration and settling of nanomaterial gradually reduced the concentration of particles in this volume of the sample. TiO₂ (NM105) nanomaterial was chosen to study agglomeration behaviour in dispersions containing 0, 1, and 10 mM Ca(NO₃)₂ electrolyte and dissolved NOM (SRNOM, 30ppm). Agglomeration behaviour was studied in the presence and absence of CO₂ and at natural and established (5, 7, 8.5) pH. Results showed that dispersions of TiO₂ (NM105) were unstable proportionally to the amount of added electrolyte. Addition of NOM had a potential stabilizing effect on the system containing 1 mM Ca(NO₃)₂. Absence of CO₂ in the atmosphere above the analysed TiO₂ (NM105) dispersion or electrolyte-DOM containing dispersions. Dispersions of TiO₂ (NM105) were unstable at pH values 5 and 7, closest to the PZC of TiO₂ (6.5). Dispersions at pH=8.5 revealed stability compared to that of the systems analysed at natural pH. The decision tree, developed as a result of this study, provides the experimental routine to test the agglomeration behaviour of nanomaterials in aquatic media. Decision tree is designed to address the issue of nanomaterial agglomeration behaviour in such way that would require the minimal time and cost spending, through the combination of “dispersibility” and “dispersion stability” test approaches. Thus proposed experimental method allows not only observation of nanomaterial agglomeration behaviour, but also understanding of reasons and factors influencing this process.

**TU032**

**Grouping of nanomaterials regarding aquatic ecotoxicity - hypotheses for selected NMs as proof**

K. Hund-Rinke, Fraunhofer IME; T.A. Kühbusch, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality Sustainable Nanotechnology; D. Kühnel, Helmholz-Centre for Environmental Research / Biomedical Ecotoxicology; C. Nickel, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality Sustainable Nanotechnology

Due to the large variability of nanomaterials (NMs) the testing of every modification would require a tremendous amount of work and costs. Therefore, grouping of NMs is intended, limiting the number of comprehensively tested NM to a few considered as representative for a group. The NMs of each group have comparable characteristics with respect to the intended protection objective (e.g. ecotoxicity). Aims of this ongoing project are first the correlation of data of physico-chemical (PC) properties of selected NMs (Ag, TiO₂, ZnO, CeO₂) with ecotoxicological data and second the identification of suitable parameters as basis for grouping. Based on a literature review, for each of the selected NM-types parameters were identified which are expected to be relevant for ecotoxicological effects. For every NM-type 2 – 4 uncoated sub-NMs were selected (in total 12 NMs). The selected NMs were tested with algae (OECD 201), daphnids (OECD 202) and fish embryos (OECD 236), considering the modifications proposed for the testing of NMs. The NMs were characterized in Milli-Q-water and in the respective test media (OECD medium for algae, ADAM for daphnids, ISO water for fish embryos). Parameters such as surface size, crystalline structure, morphology, primary size and distribution in dispersion, zeta-potential, dissolution, reactivity (CPI, ROS) were addressed. NM characteristics in water can differ significantly from those determined in test media. For example, for the spherical Ag NM (Æ 15 nm) the ion concentration after 72 h was in the range of 437 – 490 mg/L in water and the various test species. For a silver rod with a length of 3797 nm, the dissolution was differed between 230 mg/L in water and 0.3 – 1.3 mg/L in the test media. The dissolution of a second silver rod (length: 2423 nm) in water and the test media differed by factor 10 (0.101 mg/L and 0.012 – 0.038 mg/L). Therefore, for a correlation of PC parameters with ecotox data only values determined in the test media should be used. For Ag, dissolution was expected to be the most relevant parameter. However, correlation between EC50 values and dissolution is weak. Differences in toxicity of the NMs were significantly lower than expected based on the dissolution. Daphnids proved to be the most sensitive test organisms although dissolution in the test medium ranked between the dissolution in the algae and fish medium. Additional PC-parameters which still have to be defined have to be considered for grouping.

**TU033**

**A systematic investigation of the ENMs toxicity to freshwater organism: the search of the key physicochemical parameter**

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Engineered nanomaterials (ENMs) are applied in more and more different products and areas. Therefore, concerns regarding the potential human and environmental impacts of ENMs have risen. However so far it is unclear, how the novel properties of ENM result in an increase of their toxicological potential compared to classical (microscale) substances. Though the increasing research has been taking place to investigate the potential risks of these emerging contaminants, a full characterization of the potential human and environmental impacts of these materials is still missing. As well, the physicochemical parameter which determines the toxic effect is still far to be clearly declared. A bibliographic survey concerning toxicity studies on the freshwater organisms representative for the trophic levels of Algae, Crustaceans, and Fish respectively, have been performed. Conducted for MWCNT, SWCNT, and fullerene respectively, have been performed. Conducted for MWCNT, SWCNT, and fullerene respectively, different physicochemical properties have been found to have a significant effect on toxicity by the ANOVA analysis. The matrix of correlation coefficients approach shows, e.g. for SWCNT-crustaceans, that the exposure time and the length have a positive and strong correlation with the EC50 values whereas a negative and weak relationship with EC50 values. The lack of data in term of characterization of the ENM both in the media or in the commercial available form were the main limitations to the investigation. On our knowledge the approach here proposed is for its first time proposed and it might be useful as a first basic investigation for the identification of key parameters of relevance for the assessment of ENMs.

**Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (P)**

**TU034**

**Potential on the application of multi-luminescent bacteria for ecotoxicological screening of nanomaterials**

S. Baik, KIST Europe / Environmental Safety Group; Y. Jung, KIST-Europe; Y. Kim, KIST-Europe / Environmental Safety Group

A number of studies has investigated toxicological effects of nanomaterials with various methods and addressed the potential toxicity on different systems. Among the groups of nanomaterials, silver nanoparticles (AgNPs) are of concern for their biocidal effects of both ionized and particulated forms. In this study, four different surface-coated AgNPs, coating of citrate (Cit), tannic acid (Tan), pothelyene glycol (PEG), and branched polyethyleneimine (BPEI), were selected to investigate toxicological effects. A luminescent microbial array for toxicity risk assessment (LumiMARA) using multi-species of luminescent
bacteria was used to evaluate for those AgNPs. To date, various acute toxicity biosays using bacteria using toxicity screening have widely been applied to understand the ecotoxicological impacts of pollutants on aquatic organisms due to their advantages such as simplicity, rapidity, and cost-efficiency. Using single-strain of bacteria, however, may have some limitations of the various sensitivity ranges depending on microbial species. To reduce this limitation, application of multi-species of luminescent bacteria as the potential ecotoxicological screening tool for surface-coated AgNPs was investigated. Different time-dependent inhibition rates for all tested AgNPs exposed to each luminescent bacteria were achieved and used for dose-response curves to calculate the values of the 50% effective concentration (EC50) of these values of EC50 for BPEI-AgNPs (1.57 to 5.19 mg/L) were lower than those for the other surface-coated AgNPs (i.e., Cit-AgNPs, Tan-AgNPs, and PEG-AgNPs). It appears that the toxicity of AgNPs could be activated by the interaction of positively charged AgNPs with the negatively charged bacterial cell wall. Effects of the sole control materials were also investigated and achieved similar toxicological trends to surface-coated AgNPs, and consequently, coating materials may induce toxic effects of surface-coated AgNPs on the exposed luminescent bacteria. The short exposure time of LumiMARA, 15 min, would be drawback for the applicability as the toxicological assessment of nanoparticles, which may have slow toxicity reaction. However, the advantages in simplicity, rapidity, cost-efficiency, and reproducibility of this biosay may lead to have a potential applicability as an acute toxicity pre-screening tool for the environmental sample containing nanoparticles.

TU035
The effect of surface oxidation on the dispersion and biotoxicity of carbon nanotubes
L. Zhang, Zhejiang University / Environmental Science; R. Deng, D. Lin, Zhejiang University / Department of Environmental Science
Carbon nanotubes (CNTs) are employed in a variety of applications due to their unique and extraordinary properties, causing increased discharge of CNTs into aquatic environments and thereby threatening aquatic organisms. However, the severe homogenous of carbon nanotubes (CNTs) due to their high hydrophobicity strongly limits their efficient utilization. Surface oxidation is considered to be an effective way to improve the dispersity of CNTs. In this study, we treated multiwall CNTs (MWCNTs) with concentrated H2SO4/HNO3 to prepare surface oxidized MWCNTs (o-MWCNTs), investigated changes in surface properties of the MWCNTs after oxidation, and evaluated the effect of oxidation on the suspension stability and biotoxicity of the MWCNTs. The contents of oxygen and oxygen-containing groups including carbonyl, hydroxyl, and ether groups of the MWCNTs increased and the point of zero charge of the MWCNTs decreased with increasing oxidation time. The specific surface area of the MWCNTs increased as oxidation time increased due to the disclosed tube ends and the untied CNT bundles. The concentration of stabilized MWCNT suspension increased with ultrasonication time, and the o-MWCNTs had much higher stability than the pristine MWCNTs. Changes in surface property and colloid behavior of the MWCNTs by the oxidation substantially altered their biotoxicity. The o-MWCNTs exhibited significantly higher toxicity toward the algae (Chlorella Pyrenoidosa) and bacteria (Escherichia Coli) than the pristine MWCNTs, and higher toxicity was observed for the o-MWCNTs with higher degree of oxidation. Keywords: Carbon nanotubes; oxidation; dispersion; biotoxicity

TU036
Effects of cationic polystyrene nanoparticles in the hemocytes of the marine bivalve Mytilus: role of soluble hemolymph factors
C. Cucci, University of Urbino; R. Fabris, T. Balbi, University of Genova / DISTAV; K. Cortese, university of genou; E. Boregani, University of Siena; M.P. Monopoli, University College Dublin / Centre for BioNano Interactions School of Chemistry and Chemical Biology; K.A. Dawson, University College Dublin / Centre for BioNano Interaction School of Chemistry and Chemical Biology; I. Casini, University of Genova / DISTAV - Nanotechnology for Earth and Environmental Sciences; L. Canesi, university of genou / DISTAV - Nanoparticles (NPs) released into the environment can undergo considerable transformations before reaching the target biological system: however, the evaluation of the biological effects of NPs requires additional understanding of how these nanomaterials interact with aquatic species and the consequences of such interactions in a physiological environment. In mammalian models, interactions of NPs with plasma proteins originate a coating known as protein corona (Monopoli et al., 2011, J Am Chem Soc 133:2525). The corona proteins control the specific cellular receptors for particle recognition, the internalization pathways, and the immune responses. The protein corona has also been termed the artificial corona and freshwater invertebrates; however, no information is available in marine species. PS NPs can occur in the marine environment as degradation products of macro- and microplastics. Recent studies on the marine bivalve Mytilus showed that cationic PS NPs (PS-NH2, 50 nm) affect different functional parameters of the immune cells, the hemocytes. In vitro exposure to PS-NH2 in A. culmorum, Faber, exposition to PS-NH2 resulted in lytic activity, ingested particle release, extracellular oxyradical and nitric oxide production, with maximal effects at lower concentrations (1.5 g/mg). Only at higher concentrations (50 g/mg) lysosomal damage and induction of pre-apoptotic processes were also observed (Canesi et al., 2015, Mar. Environ. Res. 111:34). In this work, the effects of mussel hemolymph serum on the interactions between PS-NH2 and hemocytes were evaluated. Cells were exposed for 5 - 60 min to PS-NH2 suspensions in hemolymph serum (1, 5, 50 g/mg). In the presence of serum, PS-NH2 increased lysosomal damage and extracellular oxyradical production with respect to ASW medium. These effects were associated to an opposite trend in the activation state of p38 MAPK, a key component of immune signaling, in serum and ASW, respectively. Moreover, TEM observations indicate a general increase in cellular damage induced by opsonized PS-NH2. The results show that soluble serum components can affect interactions of PS-NH2 with Mytilus hemocytes, and suggest that a NP-protein corona might also be formed in biological fluids of mussels. The results underline the need of understanding how the formation of a NP protein corona may affect the biological outcome of in vivo NP exposure in marine invertebrates.

TU037
Exposure to sublethal concentrations of metal nanoparticles may lead to disturbed feeding behavior and elevated metal body burden of Daphnia magna
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In ecotoxicological studies of metal nanoparticles (MNPs), lethality is the most often addressed acute toxicity endpoint and aquatic crustaceans are often used as test species. Freshwater biota and Daphnia magna in particular, is one of the most vulnerable targets of MNPs mainly due to high sensitivity of D. magna to heavy metal ions, released from the MNPs, but also due to its physiology (e.g. feeding). Though significant for viability of the zooplankton population and the ecosystem health, sublethal effects of MNPs such as affected feeding are remarkably less studied. The aim of the current study was to evaluate whether the exposure to sublethal concentrations of MNPs affects D. magna feeding on microalg Chlamydomonas reinhardtii later on and whether there exists a risk for trophic transfer of MNPs (from D. magna to higher organisms). To evaluate the solubility-related effects of the studied MNPs, the respective soluble metal salts were studied in parallel. To obtain dose-response data, D. magna was exposed to Ag, CuO, CoO, and Mn2O3 NPs and the respective metal salts for 48h according to OECD202 guidelines. The tested concentration range for Ag NPs was 0.1-20 µg/L and for other MNPs 0.01-100 mg metal/L. After the 48h chemical exposure, D. magna (from sublethal exposure concentrations) feeding on microalgae was assessed by algal autofluorescence in flow cytometry. In parallel, trophic transfer potential of metals upon exposure to sublethal concentrations of the studied chemicals was evaluated by quantifying the total body burden of metal in NSI salt-exposed daphnids i) immediately after exposure and ii) after post-exposure feeding using Total Reflection X-ray Fluorescence spectroscopy (TXRF). The 48h toxicity for D. magna decreased in the following order: Ag>Co>Cr2O3>Co3O4 and AgNO3>Co3O4>Cr2O3>Co3O4>Mn2O3>NP with the soluble salts, respectively. Co2O3 and Mn2O3 NPs were non-toxic up to 100 mg metal/L. Flow cytometry and TXRF proved suitable tools for studying the compound endpoints. Results of the post-exposure feeding assessment showed decreased feeding of previously MNP-exposed daphnids compared to the metal salt exposed ones. TXRF analyses of MNP-exposed organisms showed that post-exposure feeding significantly decreased the total metal body burden nevertheless it remained elevated compared to that of unexposed control daphnids. Acknowledgements: This study was supported by Sciex NMS™ project 13.143 (MH), by ETF93/47 and by IT223-5.
Toxicity of engineered silver nanoparticles (NPs) is enhanced by co-exposure with clay particles in zebrafish larva.

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Naturally occurring particulates within the nano-size range are very abundant in surface waters and among these are mineral clay particles. Engineered nanoparticles (NPs) that are released into surface waters will undoubtedly interact with naturally occurring particulates; however, investigations of the effects of these interactions on NP toxicity have not been conducted. Clay minerals consist of silica tetrahedral (T) and alumina octahedral (O) layers in a lamellar structure that results in charge heterogeneity with both positive and negative charges on their surfaces. The presence of both polarity charges on the surface of clay particles suggests that interactions with silver (Ag) NPs with a negative zeta potential and dissolved free silver ions (Ag⁺) will occur. We hypothesised that interactions between Ag-NPs and clays would alter aqueous phase Ag-NP toxicity in zebrafish larvae (ZFL). To test this hypothesis, three agarose gel electrokinetic tests (EKT) were performed with and without 20 mg/l of Kaolinite clay suspension, and zebrafish larvae were exposed for 96 h (age 72-168 h post fertilization). Consecutive experiments were conducted with increasing concentrations of clay and humic acid (0-100 mg/l) and fixed concentrations of Ag-NPs (0.75, 1, 1.5 and 2 mg/l), and mortality of larvae was recorded. The clay exposed controls showed no toxicity from clay alone. Mortality of ZFL significantly increased when fish were exposed to both Ag-NPs and clay. The toxicity of 1 mg/l Ag-NPs increased with clay concentration by a maximum increase of 0.4 fold with 60 mg/l of clay, relative to 1mg/l of Ag-NP only. The 96-h Ag-NP exposure concentration predicted to kill 50% (LC50) of ZFL with and without clay (20 mg/l) was 0.73±0.1 mg.L⁻¹ and 0.73±0.1 mg.L⁻¹ respectively, which is a 0.66 fold increase in toxicity within the clay and Ag-NP co-exposure. Humic acid significantly decreased toxicity of Ag-NPs (consistent with previous reports); however, the presence of humic acid did not eliminate the increased toxicity of Ag-NPs when co-exposed with clay. The observed increased toxicity of Ag-NPs in ZFL when co-exposed with clay is environmentally relevant and a novel result that suggests an alternative facet regarding the environmental effects of engineered nanoparticles.

TU041

title: Environmental effects of silver nanoparticles on Hydra attenuata

demonstrate that GO currently do not pose a serious risk, it may exert some risks when they are released into the environment. Graphene and its derivatives are nanomaterial found in commerce today. It is expected that these materials will end up in the aquatic environment. This study investigated the environmental effects of different GO concentrations lower to 10.0 mg L⁻¹. Even though the results apparently showed a comparison. Chemical analysis of the sediment, overlying water and test organisms demonstrated that AgNP contaminated biosolids eliminated the silver back to background levels. Therefore there is uncertainty if the silver detected was due to bioaccumulation in the test organisms or the AgNP toxicity assessment. The study used a direct spiking technique rather than using a bioassay methodology that has been used in the past. The authors concluded that AgNP test organism choice was important in this study. They also concluded that AgNP toxicity assessment is important in the environmental risk assessment and should be included in future studies. The authors recommended that further studies are needed to understand the toxicity of AgNP in different environmental conditions and to develop a better understanding of the environmental fate of AgNP.

TU042

Tissue specific responses of the Cape river crab, Potamonauta perlatus, to silver nanoparticles and temperature

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Silver nanoparticles (AgNPs) are incorporated into an increasing number of consumer products. However, the potential toxicity of AgNPs to aquatic organisms is largely unknown. The behaviour of AgNPs is influenced by several factors, such as the inherent properties of the NP (size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution) and environmental properties of the dispersed media (temperature, pH, ionic strength, salinity, organic matter). In the aquatic environment, the exposure to AgNPs can be affected by environmental factors such as temperature, pH, and ionic strength. The effects of AgNPs on the growth, survival, and reproduction of Cape river crab Potamonauta perlatus are currently being investigated. The authors conducted a series of experiments to determine the effects of AgNPs on the growth, survival, and reproduction of Cape river crab Potamonauta perlatus. The experiments were conducted in a controlled laboratory environment with different temperatures and AgNP concentrations. The results showed that AgNPs had a significant effect on the growth, survival, and reproduction of Cape river crab Potamonauta perlatus. The authors concluded that further studies are needed to understand the mechanisms by which AgNPs affect the growth, survival, and reproduction of Cape river crab Potamonauta perlatus.
Bioaccumulation of Silver and Silver Nanoparticles in Earthworms

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Engineered silver nanoparticles (AgNP) are the most common form of nanomaterial found in commerce today. It is expected that these materials will end up in soils via the waste water stream through the application of biosolids to agricultural soils. Earthworms are an important part of the soil ecosystem and effects on their health and their ability to feed on organic material and subsequent trophic transfer is of concern. Three bioaccumulation tests were performed with Eisenia andrei in an agricultural sandy-loam soil: poly-disperse Ag nanoparticle comprised of 2 nm particles (0.3% PVP), silver nitrate (AgNO₃), and dispersed 40 nm AgNP (80% PVP) aged in biosolids prior to mixing with soil. For each test, E. andrei were exposed to the contaminated soil for an uptake period of 21 days and then transferred to a clean soil for an elimination period of 21 days. The results of the bioaccumulation indicate bioaccumulation factors (BAF) of AgNP > AgNO₃ > AgNP in biosolids (in order of significant bioaccumulation). In the cases where the soils were not amended with biosolids, the measured silver in earthworm tissue remained significantly greater than background after the elimination period, whereas E. andrei in soils amended with AgNP contaminated biosolids eliminated the silver back to background levels.

TU044

Influence of surface characteristics on graphene oxide toxicity for zebrafish embryos

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Despite nanotoxicology studies have been intensified in recent years, gaps remain in the methods used to assess the nanotechnology risks. These gaps are due to the complex nanomaterials behavior in the environment, especially in the presence of organic matter and depending on the nanomaterial characteristics. Studies indicate that humic acid present in the aquatic environment can increase the stability of nanomaterial dispersions and may change its toxicity to aquatic organisms. The adsorption of oxidative debris (carboxylated carbonaceous fragments - CCFs) has also been discussed as an important factor influencing GO properties and behavior. The surface characteristics of GO can influence its biotransformation application as well as its toxicological effects. The aim of this study was to evaluate the influence of the presence of oxidative debris and humic acid in the toxicity of graphene oxide (GO) utilizing Fish Embryo Toxicity Test (FET test). GO (Sigma Aldrich) was refluxed with NaOH (0.11M, 1h, 90°C) and HCl (0.1M, 1h, 90°C) to produce GO without debris (GOWd). GO And GOWd were characterized through spectrophotometry, dynamic light scattering and atomic force microscopy. Zebrafish embryos (Danio rerio) were exposed during 96 h to 100 mg L⁻¹ GO or GOWd, in presence or absence of humic acid (HA, 20 mg L⁻¹). A control group exposed to reconstituted water was also performed. At the end of the exposure period, the larvae were measured and frozen at -20°C for subsequent evaluation of acetylcholinesterase activity (AChE). We also performed an in vitro test to evaluate direct effect of the nanomaterial in zebrafish AChE activity. Homogenates from three larvae were mixed with GOWd at 30 mg/L (20 min, 30°C) before AChE assay was performed. Both GO agglomerated and precipitated quickly in reconstituted water. The presence of HA in the medium stabilized the GO suspensions similarly to that occurred with GO in ultrapure water. There was no difference between groups related to the occurrence of embryo malformation or mortality. Larvae exposed to GO were shorter and showed lower AChE activity than control and group exposed to GOWd. The in vitro test showed that the nanomaterial did not inhibit AChE activity. The nanomaterial showed low toxicity to embryo, but the reduction in total length and AChE activity in the organisms can be due to indirect effects in zebrafish development. More experiments will be performed to understand those effects.

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TU045

Critical assessment of a 10 day sediment toxicity and bioaccumulation study of silver and silver nitrate using the polychaete Arenicola marina.

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There is an increasing use of Silver Nanoparticles (Ag-NPs) in various industry sectors, which are entering the marine environment via sewage treatment plants. Marine and estuarine sediments are thought to be a sink for the Ag-NPs as well as a food source for the polychaete worm Arenicola marina. Objectives of this study were to measure the bioaccumulation and toxicity of Ag-NPs to Arenicola marina and to assess the methodology used for sediment exposures for nanoparticles (NPs). Survival and cast production were assessed over the 10 day test duration. The toxicity and bioaccumulation of ionic silver was also assessed as a comparison. Central analyses of the survival and cast production of the organisms was conducted to detect silver concentrations using ICP-MS. The sediment silver concentrations ranged between 116 and 146% of nominal concentrations for the Ag-NPs tested. This shows that the particles remain in the sediment portion of the test system and are available to the test organism via ingestion of sediment. Silver was detected in the A. marina exposed to the higher concentrations tested, these treatments were also associated with low survival. Therefore there is uncertainty that silver detected was due to bioaccumulation in the tissues or to sediment within the gut of the worms as depuration was not possible. The median lethal concentration (LC₅₀) is defined as the concentration resulting in 50% mortality of the A. marina. The Ag-NPs LC₅₀ value for survival was 25.2 (22.5-28.3) mg/kg compared to 8.7 (5.2-14.5) mg/kg for ionic silver. The higher LC₅₀ value for Ag-NPs suggests that they were less toxic than the ionic silver in this study. The Ag-NPs EC₅₀ value for cast production was 13.9 (11.6-16.6) mg/kg compared to 10.4 (8.4-12.9) mg/kg for ionic silver. The second objective of this study was to assess the methodology and its suitability for nanoparticle toxicity assessment. The study used a direct spiking technique rather than solvent spiking to a coating, which is often used by other researchers. The spiking method was successful for this study, shown by the measured concentrations detected relative to the target values. However nanoparticles are produced in many different sizes, with and without coatings, in solid or liquid state and can agglomerate. All of these factors can pose problems when trying to spike sediments for toxicity testing therefore the specific approach may need to be adapted on a case by case basis.

TU046

Uptake of sediment-associated Ag nanoparticles in oligochaetes - a first step towards trophic transfer

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Metal-containing engineered nanoparticles (Me-ENPs) are used in a wide range of products worldwide, such as inks, plastics, consumer products, lubricants, electronics and biomedical applications. Their release into the environment can affect aquatic organisms. This study is the first in a series of experiments leading to the identification of trophic transfer of metal-based ENPs in freshwater ecosystems. The main outcome of the study is the determination and quantification of ENP bioaccumulation in sediment-dwelling organisms, which will add to our understanding of this first step in the benthic food chain and thus of the potential trophic transfer of ENPs. Preliminary studies have shown that the oligochaeta Tubifex tubifex accumulates Ag-ENPs from sediment after long-term exposures (28days). In this study, we investigate whether this bioaccumulation pattern also occurs after acute exposure (72-96h) to sediment spiked with different Ag-ENP concentrations. Ag-ions and a particular reference are used to be able to pin-point potential nano-specific effects. The overall aim of the study is to determine the first step in food chain transfer of Ag-ENPs, using sediment as primary contaminant source. Considerations regarding acute vs. chronic exposure durations is made in relation to experimental design and bioaccumulation. ICP-MS is used to analyze the degree of uptake in T. tubifex, estimated based on total metal uptake in organisms after exposure. The concentration range of Ag-ENPs is covering both low, environmentally realistic and higher, more response-inducing concentrations. Information on experimental setup, preliminary results and ideas for future studies including the next steps in the food-chain transfer investigation will be presented. We believe that by knowing if and to what degree aquatic organisms accumulate ENPs, a greater understanding of the actual risk and trophic transfer potential of these contaminants will be achieved.

TU047

Silver nanoparticles inhibit the embryonic development of Daphnia galeata

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Silver nanoparticles are widely used in various commercial and industrial applications; therefore, it is of high importance to study their adverse effects to aquatic organisms. This study investigated the adverse effect of silver nanoparticles to the embryonic development of water flea. Test species is Daphnia galeata, which inhabits Holartic lakes and rivers, and have sharp head, are smaller in size than Daphnia magna.Embryos were extracted from Daphnia galeata adult female, and various concentrations of Ag-NPs were selected, and exposed to a series of Ag-NPs concentrations for 72 hours. We measured a range of embryonic endpoints including antennae, eye, rostrum, heart, carapace, postabdominal claw, malpighian tube, sensory bristles, and tail spine. In addition, Daphnia galeata neonates were exposed to 50 nm of AgNPs for 48 hours. As a result, the EC50 value for neonate immobilization was estimated to be 38.42 ug/L. The 50% of AgNPs of 0.04 ng/L had a serious inhibition of embryonic development. Further research is needed to investigate multiple species of water fleas which are important consumers in aquatic food chain. Acknowledgements. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A201105013).

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Nano Copper Oxide on Plants, Collembola and Earthworms in Soil

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Among cosmetics and personal care products, sunscreen products are of emerging consideration from a regulatory perspective due to their potential impact. Once used, nanomaterials are typically disposed off or via the wastewater stream. The use of sunscreen products can result in the release of nanoparticles and their presence in environmental samples. The aim of this study was to assess the potential toxicity of TiO₂ and CuO nanoparticles to zebrafish embryos. Two different species of TiO₂ nanoparticles (cube 35 nm, rod 250 nm), and Ag₀ (PVP coated and SiO₂ coated) were used at various concentrations to assess their impact on embryo development and survival. The results showed that TiO₂ nanoparticles had a negative impact on zebrafish development, with concentrations of 50 and 100 mg/L causing significant reductions in survival and developmental endpoints. Ag₀ nanoparticles had a more pronounced effect on embryo development, with concentrations of 50 and 100 mg/L resulting in complete embryo mortality. CuO nanoparticles had a less pronounced effect, with concentrations of 50 mg/L causing a significant reduction in embryo survival. The findings of this study suggest that TiO₂ and CuO nanoparticles may poses significant risks to aquatic ecosystems, and further research is needed to understand the ecotoxicological effects of these materials.
The treatments at 150 mg/kg show that there is no effect of size and shape of TiO2 on titanium flux in plant. Furthermore, there is no significant difference of flux considering the type of soil.

TU053
Physiological and biochemical responses of polychaete Diapatria natapolitana to Graphene oxide
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In the recent years there is an increasing concern about the large number of emerging pollutants and nanoparticles (NP) that are being released into the environment without yet being regulated, such as carbon based nanomaterials (CBN). Graphene Oxide (GO) is one of the most important CBN that has been extensively used but, such as for most of the CBN, limited literature is available regarding the impacts induced in aquatic organisms by this pollutant. The polychaete Diapatria natapolitana has frequently been used to evaluate the impact of environmental disturbances in estuarine systems due to its ecological and socio-economic importance but, to our knowledge, no information is available on D. natapolitana physiological and biochemical alterations due to CBN exposure. Thus, the present study aimed to assess the toxicity effects of different concentrations of GO (0.01; 0.1; 1.00 mg/L) in D. natapolitana physiological (reproductive capacity) and biochemical (metabolic stress related biomarkers) performance, after a 26 days exposure period. The results obtained revealed that the exposure to GO induced negative effects on the reproductive capacity of polychaete specimens, with individuals exposed to the highest GO concentration (1.00 mg/L) presenting the lowest capacity to regenerate their body and lowest oxygen levels. Furthermore, with increasing of GO concentration, D. natapolitana display increased lipid peroxidation (LPO), Glutathione S-transferase activity (GSTs) content and decreased the ratio between reduced (GSH) and oxidized (GSSG) Glutathione. The activity of the antioxidant enzyme catalase (CAT) was not significantly affected by GO changes, although a slight increase was noticed at the highest GO concentration. The activity of the antioxidant enzyme superoxide dismutase (SOD) was significantly higher at the 2 lowest GO concentrations, compared to the remaining conditions. Overall, the obtained results indicated that GO induced alterations in the reproductive ability of D. natapolitana. In addition, GO induced oxidative stress responses in this species, evidenced by changes in indicators of cellular damage and antioxidant defenses, specially noticed at the highest GO concentration. Thus, D. natapolitana demonstrated to be a good bioindicator to monitor emerging pollutants such as GO.

TU055
Cosmetic exposure to titanium dioxide nanoparticles does not affect cadium toxicity in radish seeds (Rafanus sativus)
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Nanoparticles (NPs) are currently present in a variety of industrial products and their use, disposal and fate (degradation and/or persistence) into the natural environment require urgent investigations. Recent developments on environmental fate models indicate that as nanowaste they could reach both terrestrial and aquatic ecosystems thus potentially affecting environmental and human health. Plants can be therefore exposed to NPs but controversial toxicity results in terms of fate and toxicity are currently available. Furthermore, there is a current lack of information on complex interactions/transformations of the NPs, which might occur in the natural environment as for instance interaction with existing toxic compounds. As a consequence the prediction and assessment of NPs behaviour, exposure and biological effects represent a current major challenge. In the present study, radish seeds have been exposed to titanium dioxide NPs (n-TiO2)(1-1000 mg/L)(P25, Aerioxide) and cadmium chloride (1-250 mg/L) alone and in combination using a seed germination and seedling growth toxicity test in radish, Rafanus sativus (OECD 208). Seed germination was not affected by n-TiO2 alone but a significant increase in root elongation was observed but not dose-dependently. On the opposite, CdCl2 cause a dose-dependent inhibition on root elongation and a complete abolishment of seeds germination at the highest concentration of 250 mg/L. Co-exposure clear showed no interaction of n-TiO2 in Cd effects since both Cd2+ and n-TiO2 alone (100 mg/L of CdCl2 and 10 mg/L of n-TiO2) do not cause the observed increase in seed germination. The presence of n-TiO2 seems not affecting the bioavailability of CdCl2 to seeds and roots so thus the toxic effects were still evident. Based on literature, n-TiO2 (anatase) is known to adsorb Cd on the surface of the NP and thus potentially increase its bioavailability. According to our knowledge, this was the first time where the combined exposure of Cd and n-TiO2 seeds were in the presence or absence of n-TiO2. DLS analysis showed that n-TiO2 tested concentrations were quite dispersed in Milli-Q water reaching NPs size of ~180 nm (Z-average) and primary Z-potential (−31.7 mV) and Pdl (0.3). Seeds exposed to n-TiO2 showed a clear uptake n-TiO2 in particular on young leaf and inside the root. Our results underline that further studies are needed in order to address any potential interactions of NPs with toxic pollutants present in soils able to cause phytotoxicity in plants.

TU056
A network perspective on experimental data of organic matter and inorganic nanoparticles in aquatic media
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Understanding the influence of natural organic matter (NOM) on the stability of nanoparticles in water. In this network, we have studied the behavior of ENPs in the natural environment, since NOM can stabilize ENP dispersions or enhance the aggregation of ENPs. The effects depend strongly on the physicochemical properties of the NOM and the surface characteristics of the ENPs. Therefore, to improve the mechanistic understanding of ENP-NOM interactions, experiments should study diverse combinations of NOM and ENPs, as pointed out by recent reviews. However, many of the numerous experiments carried out up until now repeat already well-studied NOM and ENPs combinations; rather few experiments investigate unique ENP-NOM combinations not studied before or after. This tendency leads to an overall low diversity of ENP-NOM combinations investigated, contrary to what is required. In this network, we assess the potential of this experimental approach in order to identify knowledge gaps and possible research needs. Using the fact that several NOM and ENPs are common between the different experiments, we connected the materials that were investigated in 255 peer-reviewed papers that studied the influence of NOM on ENPs in aquatic media in a network. In this network, each node represents either an NOM or ENP of a given type, and the link connecting any two given nodes means that the two were used in the same experiment. In this network, many high-degree nodes are linked to low-degree ones, resulting in segregation of the data; in many cases, a given NOM or ENP is found to be tested with a set of ENPs or NOM types that is almost unique to it. The most central nodes are by far the Suwannee River Humic Acid for NOM and TiO2 and Fe3O4 for ENPs. This indicates that these constituents are investigated in combination with the largest number of corresponding constituents, in a large number of experiments. Temporal changes in the network’s topology show a decrease in the global diversity over the last 25 years. In particular, we observe a decrease in the diversity of the NOM types employed in the experiments, which indicates that the focus is mainly on the ENPs studied. Overall, the trend observed implies that the empirical basis for a better understanding of NOM effects on ENPs remains rather limited, since insights from new studies are obtained for increasingly similar combinations of materials.

TU057
Biocompatible hydrogel nanocomposites for new generation drug design
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The use of nanotechnology in medicine is the most exciting area for nanotechnology application even in such pivotal field of healthcare as cellular level fight against tumors. Despite its advantages, nanotechnology causes concerns, as nanoparticles can cause harm if applied in some particular fields. Dangers are connected to development of artificial materials with new properties. It is still unknown: how these new materials will “behave” in the human organism, as genetic after-effects might be displayed in the long-term perspective. Biocompatible hydrogel nanocomposites were synthesized by the method of frontal polymerization (FP) on the base of acrylamide and colloid solutions of locally available fossils: bentonite, diatomite, etc. Polydisperse benzone powder was treated with solution of linear polyacrylamide as a surface active substance enveloping the nano-scale particles with their colloid state subsequent emission from polydisperse suspension. Such treatment allowed to synthesize polyacrylamide nanocomposite hydrogels with benzone nanoparticles. The resulting nanocomposite hydrogel possessed appropriate adsorption properties and prolonged desorption of medicinal preparations according to preset programme. Adsorption and desorption optimal conditions were studied in such drugs as C7Hglenor, Voltaren, Naltrexone at appropriate temperature and pH corresponding to acidity of human stomach and gastrointestinal tract. This research was contributed to obtain conformity with established requirements. The most important advantage of synthesizing mentioned nanocomposites in FP mode is their absolute non-toxicity due to specific features of frontal method of synthesis. Unlike the globally used technology, due to specifics of FP, not only ecological compatibility is ensured at PAHs synthesis process, but toxic acrylamide is absolutely absent in obtained hydrogel. Due to their n(EENPs) is essential for wide use in various fields of human activity is created. The PACCs are cross-linked polymeric structures capable of...
of swelling with absorption of a large amount of moisture and active substances without destroying the original shape. A specific advantage of PAs is ability to release absorbed substances into the environment. It is noteworthy that this method has great advantages not only from the point of view of its high efficiency, ecological safety, possibility to control initial process parameters for the purpose to regulate properties of the obtained product.

TU057 Dispersion and aggregation of carbon nanotubes in aqueous solutions of anionic surfactants

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Dispersion and aggregation of CNTs in the aqueous environment are critical behaviors affecting their fate, bioavailability, and potential environmental and health risks. Bulk CNTs are generally aggregated and cannot be dispersed significantly in water or even in organic solvents. Surfactants are widely employed as surfactants to prevent or disperse the aggregates in industrial applications. Therefore, for a better understanding of the mobility of CNTs in the environment, this work examined (i) whether bulk CNTs can be dispersed and stably suspended in the natural environment and (ii) whether surfactant-suspended CNTs can retain their stability in the natural environment. We observed that CNTs can be stably suspended in sodium dodecylbenzene sulfonate (SDBS) solution but not in water with assisted sonication. Moreover, CNTs could not be dispersed and stably suspended in water and SDSS solution by shaking at 140 rpm, a mild agitation representative of the natural aqueous environment. Therefore, both SDSS and sonication play important roles in the dispersion of CNTs, with sonication breaking down large aggregates of SWCNTs, while SDS adsorption on SWCNTs inhibits the aggregation and aggregation to maintain the stability of the suspension in water. Concentration of dispersed CNTs in the SDSS solution depended on the sonication energy, but not the sonication time or output power of the sonicator alone. The amount of dispersed CNTs was positively correlated with the concentrations of SDS and CNTs, and the length of the CNTs. The optimal energy, i.e. the minimum energy supplied by sonication to achieve a saturated suspension of dispersed CNTs in the SDSS solution, was CNT diameter-dependent. Stably suspended CNTs in SDSS do not remain stable at the presence of cations (e.g., Na+, K+, Ca2+, and Mg2+) after dilution. These observations suggest that CNTs will not travel long distances in significant concentrations in natural environment. Moreover, re-aggregation of suspended CNTs in the presence of cations was dependent on SDSS concentration but not CNT concentration.

TU058 Fate and uptake of nanoparticles in soil-earthworm systems

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Over the past few decades a significant body of work has been done to understand the ecological and health risks of synthetic pyrethroid pesticides (SPs). Recently the use of nano-encapsulated SPs has been proposed. As nanoparticles can behave very differently from dissolved chemicals, it is possible that the environmental fate, uptake and effects of these nano-encapsulated pesticides could be very different from the conventional SPs. This study therefore investigated the effects of nano-encapsulation on the fate and uptake of bifenthrin, a widely used third generation synthetic pyrethroid, in soil systems. Studies were performed, using OECD Guidelines 213, to determine how nanoparticles (titanium dioxide and carbon toluene) to determine dissipation half-lives in soil, soil-water partition coefficients and uptake and depuration in the earthworm Eisenia fetida using analytical grade bifenthrin, a conventional bifenthrin formulation (Capture LFR) and two nano-formulations. Persistence, sorption and uptake behaviour of all the study materials varied across soil types. Generally, the persistence, sorption and uptake of bifenthrin in the conventional formulation were similar to the behaviour of the non-formulated active ingredient. However, nanoencapsulation significantly affected the behaviour of the bifenthrin. Results for the two nanoformulations were similar to each other but these showed enhanced persistence, decreased sorption and increased rates of uptake and depuration in the earthworms compared to the analytical grade material and the conventional formulation. We therefore anticipate that the distribution and impacts of the nanoformulation in natural soil systems will be different from currently used formulations. The observed differences in persistence and sorption behaviour are possibly due to the polymer capsule protecting the active ingredient from microbes and soil binding sites. Differences in uptake might be explained by differences in distribution of the bifenthrin within the organism (i.e. the nanoformulation is accumulating in the earthworm gut while bifenthrin in the conventional and non-formulated treatments is being internalised). Keywords: Synthetic pyrethroids; Nanoformulations; Bifenthrin; Earthworms

TU059 In vitro and in vivo effects of copper oxide nanoparticles and copper ions in Zebrafish (Danio rerio): Effects on Cells, Embryos and Fry

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The use of engineered metal nanoparticles (NPs) is continuously increasing and so is the scientific body of evidence regarding toxicity of these particles. However, further information on toxic effects of metal NPs is still needed to assess whether these particles are more or less toxic compared to their ionic counterparts. Here, we compare the toxicity of CuO NPs with ionic Cu to zebrafish hepatoma cells (ZFL), embryos and fry. Both CuO NPs and CuCl2, at concentrations that do not induce cell death (e.g., decreased metabolic or cell membrane integrity) was detected for Cu ions or CuO NPs, though both Cu forms caused significant effects on production of reactive oxygen species (ROS) compared to the control. However, results show that both Cu ions and CuO NPs are toxic to zebrafish embryos and fry. Embryo mortality increased in both Cu ions and CuO NP treatments. 24 h LC50 was about 10 ng L−1 (±0.6 mg Cu L−1) for both treatments, whereas exposure to Cu ions at 50 µM (±3 mg Cu L−1) caused significantly higher mortality than CuO NPs (≈10% and ≈70% respectively). Only Cu ions showed effect on fry mortality (24 h LC50 about 30 µM, >2 mg Cu L−1 for Cu ions and >200 µM, 12.7 mg Cu L−1 for CuO NP). Both Cu treatments affected the biotic environment and potentially threaten plankton like algae. Exopolymeric substances (EPS) produced by algae may play an important role in the bio-nano interaction and nanotoxicity, which warrants to be investigated. We synthesized citrate- and PVP-stabilized AgNPs suspensions (C-AgNPs and P-AgNPs, respectively), extracted EPS from a green alga (Chlorella sp.), investigated interactions between the AgNPs and the EPS, and specifically evaluated effects of the NP/EPS interaction on the algal accumulation and toxicity of the AgNPs. Results show that the algal EPS contained many active functional groups and strongly interacted with AgNPs as evidenced by the variation of UV and IR spectra, the increase in hydrodynamic sizes and zeta potentials, and the reduction of particle concentrations. The developed methods can be applied to marine water and sandworm extraction to assure the quality of the data. This study highlights the importance of understanding the role of EPS in the bioaccumulation and toxicity of nanoparticles.
concentration: 1 mg Ce kg⁻¹. Canola plant (Brassica napus) was grown one month on this soil amended with the sludge without NMs or with biosolid enriched Ce NMs. Bulk Ce L-edge X-ray absorption spectroscopy (XAS) was used to study Ce speciation in the sludge before culture in order to evaluate the NPs transformation in the reactor. After the culture, elemental concentrations were measured in the plant parts by ICP-AES, and their distribution in roots by laser ablation ICP-MS. The first results indicate that the amendment of a biosolid polluted with CeO₂ NPs, performed at realistic concentration and after aging in a laboratory-cage reactor has an effect on the plant nutrients concentrations. Extensive analysis of elemental distribution in roots as well as effects on soil microbiota (microbial activities, bacterial community structure) are being performed in order to understand the impact of the enriched Ce biosolid and will be presented.

State of the science on poly- and perfluoroalkyl substances (PFASs) in the environment and humans (P)

TU062 A fast screening strategy for the analysis of poly- and perfluoroalkyl substances (PFASs) in solid matrices using laser diode thermal desorption coupled to high resolution mass spectrometry

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In recent years, concerns have arisen about the widespread environmental occurrence of poly- and perfluoroalkyl substances (PFASs) due to their potential for persistence, bioaccumulation and toxicity. Since the first specific analytical methods were published, significant advances have been made in the analysis of PFASs in complex matrices. However, conventional methods are relatively time-consuming requiring labor-intensive extraction and clean-up procedures, as well as a significant amount of sample material. The purpose of the present work was to develop a fast screening strategy for the analysis of legacy and newly-identified PFASs in solid matrices, using a reduced sample amount (< 200 mg) and a simple preparation procedure prior to instrumental analysis by laser diode thermal desorption atmospheric pressure chemical ionization (LDTD/APCI) coupled to high resolution mass spectrometry (Orbitrap-MS). The development of the analytical procedure was conducted with the help of experimental designs to identify optimum operating conditions, including the choice of a suitable extraction solvent, the amount of graphite used for sample clean-up, and ULDTD instrumental settings. Following the addition of internal standards to the samples, an ultrasonic extraction step was performed using a small solvent amount (< 1 ml of methanol). For sample clean-up, graphite was then added (100 mg, batch) and the supernatant was recovered following centrifugation. 7 µl aliquots of sample were then analyzed by LDTD/APCI-Orbitrap-MS. The reduced sample preparation (no need of SPE manifold, no concentration step) and ultra-fast capability of the LDTD/APCI-Orbitrap-MS interface (acquisition time < 9 s per sample) imply that total analysis time (preparation + instrumental analysis) could be reduced to < 1 hour for 12 samples. The method was validated on fortified sediment and biota samples (i.e., assessment of detection limits, matrix effects, recovery, accuracy, and precision); when reference materials were available, method trueness was also evaluated. This allowed for the quantitative or semi-quantitative analysis of anionic, neutral and cationic PFASs in sediments and fish collected across Quebec at potentially-impacted sites (e.g., downstream from wastewater treatment plant effluents or aqueous film forming foam spills). Samples were also re-analyzed by LC/ESI-Orbitrap-MS, thus providing for a comparison with the newly-developed LDTD/APCI-Orbitrap-MS method.

TU063 Analysis of zwitterionic, cationic, and anionic fluoroalkylated surfactants in sediments by liquid chromatography polarity-switching electrospray ionization coupled to Orbitrap mass spectrometry

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The environmental prevalence of poly- and perfluoroalkyl substances (PFASs) in aquatic environments is rapidly increasing requiring labor-intensive extraction and clean-up procedures. To date, perfluoralkyl acids (PFAs) such as perfluorooalkyl carboxylates (PFCAs) and sulfonates (PFOSs) have garnered the most attention of PFAS monitoring surveys. However, recent reports indicate that a considerable portion of the total organic fluorine in environmental samples may be attributed to PFAS precursors and other unknown PFASs. Here, we describe a screening strategy for the identification and quantification of a wide range of PFASs in sediments. A total of 28 model PFAS analytes were selected for optimization and validation purposes, including 21 legacy PFASs and 7 novel cationic and zwitterionic PFASs. Instrumental analysis was conducted by ultra-high-performance liquid chromatography coupled to a Q-Exactive Orbitrap through a polarity-switching ionization source, allowing simultaneous acquisition of negative and positive mode PFASs within a single run.

The extraction/purification step was optimized to maintain a common preparation procedure for all PFASs at once, and adequate whole method recoveries were obtained (60–110 % for 28/28 model PFASs). Method validation included assessment of blank contamination, linearity, detection limits, matrix effects, recovery, accuracy and precision. The newly-developed method was subsequently applied to a selection of riverine or lacustrine sediment samples collected at large spatial scale in mainland France covering the six French Water Basins. In addition to the 28 model PFASs used for optimization and validation purposes, these samples were screened for more than 60 infrarequently reported anionic, zwitterionic or cationic PFASs. Perfluorooctane sulfonate (PFOS) generally prevailed over other PFASs (concentration range of PFOS: 0.84–23 µg kg⁻¹ dry weight). Fluorotelomer sulfonamide amines (FTAs) and fluorotelomer sulfonamide betaines (FTABs) were also particularly prevalent in these samples. Hot spots of zwitterionic/cationic PFASs (estimated ZPFA = 8.9–27 ng g⁻¹ dry weight) were associated with low-flow watercourses in the close vicinity of airports, suggesting the presence of firefighting activities at these sites. Treatment aqueous film forming foam (AFFF) formulations. In the long run, FTAs and FTABs could degrade to more environmentally persistent PFASs such as PFAs.

TU065 Development of Polar Organic Integrative Sampler for quantitative analysis of PFAS in surface waters

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Since the beginning of the 1990s, the development of passive samplers to analyze organic micropollutants in surface waters has been the subject of a large number of studies. They offer a complement to grab sampling, allowing for a better quantitative and qualitative analysis of a wide variety of contaminants. For instance, the Polar Organic Chemical Integrative Sampler (POCIS) has gained particular attention in recent years as a tool for the analysis of pesticides, other organic pollutants, pharmaceuticals. However, the application of this tool to poly- and perfluoroalkyl substances (PFASs) has been rarely reported, in spite of the ubiquitous character of these chemicals in aquatic environments. In this context, the present work consists in the development of a POCIS-like sampler allowing for the PFAS analysis in water after a two-step approach. First of all, the selection of a suitable configuration was realised under controlled conditions for 12 PFAS (9 carboxylates and 3 sulfonates). Four configurations based on Strata-XAW or Oasis HLB sorbents enclosed between Nylon or PES membranes were compared. The second step consisted in the in situ calibration of selected configurations with a two-fold objective: to confirm the analytical performance of the POCIS-like sampler and to evaluate the environmental parameters that could influence the sampling rates Rs (necessary for quantitative analysis). The reliability of the tools for the quantitative analysis was evaluated in a last in situ experiment (7 days of exposure). The first experiment showed that only the Strata X-AW sorbent accumulated the target 12 compounds (in agreement with previous report, PFAA was not accumulated by the HLB phase for the highest Rs) at levels of 80% recovery for all compounds (Rs = 0.02–0.45 L/d). However, a lag-effect was observed with PES membranes...
because of the PFAS affinity for this material. Better linearity of PFAS accumulation (and, as a consequence, better quantitative analysis) was observed for long-chain carboxylates and sulfonates with the Strata X-AW / Nylon configuration (Rs = 0.08-0.29 L/d). The latter configuration was therefore elected, and subsequently calibrated in a perirarian river near Bordeaux. 22 PFAS were detected in grab and passive samplers. Sampling results similar to those obtained in a laboratory, ranged from 0.07 to 0.42 L/d and the linearity period was in the range 2.5-15 days. In situ assessment showed good agreement between concentrations obtained with grab and passive samplers.

**TU066**
Multianalyte profiling of per- and polyfluoroalkyl substances (PFASs) in liquid commercial products

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The specific chemical properties of per- and polyfluoroalkyl substances (PFASs) make them widespread for use in a number of industrial and commercial products. For instance, PFAS are known to occur in consumer products and are added to confer water and oil-repellency characteristics (e.g. waterproof, anti-stain) and to reduce surface tension in aqueous-film forming foams (AFFF). Some perfluorooalkyl acids (e.g., carboxylates and sulfonates), are known to cause significant human and ecological environmental impact. Of current knowledge on the occurrence and content of specific PFAS in commercial products remains very scarce due to limited information available, thus impeding any precise assessment of human exposure and environmental release upon use. This study aimed at collecting 194 samples in Switzerland, and included a wide variety of liquid products likely to contain PFASs. Liquids tested included: impregnating agents, lubricants, cleansers, polishes, fire-fighting foams and other industrial products. By means of LC- and GC-MS/MS analytical techniques, a total of 24 different PFASs that comprised of over 41 targeted compounds were detected and quantified in 55% of 194 samples. PFAS quantification and compound profiling was found to be consumer-product specific. PFASs were mainly found in AFFF (90% occurrence) and impregnating agents (60% occurrence) with more than 100 ppm of targeted PFASs in both cases. Mainly ionic (carboxylates, sulfonates) and neutral (fluorotelomer alcohols, acylates) species were detected for AFFF and impregnating agents. Further investigation by Fast Atom Bombardment Mass Spectrometry (FAB-MS) on a set of AFFF samples allowed the characterization of 8 additional PFAS classes as major components in commercial AFFF formulations. Altogether, these results demonstrate the significant presence of numerous known and emerging PFASs in specific commercial products, which provide a source of further human exposure and environmental release.

**TU071**
Perfluorinated compounds (PFASs) in Northern Spain municipal solid waste landfill leachates

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PFASs comprised of over 41 targeted compounds were detected and quantified in the leachates of Northern Spain municipal solid waste landfill leachates. Compounds like PFHpA, PFDA and L-PFBS were measured in products likely to contain PFASs. Liquids tested included: impregnating agents, lubricants, cleansers, polishes, fire-fighting foams and other industrial products. By means of LC- and GC-MS/MS analytical techniques, a total of 24 different PFASs that comprised of over 41 targeted compounds were detected and quantified in 55% of 194 samples. PFAS quantification and compound profiling was found to be consumer-product specific. PFASs were mainly found in AFFF (90% occurrence) and impregnating agents (60% occurrence) with more than 100 ppm of targeted PFASs in both cases. Mainly ionic (carboxylates, sulfonates) and neutral (fluorotelomer alcohols, acylates) species were detected for AFFF and impregnating agents. Further investigation by Fast Atom Bombardment Mass Spectrometry (FAB-MS) on a set of AFFF samples allowed the characterization of 8 additional PFAS classes as major components in commercial AFFF formulations. Altogether, these results demonstrate the significant presence of numerous known and emerging PFASs in specific commercial products, which provide a source of further human exposure and environmental release.

**TU073**
Perfluorooctane sulfonate (PFOS) body burdens. Such PFOS-precursor compounds include perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamidoethanol (3M) in 2000-2002, trends in direct exposure to PFOS have been reported to be rapidly, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery
South African environment. This study focused on the analysis of selected PFCs from multiple environmental matrices in the Orange-Senqu River basin, one of the largest river systems in South Africa. Extraction and clean-up methods employed, varied for each matrix. Abiotic samples were extracted using sodium hydroxide followed by weak anion exchange solid phase extraction (SPE). Biotic samples were extracted using methanol and a combination of ultra high performance liquid SPE clean-up. Thereafter, the extracts were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS). The mean recoveries for PFCs varied between 18 – 105% for water; 33 – 122% for sediment, soil and tailings dam waste; and between 45 – 137% from bird eggs. Perfluorohexanoic acid (PFHxPA) and perfluorohexanesulfonic acid (PFHxSA) were not detected. Only 8% of water samples analysed had quantifiable PFCs, while soil, sediment, and tailing matrices also showed a low frequency of occurrence with only 24% of samples containing PFCs. Measurable concentrations within the aquatic environment were expected where the wild bird eggs were collected. Ninety percent of the bird eggs analysed had quantifiable concentrations of PFCs. PFOS had the highest prevalence, followed by perfluorodecanoic acid (PFDA) and perfluororononanoic acid (PFNA). The concentrations of PFOS in eggs of the same species from the same site were quite variable, probably due to their wide feeding ranges. This variability was further investigated by ensuring the validity of analytical identification, as well as looking at unique environmental exposure routes. PFOS identification was confirmed by retention time, mass transition, and isotope modelling against an authentic PFOS standard. Acknowledgements: Financial assistance was provided by the Department of Trade and Industry, the United Nations Office for Project Services and the National Research Foundation, South Africa.

TU071
PERFLUORALKYL SUBSTANCES (PFSAs) IN REMOTE HIGH ALTITUDE AREAS AROUND THE GLOBE
A. Dreyer, Eurofins GfA GmbH / Air Monitoring; E. Neugebauer, Eurofins GfA Lab Service GmbH / Research Development; M. Wang, K. Bridgen, L. Labunshka, D. Santillio, Greenpeace Research Laboratories; M. Greenpeace Perfluorinated alkyl substances (PFSAs) are compounds of environmental concern. Of the numerous PFSAs investigated in environmental samples, perfluorooctane sulfonate (PFOS) is listed as persistent organic pollutant and recently the German Environment Agency submitted a proposal at ECHA to restrict perfluorooctanoate (PFOA). In the past, several perfluorinated acids have been detected in snow or firm of high altitude regions in Central Europe. In the present study, we investigated the presence of these compounds in snow and water samples at altitudes up to 5000 m a.s.l. at 8 remote sites. These were located in South America, Asia, Northern and Central Europe. Snow and lake water samples were taken in duplicate and one field blank was taken per site. Whenever the amount of melted snow was too small, samples were combined. PFASs were detected at all sites in water and/or snow samples. Highest PFAS snow concentrations were observed in Slovakia (High Tatras) and Italy (Monti Sibillini) with perfluorononate (PFNA) being the compound which contributed the most. Lowest concentrations were observed in China. Highest PFAS concentrations in lake water samples were observed in Switzerland (Macun Lakes) followed by Russia (Altai) and Slovakia (High Tatras) usually with PFOA contributing the most. The occurrence of PFASs at high altitude lake and snow samples demonstrates the ubiquitous distribution of these compounds and indicate their atmospheric transport to remote regions, probably by degradation of atmospherically transported volatile precursors.

TU072
Accumulation and temporal trends of PFCs in finless porpoises (Neophocaena asiaeorientalis) collected from Korean coastal waters between 2003 and 2010
Y. Jeong, A. Shen, HanYang University; J. Lim, HanYang University; J. Lee, S. Lee, HanYang University; Y. An, Cetacean Research Institute (CRI), NRFRI, H. Moon, HanYang University / Marine Sciences and Convergent Technology Perfluorinated compounds (PFCs) are ubiquitous contaminants in marine environment, and there have been reports as severe sediment, and Suren, Grever, limited information is currently available on temporal trends of perfluorinated compounds (PFCs) in marine mammals from Korean coastal waters. In our study, accumulation and temporal trend of perfluorinated compounds (PFCs) were examined in liver samples of finless porpoise (Neophocaena asiaeorientalis) collected from Korean coastal waters between 2003 and 2010. Total concentration of PFCs (ΣPFC, sum of 3 sulfonates, and 12 carboxylates) ranged from 123 to 1322 ng/g wet weight (ww) (median: 447 ng/g ww) for 2003 (n=27), and 245 to 3690 ng/g ww (median: 840 ng/g ww) for 2010 (n=77). In both years, PFUnDA (C11) showed the highest contribution, followed by PFOS (C10) contributed collectively over 70% of the ΣPFC concentrations. Other longer-chain carboxylates such as PFHxS (C12), and PFTDA (C14) also had relatively higher contributions than other PFCs. To investigate age-, sex-dependent accumulation pattern, specimens in 2003, and 2010 were divided into 4 groups (immature male/female, and mature male/female). The median concentrations of PFCs were higher in immature female followed by immature male, mature male, and mature female. The concentration in immature male and female increased, the concentration of PFCS showed decreasing trend (p < 0.05), suggesting biodilution effect of PFCs. The ΣPFC concentrations of mature females in both years were lower than immature male/female, suggesting the excretion of PFCs via placental transfer and lactation. Compared to 2003, the ΣPFC concentrations were significantly increased in 2010 (p < 0.0001). Among major compounds (which contributes >5% to ΣPFC concentration), PFOS (182%) increased the most, followed by PFHxS (113%) and PFDA (78%). Our results suggest that a certain time would be needed for phase-out of PFCs in marine mammals due to the on-going use of products containing PFCs.

TU073
Particle-Induced Gamma-ray Emission (PIGE) as a Novel Screening Method for PFASs in Groundwater
G.F. Peaslee, Hope College / Chemistry; D.F. Lunderberg, Hope College / Chemistry Dept; E. Kitter, UMP Analytical LLC; W. DiGuiseppi, CH2M Inc; D. Bocek, Cornell AECOM; K. Fjeld, Department of Environmental and Molecular Toxicology, K. Barzen-Hanson, Oregon State University Because of the environmental persistence of per- and polyfluoroalkyl substances (PFASs), their ability to bioaccumulate, and their suspected human toxicity, new methods to identify these chemicals at trace levels in groundwater are needed. Particle induced gamma-ray emission (PIGE) spectroscopy is an established ion beam analysis technique that has been used to quantitatively measure light elements, including fluorine, in diverse target materials. An ex-vacuo PIGE method has been developed and is shown to be an effective and sensitive tool to determine the total organofluorine content in groundwater. Solutions of various concentrations of perfluorinated carboxylic acids and sulfonates were extruded onto the surface of a weak anion exchange solid-phase extraction (SPE) cartridge. This pre-concentration method was sufficient to use realistic volumes (40 - 80 mL) of distilled water to measure total fluorine. The total fluorine measured on these cartridges by PIGE correlates well with the initial PFAS concentrations for a broad range of anionic PFASs. Subsequent experiments with actual contaminated groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery tests have been performed as well as a post-extraction method to remove fluoride ions from the cartridges. This novel spectroscopic method for detection of PFASs can be adapted to detect environmentally-relevant PFAS concentrations in groundwater and may be very suitable as a pre-screening method to determine the presence or absence of PFASs in groundwater samples.

TU074
What do we know about indirect exposure to PFOS? In vitro metabolism of MeFOSE and MeFOSE A. Miralles-Marco, University of Birmingham / School of Geography Earth Environmental Sciences; L. Lucattini, A.M. Ballesteros-Gómez, Vrije Universiteit Amsterdam / Institute for Environmental Studies IVM; S.J. Harrad, The University of Birmingham / Division of Environmental Health and Risk Management College of Life and Environmental Sciences; P.E. Leonardis, Vrije Universiteit Amsterdam / Institute for Environmental Studies Perfluorooctane sulfonate (PFOS) is a widely known Persistent Organic Pollutant (POP) with an extensive historical use, until its restriction in the early 2000s. Due to its proven toxicity and health concern it was incorporated in the Stockholm Convention on POPs in 2013. After the phase out by the principal manufacturer (3M) in 2002-2003, trends in direct exposure to PFOS have been reported to decline. However, the indirect exposure through PFOS-precursors that are metabolized to PFOS has been suggested in recent literature. This PFOS indirect exposure could be an increasingly important contribution to the levels of PFOS in biological samples: estimates derived from modelling studies range current exposure from these PFOS-precursor compounds between 10% and 40% of total PFOS body burdens. Such PFOS-precursor compounds include perfluorooctane sulfonamides (FOSAs) and sulfamidethioethanes (FOSEs), which historically were synthesized to produce polymeric materials and phosphate esters, for their later use in surface coatings for textiles and paper products. However, there are still several uncertainties associated with these estimates and metabolism of not all PFOS precursors have been investigated so far. So, further in silico, in vitro and in vivo studies of these compounds are strongly required, as they would render the margin of safety between the current exposure limits and estimates of external exposure to PFOS alone. The work intends to define the metabolic pathways to human liver microsomes (HLM) and cytosol (HLCyt) of two of these “PFOS-precursors” (N-methylperfluoro-1-octanesulfonamido)-ethanol (MeFOSE) and N-methylperfluoro-1-octanesulfonamide (MeFOSEa) were investigated. Results show at least 4 expected metabolites for MeFOSE and 5 for MeFOSEa (e.g. PFOS, FOSA, hydroxylated metabolites, breakdown products and conjugations).

TU075
The remediation of PFAS contaminated airport soils S. Hale, NGI; H.Aassen, G. Stinde, Norwegian Geotechnical Institute; H. Arp, NGE Environmental Engineering; G.D. Broedvel, NGI / Department of Environmental Engineering; P. Cappelen, E. Wade, Norwegian Geotechnical
Institute; B. Straith, K. Moe, M. Jartun, Oslo Lufthavn AS

AFFF has been used historically at Norwegian airports and as such has resulted in contamination of ground water and soils with a multitude of per- and polyfluoroalkyl substances. The behaviour of PFASs in the unsaturated soil zone and the way in which this affects contamination of underlying groundwater is relatively unknown. This order suggests suggesting that comply with environmental legislations it is important to obtain an understanding of this behaviour. In Norway very low target values for the concentration of PFOS that are permitted in water and soil drive remediation efforts. Cleaning up to such low concentrations is technologically, logistically and chemically challenging. However, current data indicate that the need for the remediation of a soil from a Norwegian airport and contaminated with PFOS. Soil samples were taken from a currently used firefighting training facility and the concentration of a variety of PFASs was quantified. Concentrations of PFOS ranged from 6.4 μg/kg up to 2600 μg/kg and all of the soil samples were sandy in nature. Lab experiments were carried out to investigate leachability using a shaking test. The standard shaking test involves mixing the soil with water at a 1:5 ratio of 10 and revealed that the leachability of PFOS was up to 100 % of the total concentration. For both the total concentrations of PFOS and the leaching there were no clear trends with soil properties (Kw, organic matter content, Ca content, CI content, pH, Mn content, Fe content or SO4 content). Soil-water partitioning coefficients were calculated and were low for these samples (2 to 50 L/kg). Remediation trials were tested in the lab for a possible field clean-up effort and they consisted of soil washing to remove a great deal of PFOS and stabilisation to immobilize the remaining PFOS. Activated carbon, a clay and an organic matter rich compost soil were tested as the sorbents for stabilisation. Soil washing involves the addition of vast quantity of water and a removal of the contaminated fraction. The results of these trials will be instrumental in order to suggest the most feasible site remediation options in collaboration with the Norwegian Airport Authority and the Norwegian Environment Agency.

TU076 Performance and hazard assessment of fluorinated and non-fluorinated state-of-the-art DWR-polymer
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Durable water repellent (DWR) impregnation is applied in textile finishing to impart water and, depending on impregnation chemicals, oil and stain resistance to textiles. Following the phase-out of the most effective and predominating DWR-technology based on long-chain per- and polyfluoroalkyl substances (PFASs), the textile industry had to find suitable alternatives. This phase-out has resulted in a market where both fluorinated and non-fluorinated DWRs are available, divisible into three broad groups: short chain PFAS-based, silicone-based and hydrocarbon-based polymers. During our research in the SUPFES (Substitution of prioritised poly- and perfluorinated chemicals to eliminate diffuse sources) project, the alternative DWRs were assessed with regard to (i) their properties and connected performance; (ii) degradation processes resulting in diffuse environmental emissions, and (iii) hazard profile for selected emitted substances. We worked with DWR-chemistry and raw material producers to appropriately treat two commercially relevant types of fabrics with the DWR alternative chemicals (fluorinated and non-fluorinated). We compared the performance of the treated fabrics developed in the project by testing the following properties using industrial standardised methods: general properties, physical properties, DWR properties and stabilities of properties considering relevant stress-parameters. We demonstrated that non-fluorinated alternatives can have a competitive water repellency in comparison to short-chain DWR-technology based on long-chain per- and polyfluoroalkyl substances (PFOS). Persistent Organic Pollutants (POPs) in 2009. Several studies have been published reporting environmental or dietary exposure data, biomonitoring levels and health concerns. Notwithstanding reports that absolute levels of PFOS in human biomonitoring studies are declining; recent published studies of environmental, dietary exposure data and biomonitoring levels, highlight that uncertainties in terms of exposure and metabolism still remain, as the levels are decreasing slower than predictions suggest. To illustrate, indirect exposure to PFOS-precursor compounds followed by in vivo metabolism to PFOS has been highlighted as a potentially important source of current levels of PFOS present in biological samples. Current modelling studies estimate such PFOS-precursor exposure after the 5M phase out contributes between 10 and 40% of total PFOS body burdens. This evidence is supported by a small number of in vivo and in vitro studies, which report conversion rates up to 20% (e.g. EFOSE in long term exposed rats). As a consequence of this evidence, further study of the external exposure to these PFOS-precursors substances is required. In an effort to enhance understanding of why levels of PFOS are not decreasing as expected, this study reports dietary exposure to the following PFOS-precursor compounds: sulfonamides (FOSAs), sulfonamidoethanols (FOSEs) and sulfonamide alcohols (FOSAAs). This is achieved via analysis of 24 h duplicate diet samples from 61 Norwegian adults in 2014. These data will be linked to food questionnaires, to help identify which groups of food are the main contributors to dietary exposure to these compounds for this cohort.

Alternative approaches to animal testing for ecotoxicity assessments (P)
T.J. Norberg-King, U.S. EPA/ EPA MidContinent Ecology Division; M.R. Enomoto, ILSI Health & Environmental Sciences Institute; S.E. Belanger, The Procter & Gamble Company; J. Oris, Miami University / Department of Biology; K. Ryder, University of Heidelberg / Centre for Organismal Technical Committee’s “Concepts, Tools, and Strategies for Effluent Testing: An International Survey” facilitated by the ILSI Health and Environmental Stewardship and Sustainability Organization; T. Braunbeck, University of Heidelberg / Centre for Organismal Toxicology and Chemistry Laboratory; P. Dorn, PB Dorn and Associates; B. Farr, ILSI Health and Environmental Sciences Institute (HESI) / Health and Environmental Sciences Institute HESI; P.D. Guiney, Retired- S C Johnson & Son, Inc / Molecular and Environmental Toxicology Center; S.A. Hughes, Shell Health Americas / Global Discipline Lean and Manager Ecotoxicology; M.K. Sellin Jeffries, Texas Christian University / Department of Biology; R. Journel, SANOFI; M. Leonard, IOREAL SA; M.E. McMaster, Environment Canada / Health Americas / Global Discipline Lean and Manager Ecotoxicology; M.K. Syenac, SANOFI; G.J. Van Der Kraak, University of Guelph / Department of Integrative Biology; P.W. Wilson, Sanofi U.S., Inc / Health Safety and Environment; G. Whale, Shell Health / Shell Health

Whole effluent testing (also called Direct Toxicity Assessment) remains a critical long-term assessment tool for aquatic environmental protection. Use of animal alternative approaches for wastewater testing is expected to increase as more regulatory authorities routinely require fish and invertebrate tests for effluent assessments or as dischargers seek means to reduce overall animal (fish) use. To address the current state of the science associated with the use of alternative (3R) methods in effluent testing and assessment, a survey was conducted to identify the numerous and varied testing strategies to assess the potential environmental impact of effluents. This survey was aimed at summarizing the breadth of effluent testing approaches that are currently used in various regions and countries. Several questions were asked about the types of regulatory programs used to control the discharge of toxic chemicals, and at what level wastewater discharges are regulated. Specific information on what testing methods and species are used was requested, as well as whether non-animal alternative methodologies are used or considered for effluent/wastewater biological assessments. Finally, several questions included inquiries about the performance for various types of dischargers and what, if any, regulatory requirements or regulatory monitoring exists to regulate toxics wastewater. This survey provides the baseline knowledge to assess the role of biological testing for wastewater streams and identify opportunities to use novel strategies in an integrated manner to both optimize testing approaches and reduce reliance on animal tests on a global scale. The results of the survey were one of the cornerstones of the International Workshop titled “Concepts, Tools, and Strategies for Effluent Testing” facilitated by the ILSI Health and Environmental Sciences Institute (HESI) Animal Alternatives in Environmental Risk Assessment Technical Committee. Disclaimer: The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of Environment Canada or the US Environmental Protection Agency

TU079 How the integration of sub-lethal criteria and of genotoxicity assessment can improve the Fish Embryo Toxicity test sensitivity

210 SETAC Europe 26th Annual Meeting Abstract Book
It's restriction in the early 2000s. It was listed in the Stockholm Convention on Environmental Sciences; S. Poothong, Norwegian Institute of Public Health; S.J. fluorocarbon-based polymers. Future work will include risk assessment and life alternatives can have a competitive water repellency in comparison to short-chain considering relevant stress-parameters. We demonstrated that non-fluorinated testing the following properties using industrial standardised methods: general state-of-the-art DWR-polymers quantified. Concentrations of PFOS ranged from 6.4 of these samples (2 to 50 L/kg). Remediation trials were tested in the lab for a which report conversion rates up to 20% (e.g. EtFOSE in long term exposed rats). –

**TU080 Environmental Effects Monitoring as a tool for Effluent Testing**

M.E. McMaster, Environment Canada / Water and Science Technology Directorate Aquatic Contaminants Research Division; J. Parrott, Environment Canada / Water Science and Technology Directorate; M. Hewitt, Environment Canada / National Water Research Institute; K.R. Munkittrick, COSIA As part of the HESI Animal Alternatives in Environmental Risk Assessment Technical Committee's "Concepts, Tools, and Strategies for Effluent Testing: An International Workshop" various Case studies were presented on methods used in different countries to monitor effluent. In Canada starting in 1992 an Environmental Effects Monitoring (EEM) program was developed for the Pulp and Paper industry. This program requires each discharger to monitor fish and benthic invertebrates both upstream and downstream of its discharge to evaluate effects it may have on the receiving environment. The Government of Canada than uses this information Nationally to determine whether its existing discharge limits for the industry are protective of all receiving environments. The program is cyclical in nature with upcoming cycles dependant on results of the previous cycle on a mill specific basis. Effects in a receiving environment are confirmed in the next cycle, following confirmation the extent and magnitude of the effects are evaluated. Once effects are documented, investigation of cause and solution are then implemented. The pulp and paper program is now entering its 8th cycle and has implemented solutions to some of the effects documented. The EEM field program is then used to confirm improvements in the receiver following implementation. The program is aimed at protecting all metal mining operations in Canada 2002 and has considered implementation for municipal waste water discharges. It is also the same methodology implemented for the fish program in the Alberta Oil Sands Area. This program has been used or applied in a number of countries including Chile, Brazil and Uruguay. Discussions will include how this tool has been used for effluent testing in Canada and if it may be used as an alternative for laboratory effluent testing in certain cases.

**TU081 Implementation of ecotoxicological tests using freshwater planarians: Choice of cultivation medium by using the fecundity mean index as viability marker**

A. Weihe, B. Sones, University of Duisburg-Essen / Aquatic Ecology; T.C. Schmidt, University of Duisburg-Essen Freshwater planarians have been successfully used in several ecotoxicological methods. This invertebrate can respond to environmental contaminants from multicellular to behavioral changes. There are standard guidelines for biosysa or laboratorial cultivation. Consequently, there are problems in cultivation and in results replication and interpretation. This work aimed to use the reproductive success of two planarian species in laboratory as deciding factor for the cultivation medium’s choice. Sexual specimens of Girardia tigrina (Gt, n=45) and Schmidtea mediterranea (Sm, n=20) were donated by the Max Planck Institute for Molecular Biomedicine (Münster, Germany). During the following four weeks of adaptation all animals were maintained in artificial water. Using the donor’s medium (Münster’s tap water plus Montжу salt mix (MJ) – 1.6 mM NaCl, 1 mM CaCl₂, 1 mM MgSO₄, 0.1 mM MgCl₂, 0.1 KCl and 1.2 mM NaHCO₃, pH 7.5) but any cooccards were produced. After that, animals were split in 10 experimental groups according to specie (Gt and Sm) and cultivation medium (Eur: tap water plus and without MJ, commercial spring water and tap water without MJ and ultrapure water plus MJ). The population density was 0.025 org/ml. During the five testing weeks all vessels were held in darkness under controlled room temperature (17.9 ±0.9°C). After weekly feeding with bovine liver, the medium were completely renewed. To determine the mean fecundity index per test each the total number of collected offspring was recorded per experimental group. Each week was divided by the respective number of planarians. Then, the weekly FC’s of each group were compared using analysis of variance (ANOVA) followed by Tukey test, at significance level p <0.05. As a result, the higher and statistically significant FC values were 0.59 ± 0.4 (Gt) and 0.32 ± 0.4 (Sm) obtained in the groups just in tap water. The other groups presented lower FC values ranging from 0 to 0.3. In short, all observed FC’s are still lower than the expected for planarians. As an example, literature indicates a FC > 1 for Gt in healthy laboratory conditions. However, the fecundity mean index has been shown as a great marker to assess planarian viability in laboratory.

**TU082 A Critical Review of Mode of Action (MOA) Assignment for Ecotoxicology**

M.R. Embry, ILSI Health & Environmental Sciences Institute (HE); M.G. Barron, U.S. EPA/Gulf Ecology Division; A. Beasley, Dow / Product Sustainability Concepts, Tools, and Strategies for Effluent Testing: An International Workshop. The Program was adapted for use at all metal mines in Canada in 2002 and has considered implementation for municipal waste water discharges. It is also the same methodology implemented for the fish program in the Alberta Oil Sands Area. This program has been used or applied in a number of countries including Chile, Brazil and Uruguay. Discussions will include how this tool has been used for effluent testing in Canada and if it may be used as an alternative for laboratory effluent testing in certain cases.

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**TU083 Implementation of ecotoxicological tests using freshwater planarians: Choice of cultivation medium by using the fecundity mean index as viability marker**

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Acetylcholinesterase activity, in the orb web spider Agelenata redii (Scopoli 1763) (Araneae, Araneidae). Characterization and sensitivity to an organophosphate pesticide

C. Mazzia, University of Avignon / Biologie; T. Mazzia, University of Avignon; A. Pasquet, Université de Lorraine CNRS UMR; Y. Capoviez, INRA Institut National de la Recherche Aromatique; M. Rault, University of Avignon.

In the European context aiming at reducing the use of pesticides, biological control against pests has to be encouraged and require the protection of beneficial species. Thus studying the effects of pesticide use on current natural enemies is necessary. The spider Agelenata redii is a non-target species, present in apple orchards, and could be used for both chemical and ecological approaches. The objective of this work was to characterize the A. redii acetylcholinesterase (AChE) and to assess in laboratory conditions, its sensitivity towards Pyrinex®, an organophosphate insecticide currently used in orchards. We found that AChE is a membrane-bound activity, preferentially located in the cephalothorax, five times more sensitive towards Pyrinex® than the abdomen (19±5 and 3±0.48 μmol/g of protein respectively). This cholinesterase seems to belong to the true acetylcholinesterases as it preferentially hydrolyzed acetylthiocholine (Vmax = 19±1 μmol/g; Kcat = 0.12±0.032 mMol/L) than butyrylcholine (Vmax = 10±4 μmol/g; Kcat = 4.5±3.2 mMol/L) and exhibited a strong inhibition for high acetylcholine concentration. Moreover, the A. redii AChE is very sensitive to eserinine hydrochloride (selective ChE inhibitor) and is selectively inhibited by BW284c51 (a specific inhibitor of AChE) at concentrations in range of 10⁻³ to 10⁻⁴ M. No inhibition was observed using iso-OMPA, a selective inhibitor of pseudocholinesterases. After one-week exposure to chlorpyrifos-ethyl (Pyrinex®) at 0.33 times the normal application rate, a significant inhibition of AChE activity was observed in the cephalothorax, with a loss of 30% compared to the control. These results suggest that the AChE is a relevant biomarker to study the effect of Pyrinex® on A. redii because of its high sensitivity to organophosphate following pesticide exposure. Key words: Acetylcholinesterase; Spider; Ecotoxicology; Insecticides

TU084 Stepwise screening scheme for identification of animal testing alternatives

R. Weiss, Envigo CRS (Switzerland) Ltd. / Regulatory Consulting

When seeking for registration of a substance, GLP-guideline studies are normally employed on the substance as test item to obtain the basis for an assessment as required by the applicable guidance. Nonetheless there could be other possibilities to reach a meaningful conclusion. Furthermore a systematic evaluation assures avoidance of test artefacts, which may not only produce unnecessary cost and animal consumption, but also the requirement to repeat a study. Generally the following options should therefore be considered first: 1. Waiving an endpoint - Not assessing on an endpoint information requirement in a Short-term effect study may be unnecessary when long-term exposure results exist. In such case the acute toxicity testing may be omitted. 2. Waiving an experiment - Not testing for an endpoint information requirement in Apart from the three standard waiving arguments, i.e. testing does not appear scientifically necessary in because existing (literature) data suffice, testing is technically not possible and exposure based (below adequate Threshold of Toxicological Concern), assessment of one endpoint by data from a different one is possible: Endpoint-Analogy Read-Across: ‘n e.g. chronic fish toxicity from a carcinogenicity study using fish

TU090 Structural Alerts to Predict Excess Toxicity in the Pimephales promelas assay

J. van Oost, Department of Ecological Chemistry; R. Kühne, R. Ebert, M. Hillebrand, G. Schüttmann, Helmholz centre for environmental research - UFZ / Department of Ecological Chemistry

Safety REACH has created a number of chemicals have been evaluated in terms of risks for environment and human health. Further animal tests should be reduced. In aquatic toxicology, Pimephales promelas has been used widely as bioassay to study adverse effects of chemicals. While the toxicity of narcotic compounds can be predicted reasonably well through simple regression equations employing the octanol/water partition coefficient as hydrophobicity parameter, this is not the case for many pesticides. Since decades. Even though fish toxicity may be interpolated by a read-across approach within a limited chemical domain [1], there are no corresponding general relationships for compounds with specific or reactive modes of action on a mechanistic base. Taking a dataset of more than 900 organic compounds with experimental values for the 50% lethal concentration within 96 h exposure time (LC50), we have developed structural rules that enable the predictive discrimination between narcotic and non-narcotic compounds directed from molecular structure with reasonable results. The discussion includes a comparison with established structural alert schemes for daphnids, other MOA-models for fish toxicity and models predicting general reactivity. So far neither the daphnia structural alert models nor the MOA-models are suitable for use in the prediction of excess toxicity in the Pimephales promelas assay. The new model will be combined with the read-across model [1] in a consensus approach, and will be made publically available in computerised form via the software system "Fish ChemProp [2]. Acknowledgement: The SOLUTIONS Project is supported by the 7th Framework Programme (FP7-ENV-2013) of the European Union under grant agreement n°308347. This paper was presented at SETAC Europe's Fish Toxicology Conference in London, September 2011. Quantitative read-across for predicting the acute fish toxicity of organic compounds. Environ. Sci. Technol. 45: 4616-4622. [2] UZF Department of Ecological Chemistry 2015, ChemProp 6.3u http://www.ufz.de/index.php?en=6738

TU083 Pitfalls in abiding-long term fish toxicity testing instead of short-term to reduce animal consumption

R. Weiss, Envigo CRS (Switzerland) Ltd. / Regulatory Consulting

With respect to animal welfare the legal text of REACH Regulation (EC) No 1907/2006 does not include any specific requirements to apply animal tests. The regulatory requirements to submit the dossier at the respective deadline (Table, 9.1.3, column 1): ‘Short-term toxicity testing on fish: the registrant may consider long-term toxicity testing instead of short-term testing’. This is based on the consideration that the short-term test can be omitted if long-term data are generated directly (without a previous short-term study) and thus using less fish. Apart from the fact that the number of fish that are required for an Annex IX endpoint for which it is generally mandatory to submit a testing proposal before conducting a study. In consequence it is possible that no fish toxicity data would be available in the submitted dossier thus making it incomplete. In result the choice when following the column 1 recommendation would be to select between non-compliance with regard to data completeness (lack of fish data) or with regard to the duty to follow the test-proposal procedure. Another conflict in fish long-term testing exists in the registration of Medicinal Products for Human use (MPH). A long-term fish early-life stage test is part of the aquatic base set requirements, which has to be conducted if the maximum daily dose (DOSEii) exceeds the action limit of 2 mg/patient and day, but the DOSEii may still be reconsidered until final submission. Independent from DOSEii, an assessment for Persistence, Bioaccumulation and Toxicity (PBT assessment) potentially including fish toxicity testing is nonetheless triggered for substances whose lipophility exceeds a Log Kow of 4.5. In this PBT assessment the employment of an alternative test for acute fish toxicity is prescribed. When the substance is found not PBT/PvB or if the risk characterisation ratio is < 1, a long-term fish test must not be performed. The poster presents the details of these regulatory dilemmas. It is considered for guidance purposes only and should not be regarded either as a personal or company recommendation or a substitute for taking legal or other professional advice.

TU087 Statistical Approach to Inform the Study Design of an Inter-laboratory Comparing of In Vitro Methods (59 Fraction and Isolated Cryopreserved Haptenocytes) to Estimate Hepatic Metabolism in Fish


In a new project the development of an OECD Test Guideline (OECD Project 3.13), a multi-laboratory ring-trial is being conducted to evaluate in vitro methods used in assessing chemical biotransformation in fish involving both trout liver subcellular fractions (S9) and cryopreserved hepatocytes. Substrate depletion methods are used to determine the rate at which both test systems metabolize a series of test chemicals. The goal of the ring trial is to compare in vitro S9 and hepatocyte performance in measuring laboratory to laboratory variation in hepatocyte performance. In order to inform the study design for the current ring
trial, we examined the results from previous multi-laboratory studies with multiple chemicals which used trout hepatocytes and 59 fractions to estimate whole-body rates of chemical metabolism. An analysis of the existing data was conducted using a linear-mixed effects (LME) model which was fit with a restricted maximum likelihood estimation procedure to determine the sources of variability in the data and to estimate the degradation rate. The LME model analysis was subsequently used to set Monte Carlo simulations (>1000) varying the parameters that contributed to the variance in the model (number of time points, replications, chemicals, and laboratories) in order to identify the optimal experimental design for the current ring trial. For each simulation we computed the coverage probability of a 95% confidence interval and mean confidence width. The final study design was developed based upon a small confidence width and greater coverage probability in order to achieve a statistically robust study while including practical considerations such as cost, availability of biological material, time, and other critical resources.

TU088 Crossing frontiers - using zebrafish and nematode models for both ecological and mammalian toxicity screening purposes

G. Whale, Shell Health / Shell Health; M. Wildwater, University of Applied Sciences of Arnhem and Nijmegen; P. Zeman, ZF-screens BV; R. Peters, Utrecht University; C.J. Snalder, Shell International B. V. / Shell Health Risk Science Team; R. Dirks, ZF-screens BV; H. Spank, Leiden University / Institute of Biology; M. Rooseboom, Shell International Limited / Shell Health Risk Science Team

Under the principles of the 3Rs (reduction, replacement and refinement) there is a need for ‘animal’ alternative toxicity screening assays to assess potential risks to man and the environment. For human health hazard assessment these screening assays need to be translatable to humans, have high throughput capability, and from an animal welfare perspective be harmonized with the principles of the 3Rs.

To avoid some of the limitations of cell culture systems (i.e. lack of cell transformations capacity, multicellular or multi organ complexity) we have used Caenorhabditis elegans (nematode) and Danio rerio (zebrafish) larvae models as alternative assays for developmental and reproductive toxicity (DART) hazard assessment of some candidate chemicals. Both models follow the guidelines on the protection of experimental animals (Council of Europe, Directive 2010/63/EU) and are counted as non-animal tests. In our studies the results of the zebrafish and nematode studies were in alignment with data obtained from conventional mammalian toxicity studies indicating that these have potential as developmental toxicity screens. Although their applicability domain has yet to be established it is apparent that there are a number of conserved genetic pathways across the species.

In addition to providing mammalian toxicity screening data we believe that, with careful selection of dosing methods and consideration of exposure mechanisms, these models can also provide relevant information for environmental hazard and risk assessment to both aquatic and terrestrial organisms. By combining both mammalian and ecotoxicity considerations into the test designs these models could provide a unique opportunity for environmental and mammalian toxicologists to interact to develop powerful diagnostic tools for a range of applications. For example, such models could have significant potential in screening product categories (e.g. to assess which products should be selected for higher tiered testing) and in screening of water and groundwater samples. In the latter examples the models could be used to provide a more holistic indication of biotransformation potential from complex mixtures. The models and potential applications will be discussed in the presentation but we recognise that the true potential of such screens will only be realised if we are not ‘shackled’ by the boundaries of our classical specialisms and embrace an interdisciplinary approach from the outset.

TU089 Score sheets as a tool to enable consistent and comparable identification of the moribund stage in exposed fish in fish acute toxicity tests

M. Zegeleer, Fraunhofer IME / Ecotoxicology; K. Kuhl, Bayer CropScience AG; A. Meister-Winninger, IBACON GmbH; D. Sacker, Enivigo; H. Rufli, Ecotossolutions

The OECD Guideline 203, Fish acute toxicity test, is currently under revision by the OECD expert groups. In recent years and particularly with Directive 2010/63/EU taking effect in September 2010 including its requirement “to reduce the number of animals in toxicological testing to the minimum possible and ensure a painless death”, there has been on-going discussion how to replace this test method or at least ensure tests are compliant with the growing demands of animal welfare legislation. For this reason, the revision process is especially focused on the stage of moribundity. If this stage can unambiguously identified in an exposed animal it should be removed from the exposure tank and humanly killed to avoid unnecessary suffering. The question is whether the inclusion of the moribund stage in the evaluation of the study endpoints, is part of the expert discussion during guideline revision. One of the concerns to this approach is the high degree of subjectivity in the identification of moribundity, a process which requires experienced staff and clear-cut definition of clinical signs/sub-lethal effects to be assessed. The authors suggest implementing a scoring system, which is a standard requirement during performance of animal tests at least in Germany. In this scoring system, parameters are defined which enable different stages of suffering to be classified. The scale includes the stages of; no suffering, mild suffering, moderate suffering and severe suffering resulting in moribundity and eventually death of the fish. The parameters to be assessed include body coloration, breathing, swimming position and general appearance. Each stage is scored based on expression of expression of physiological and clinical signs. The sum of scores will induce different actions, which includes; enhanced monitoring, contacting the study director and declaration of moribund stage followed by humane killing. The authors present a harmonised scoring sheet provided by different European fish research laboratories. This presentation is intended to deliver further input into the revision process of OECD guideline 203.

TU090 The use of trout liver S9 fraction and cryopreserved trout hepatocytes in substrate depletion assays for the evaluation of xenobiotic biotransformation by<br>K.M. Johanning, KJ Scientific LLC / dba of Pura Vida Connections LLC; K.A. Fay, University of Minnesota - Duluth / Biology; M. Bernhard, The Procter & Gamble Company / Env Stewardship and Sustainability; I. Bischof, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; J.W. Davis, Dow Chemical Company / Toxicology and Environmental Research and Consulting; I. Domoradzki, Dow Corning Corporation / Health and Environmental Sciences; M.R. Emby, ILSI Health & Environmental Sciences Institute (HE); M. Halder, European Commission Joint Research Centre / DG Joint Research Centre IHCP EUR/L; J. Hu, The Dow Chemical Company / Toxicology and Environmental Research and Consulting; H. Laze, Givaudan Schwabe AG / Fragrances S. T. D. Natural Products; H. Peterson, DuPont; C. Schlechtriem, Fraunhofer IME / Bioaccumulation and Animal Metabolism; H. Segner, University of Bern / Centre for Fish and Wildlife Health; L. van der Wal, OECD; J.A. Weeks, S.C. Johnson & Son, Inc. / Environmental Safety

Acetylcholinesterase; Spider; Ecotoxicology; Insecticides

strong inhibition for high acetylcholine concentrations. Moreover, the

TU091 An intestinal fish cell line of rainbow trout (RTgut-GC) for predicting fish acetic acid toxicity of poorly biotransformed compounds.

H. Schug, Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Toxicology; M. Hülskamp, Eawag Swiss federal Institute of Aquatic Science and Technology / Department of Environmental Toxicology; F. Begnaud, Firmenich / DRAP; C. Debombeville, F. Berthaud, S. Gimenno, Firmenich; K. Schirmer, DuPont Nutrition & Health / Environmental Toxicology.

Various approaches are investigated with the goal to reduce and replace the use of animals for testing of chemical substances. Fish are being used in environmental risk assessment as well as for the testing of industrial effluents. Thereby the Fish Embryo Toxicity test (OECD 236) has already been validated as suitable alternative. Research onto the use of fish cell lines has shown as well to accurately predict fish acute toxicity. Specifically the in vitro cytotoxicity assay based on the rainbow trout (Onchorhynchus mykiss) gill cell line is suitable for a variety of organic chemicals. Other than the fish gill, the intestine of fish is considered a major route of exposure and uptake of chemicals, especially for persistent and very hydrophobic substances. When present in water, such substances may adsorb to biological material and make their way into the fish through consumption or dermal contact. The novel intestinal cell line of rainbow trout (RTgut-GC) is equally able to predict fish

SETAC Europe 26th Annual Meeting Abstract Book 213
acute toxicity for less water soluble compounds. If proven to be true, this approach may be used to test hydrophobic chemicals, such as fragrances, for regulatory purposes and to reduce the use of animals in a laboratory test. The cytotoxicity of selected hydrophobic fragrances towards the RTgut-GC cells was quantified using previously established protocols. The assays are based on the combination of three fluorescent dyes recorded at the outer cytoplasmic membrane and lysosomal membrane integrity. Fragrances were selected to cover a range of physicochemical properties. Specific extraction and quantification methods were developed and were successfully applied for samples from the cytotoxicity assays to quantify truly present chemical concentration. Dose-response curves were calculated based on nominal and measured concentrations and cytotoxicity values were expressed accordingly. Finally, effect concentrations showing 50% effect (EC50 values) were calculated and compared with concentrations showing 50% death in fish (LC50) from in vitro experiments. We were able to show a very good agreement between in vitro and in vivo values. In summary, the rainbow trout gall cell line (RTgut-GC) and the neuroblastoma cell line from rainbow trout (RTgut-GC) and applied for the testing of hydrophobic organic chemicals. As was previously shown for RTgill-W1, the RTGut-GC produced acute cytotoxicity data that are predictive of the acute fish data.

TU092

Assessment of early life stages to insecticides

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Early life stages of Senegalese sole (Solea senegalensis) were used to test the effect of triclosan (3-triclosan). The assays are based on the combination of three strategies: (1) morphological changes, (2) biomarkers to assess the effects of pesticides on earthworms. Among these mechanisms, thyroid related genes in order to assess the modes of actions of triclosan during early life stages. Our results suggest that triclosan might be disrupting chemicals, allowing an efficient method for screening thyroid disruptors.

TU093

Effects of the endocrine disrupter triclosan to early life stages of the marine fish "Solea senegalensis"

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The effects of zinc on the structure and functioning of a freshwater daphnia population. The aim of this study was to evaluate the effects of 2 insecticides, a carbamate (Pirimicarb) and an organophosphate (Lorsban), both inhibitors of acetylcholinesterase (AChE) on the enzyme activity on the NCV of Apurecostes caliginosa belonging to the genus of earthworms in the family Lumbricidae. NCV of the medial giant fibers was recorded on days 1, 2, 3, 4 and 7 following exposure to 3 insecticide doses (0.5X, 1X, 5X where X is the predicted environmental concentration based on the normal field application rate) and a control. AChE activity of A. caliginosa homogenates was measured at the end of the experiment. A significant decrease in NCV was observed on Pirimicarb on days 3, 4 and 7 for doses 1X and 5X. AChE activity was significantly decreased for the same 2 concentrations. For Lorsban, no significant changes were observed. This study shows that NCV could be an interesting new biomarker to study the impact of insecticides on earthworms. This physiobiomarker is easy to perform, cheap, quick, does not harm the earthworm and fills a missing link between other biomarkers.

TU094

Reducing repeat testing of regulatory vertebrate ecotoxicology studies through a critical assessment of Test Guideline criteria

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In practice a significant number of regulatory ecotoxicology studies do not comply completely with the corresponding OECD Test Guideline in terms of meeting the stated validity criteria and/or other parameters outlined in the guideline. This can result in much uncertainty for the decision makers evaluating the studies, and ultimately leads to requests for repeat testing when it is unclear whether these data are acceptable. Study directors may also decide to repeat studies due to a perception/uncertainty regarding whether regulatory bodies or sponsors will accept the study. In some circumstances, repetition may be completely justified if overall test performance is affected, for example where environmental parameters are not suitable for the test species. However, in other situations, deviation(s) from the guideline may not affect its scientific robustness and thus the overall test performance, but could still result in a rejection due to non-compliance with, for example, all the validity criteria set out in the relevant Test Guideline, or b) the study only being used as supportive information due to perceived shortcomings. This project, led by an expert steering group of international academics, regulators and industry scientists, aimed to explore the range of validity criteria and other required/recommended parameters within vertebrate ecotoxicology OECD Test Guidelines and other commonly used standard regulatory guidelines. The project will determine which deviation(s) (or magnitude of the deviation) fundamentally undermines study outcomes and overall test performance (hence necessitating repeat studies), and conversely which do not impact on the scientific quality of studies (thus negating the need for their repetition). For each Test Guideline the key validity criteria and other study parameters will be considered in detail. The project ultimately aims to provide robust evidence to support the expansion, where appropriate, of validity and/or other criteria within vertebrate ecotoxicology OECD Test Guidelines, to increase the utility of studies in regulatory decision making and decrease the need for scientifically robust in vivo studies to be repeated. Consideration of the criteria within the Test Guidelines that are undertaken most often and for which the majority of repeat testing occurs will be prioritised. This presentation outlines the objectives and proposed strategy to achieve the project aims.

TU095

Phase two of inter-laboratory validation of the Xenopus Embryonic Thyroid Signalling Assay

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The Xenopus Embryonic Thyroid signalling Assay (XETA) was designed as a screening assay to provide information on the potential of a test substance to alter the normal functions of the thyroid system. The XETA provides a rapid (< 72h) way to measure the response of embryonic stage tadpoles to potential thyroid disrupting chemicals, allowing a efficient method for screening thyroid disruptors. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the in vivo Amphibian Metamorphosis Assay (AMA - OECD TG231). The XETA method is based on the study of the metamorphosis of tadpoles after three weeks of exposure to a given chemical, and includes histological examination of the thyroid gland. XETA could provide an alternative test that can be performed quickly, providing information that would be useful for screening large number of molecules or testing environmental samples that couldn't be stored or sampled in large quantities. The objective of the validation study is to establish the relevance of the assay by assessing its sensitivity to detect disruption of the thyroid system by compounds active at different points within the thyroid signalling pathway. The XETA was intended to determine the performance and transferability of the assay across a range of both experienced and naive laboratories. XEETA utilizes live-fiving I. laevis embryonic-stage animals (stage 45 up to stage 47) in a multi-well format to detect modulation of thyroid receptor signalling by potential thyroid active chemicals. The assay is transcriptional-based, and uses a transgenic tadpole line containing the thyroid receptor gene under the control of a tetracycline responsive promoter with antibodies that work through various mechanisms. The phase one ring test experiments gave
the expected results for the chemicals chosen. A statistical approach was determined and a great consistency of the results was observed between laboratories. The XETA Phase I results demonstrate that the assay provides reasonable sensitivity with the chemicals tested and is reproducible, with a few exceptions, across replicates and labs. The XETA phase II started in fall 2015 and active chemicals with modes of action that were not covered in phase I are tested. Estradiol was selected as an inert chemical to challenge the assay and the statistical analysis procedure. The XETA protocol has been modified in accordance to lesson-learned during the phase one.

Interpreting Biological Effects of Metals and Their Mixtures (P)

TU096 Toxicity of equitoxic and non-equitoxic zinc and cadmium mixtures to Daphnia magna
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In contaminated ecosystems organisms are generally exposed to mixtures of many chemicals, while toxicity tests are often performed on single substances. In the sparse cases of mixture toxicity testing, almost exclusively equitoxic mixtures are tested in stead of non-equitoxic mixtures. The aim of this study was therefore to compare the toxicity of equitoxic and non-equitoxic mixtures of zinc and cadmium to Daphnia magna. In this purpose acute immobilisation tests were performed with (non-)equitoxic mixtures of Zn and Cd prepared according to the Toxic Unit concept using separately determined EC50 values for Zn and Cd. The EC50 values of Zn and Cd were 1017±127 µg/L and 127 µg/L respectively. The EC50 of the equitoxic Zn+Cd mixture was calculated to be 1.19 TU (CL: 0.81-1.75) and the Zn+Cd mixture was concentration additive. In contrast, the toxicity of the non-equitoxic mixtures of Zn+Cd ranged from more than Zn ratio to more than Zn+Cd ratio. This could be explained by differences in the slope of the concentration response relationships of the individual metals, but this was not the case since these values were additive for both Zn and Cd, respectively. An alternative explanation is that Zn and Cd possibly compete for binding sites at metallothionein-like proteins. Since non-equitoxic mixtures of Zn+Cd dominated by Cd contained less metals in total (because of the much lower EC50 value for Cd compared to Zn), the available metallothionein-like proteins pool could bind a higher part of the Cd dominated mixture than of the Zn dominated non-equitoxic mixtures. Consequently, the daphnids could withstand the Cd dominated non-equitoxic mixtures better than the Zn dominated non-equitoxic mixtures. Given the observed ratio-dependent additivity of Zn and Cd in non-equitoxic mixtures, it is concluded that deviations from equitoxicity may lead to over- or underestimation of the additivity of metals in mixtures.

TU097 Effects of metal mixture contamination on a freshwater community: can predictive mixture toxicity models be validated experimentally?
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In the EU and world-wide, the assessment and management of potential risks posed by chemicals is almost exclusively based on exposure and effect evaluations of individual substances. Previously, four different methods - combining the concentration addition (CA) model or the Independent Action (IA) model with Species Sensitivity Distributions (SSD) in four different ways - were used, compared and critically evaluated for their ability to predict metal mixture toxicity. However, the validity of these predictions needed to be further evaluated using dedicated laboratory and/or field studies. Therefore, the present study assessed the predictive capacity of these models by evaluating potential risks to a freshwater community exposed to metals occurring in ternary-mixture (Cu, Zn, Ni) mixtures. For this experiment, natural surface water and a naturally occurring planktonic community were collected from a pond in The Netherlands. These communities were exposed to metal mixtures in which each metal was present (1) at its biotic ligand model (BLM) based, bioavailability-corrected HC50 and HC50 concentration (i.e. the concentration that is hazardous to 5% and 50% of the species in a community, respectively) as well as (2) at ascending concentrations of metals with a fixed environmentally realistic metal concentration ratio of 12.6 for Zn:Cu and 2.6 for Zn:Zn (on a µg/L basis). Different community structure and function aspects including abundances of different planktonic groups and primary production rates were examined and compared to the fraction of Potentially Affected Species (PAS) per water body. The latter was calculated using CA and IA as predictive mixture toxicity models. In this way, we were able to test the accuracy of predictions of metal toxicity to freshwater communities made by these mixture toxicity models and thus contribute to the risk assessment of metal mixtures.

TU098 Evaluation of Toxicity Effects of Heavy Metal Contaminated Soils on Earthworm (Eisenia fetida) in a Mining Area of Guizhou Province
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Typical soils contaminated by multiple heavy metals were collected from a mining area in Guizhou province for 28d chronic toxicity assay. Activity of antioxidant enzymes, i.e. catalase (CAT) and superoxide dismutase (SOD), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and metallothionein (MT) in the earthworm (Eisenia fetida) were determined to evaluate correlations between these indicators and soil heavy metal concentrations. Feasibility of using these indicators to assess ecological risk of heavy metal contaminated mining soils was investigated. Results showed that the activity of CAT and SOD in earthworms increased firstly, and were inhibited thereafter during the exposure duration. This might suggest that activity of CAT and SOD in earthworm was raised to remove free radicals caused by stress of heavy metal contamination so as to adapt to changes of the environment. The accumulation of metabolites in earthworms after alleviating of metal toxicity inhibited activity of antioxidant enzymes, which led to decrease in CAT and SOD activity. When exposed to sub-lethal heavy metal concentrations, content of 8-OHdG in earthworms showed significant decrease with exposure time. With increase in exposure concentrations, more serious damages on 8-OHdG were observed. Within the exposure duration of 28 days, MT in earthworm played a detoxification role under the stress of heavy metal contamination. MT contents increased after 7 days of exposure and decrease thereafter. This study performs a comprehensive evaluation on ecological risk of typical heavy metal contaminated soils of a mining area based on toxicity assay. Results of this study will be helpful to guide remediation of heavy metal contaminated soils as well as its reuse after treatment.

TU099 Combined effects of interspecies interaction, temperature and zinc on Daphnia longispina population dynamics
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During the last decades, the earth’s climate has changed rapidly by human activity. Several studies have already investigated the effect of global warming on aquatic communities. However, most studies have, so far, focused on the effects of single stressors and ignored species interactions, while in nature, multiple stressors and species interactions are present simultaneously. Predicting and understanding the consequences of global warming and species interactions on the effects of chemicals on aquatic populations and communities are a primary goal of ecotoxicological research. The main objective of this study was to assess the combined effect of temperature, interspecific temperature and zinc (Zn) on a Daphnia longispina population. During an indoor microcosm experiment, D. longispina populations (5 adults and 5 juveniles) were exposed to 2 different temperatures (natural conditions: 16°C-19°C, global warming prospective: 21-24°C), 2 different periods of interspecific interactions of Brachionus plicatilis (0 & 2900/L) and 3 different Zn concentrations (background, HC5: 44 µg/l and HC50: 142 µg/l) and were varied in a full 2x2x3 factorial design. During the experiment general water quality (DOC, DIC, pH, and O2), and B. calyciflorus and D. longispina abundances were recorded every 3 days, for a period of 4 weeks. Interactive effects between the different stressors on the endpoints were determined by comparing observed abundance values with values predicted with the independent action model. The significance of the interactions (three-way and two-way) and the main effects were tested by using ANOVA’s (three and two-way) on log-transformed observed data. By evaluating the combined and interactive effects of temperature, interspecific interactions and Zn on a D.longispina population, it can be concluded that there are significant interactions between both stressors. Temperature had a positive effect on the D. longispina population growth in the control and HC50 treatment, but an opposite effect in the HC50 treatment. Initial analyses also suggests that the sensitivity of D. longispina populations to Zn was affected by the presence of interspecific competition. Overall, our study provides information that aids the discussion about integrating multiple stressor interactions and ecological interactions in risk assessment of chemicals.

TU100 The effects of zinc on the structure and functioning of a freshwater community: a microcosm experiment
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Combined effect of airborne heavy metals on A549 human lung cancer cells

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Metallic elements in farmed salmons, levels of toxic elements may be high in brown crabs at sampling sites exposed to potential sources of pollution. The brown crab (Cancer pagurus) is a unique model for both in vitro and in situ exposure of the human respiratory epithelium to metals. In this study, we investigated the response of human alveolar epithelial cells (A549) to mixtures of metals that are known to be toxic to human health at different concentrations. The results showed that the combined effects of metal mixtures are more toxic than the effects of individual metals. This indicates that the current risk assessment approaches for single metals may not be sufficient to assess the risk of metal mixtures. The study also revealed that the toxicity of metal mixtures is significantly affected by the concentration of metals and the type of epithelial cells used in the study. The results suggest that the combined effects of metal mixtures should be taken into account in the risk assessment of metal pollution.
(Cd) has been reported in this human food item. Due to trace contents of toxic elements, such as Cd, in aquaculture feed, or due to high excretion rates of toxic elements in farmed salmonids, levels of toxic elements may be high in brown crabs that feed in the vicinity of salmon aquaculture farms in Norway. Thus, the aim of the present study was to compare levels of elements in brown crabs caught close (ca. 300 m) to a salmon aquaculture farm in a reference farm (ca. 3000 m). Brown crabs were collected in the autumn during 2012-2015 at Mausund, Norway, and analyzed for element concentrations in the edible meat. Sea floor sediments were used for time integrated chemical characterization of the crabs’ feeding ground, and samples from the two locations were analyzed for the same suite of elements as the crabs. The study showed that in general, there were no differences in the concentrations of elements (B, Se, Cd, Sn, Cs, Hg, Pb, Al, Cr, Mn, Fe, Ni, Cu, Zn and As) in crabs from the two locations. Thus, salmon farming did not appear to affect levels of toxic element in brown crabs caught in the vicinity of salmon farms. However, relatively high levels of Cd were found in some of the crabs, irrespectively of location.

TU106 Nickel stress induces enzymatic and ultrastructural changes in the green microalga Ankistrodesmus falcatus. Implications for Ni regulation
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Martínez-Jerónimo, Escuela Nacional de Ciencias Biológicas-I.P.N. / Laboratory of Experimental Hydrobiology

Nickel is one of the main toxic pollutants discharged to the environment, at increasing concentrations in the last years due largely to industrial and mining activities. For example, in Mexico, water concentrations can be as high as 1.16 mg L⁻¹. The novelty of this study was to reveal that Ni production worldwide is about 1 500 million tonnes, among the products that contain nickel are stainless steel, coins, and batteries; for some of these products Ni cannot be substituted with other metals. Phytoplankton is an important community because it acts as primary producers, responsible of organic carbon synthesis and oxygen production (through photosynthesis), being the base of all the trophic webs in aquatic environments. Nickel is an essential micronutrient that plays an important role in cellular physiology in photosynthetic organisms, but it is toxic at concentrations higher than that required. For this reason, it is important to know how Ni affects the green microalga A. falcatus at an enzymatic level and in its ultrastructure when exposed to toxic Ni concentrations. In this study, A. falcatus was exposed to different Ni²⁺ concentrations, under continuous illumination, at 27 °C during 96 h to determine the IC₅₀ value. Considering the IC₀₀ value as basis, five concentrations were selected to make the subchronic assays: 1, 4, 8, 16, and 17 µg L⁻¹. Cell density was quantified daily and, at the end of the assay, the activity of catalase, glutathione peroxidase, and superoxide dismutase was measured; scanning (SEM) and transmission electron microscopy (TEM) observations were also done. A. falcatus was very sensitive to low concentrations of nickel, being the IC₀₀ value 17 µg L⁻¹. Additionally, catalase and glutathione activity increased when this alga was exposed to increasing Ni²⁺ sublethal concentrations. Moreover, SEM images demonstrated morphological alterations observing widened cells with abnormalities. TEM analysis revealed that nickel induced ultrastructural changes including an increased number of polysaccharide granules, reduction in the starch grains number, as well as anomalies in chloroplasts. At the lowest Ni concentration, significant damage in A. falcatus was observed; this concentration was much lower than the one established by the Mexican regulation as the allowable Ni concentration in North American waters (4 mg L⁻¹, average monthly). Results here obtained point out the need to review and modify the permissible limit to avoid toxic effects in phytoplankters.

TU107 Chronic metal mixture toxicity to Ceriodaphnia dubia: a meta-analysis and implementation in ecological risk assessment
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Although metals in the aquatic environment mostly occur as mixtures, ecological risk assessment has commonly been carried out based on a metal-by-metal approach. However, before metal mixture toxicity can be integrated into risk assessment frameworks, better knowledge on mixture toxicity effects during chronic exposure is needed. In several experiments conducted over the past years, we investigated chronic toxicity of Ni, Zn, Pb, Cu and Cd mixtures to Ceriodaphnia dubia in 6 different binary, ternary and quaternary combinations comprising in total 210 mixture treatments. In the present study, we used a meta-analysis approach to evaluate the following three risk assessment related questions: I) are mixture effects important?; II) is the concentration addition (CA) model a conservative model for mixture toxicity?; III) which of the two commonly applied model mixture reference models (CA or independent action (IA)) describes mixture metal toxicity most accurately? Overall, the mixture effect was significantly different from the effect of the most toxic metal. This indicates that mixture effects are important, and that the current risks evaluation approaches based on a metal-by-metal approach might not be protective for communities exposed to metal mixtures. In general, the CA model was the most conservative model. Additionally, very few (less than 1%) significant synergistic interactions related to CA mixture were observed. The latter result confirms that the CA reference model can be used as a conservative first tier in a tiered mixture risk evaluation scheme. Overall, the IA model described metal mixture toxicity to C. dubia more accurately than the CA model. Finally, a possible tiered approach is presented.

TU108 Mechanism of Heavy Metals Toxicity and Adaptation at Different Levels of Biological Organization in White Sea Starfish Asterias Rubens L.
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The purpose of this work is estimation sublethal effects of individuals and mixture heavy metals at different levels of biological organization on the White Sea starfish Asterias rubens L. Methods: At cellular level were estimated coelomic cells in exposure experiments in vitro and in vivo. Coelomocyte primary cell cultures was used to analyzed enzyme activity by photometric methods (α-NAR-assay) and gene expression by real-time PCR. At organism level were estimated starfish survival and behavior reaction in exposure experiments as well as metal bioconcentration and bioaccumulation in the all body without dissection. At population level were estimated size structure and abundance in points with heavy metals concentration differences. At community level was estimated association symbiotic fauna in different points also. Some of results: Studied area has high levels of As and Cd. Cd concentration in northern part of European part of Barents Sea. Copper concentration especially high in southern part of area. Asterias rubens L. has high ability of metal bioaccumulation and biomagnification. Heavy metals accumulation is combined based on low effect ratio for human health effect. Metal mixture effects are important, and that the current risks evaluation approaches based on a metal-by-metal approach might not be protective for communities exposed to metal mixtures. In general, the CA model was the most conservative model. Additionally, very few (less than 1%) significant synergistic interactions related to CA mixture were observed. The latter result confirms that the CA reference model can be used as a conservative first tier in a tiered mixture risk evaluation scheme. Overall, the IA model described metal mixture toxicity to C. dubia more accurately than the CA model. Finally, a possible tiered approach is presented.
regime plays an important role in determining the tolerance of zebrafish towards the effects of metal exposure.

TU110  
Acute toxicity of copper and zinc mixtures to the tropical freshwater cladoceran *Macrothrix flabelligera*.  
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MIXTURES of metals (such as copper, lead, zinc, etc.) and organic compounds are frequently found in industrial and municipal wastewaters, as well as in urban storm waters. All of these contaminant sources reach the aquatic receiving systems directly or indirectly, but, considering toxicological aspects, the water quality guidelines are generally obtained from acute and chronic bioassays testing individual compounds. Thus, such criteria can’t predict the interactions and effects of synergistic mixed mixtures, which is the most plausible way to find them in the environment. In an attempt to add some new knowledge to this scientific gap, the acute toxicity of copper (Cu) and zinc (Zn) was determined for the tropical freshwater cladoceran *Macrothrix flabelligera* and the association of both metals were investigated through ToxCalcMix analysis. For this purpose, the neonates were exposed to five concentrations of CuSO₄ (0.78 to 3.00 μg L⁻¹) and ZnSO₄ (27.9 to 79.4 μg L⁻¹) for 48 hours. The tests were replicated five times. Results from these acute tests were subjected to probit analysis using the PriProbit Program V 1.6.3.4 to determine EC₅₀ values. The average for the EC₅₀-48h/Cu was 1.4 μg L⁻¹ and EC₅₀-48h/Zn was 46.5 μg L⁻¹. Binary combinations of Cu/Zn had a significant effect on survival of *Macrothrix flabelligera* and this interaction was classified as additive, indicating the enhancement of adverse effects of mixtures of metals compared to the single metals. The results proved the importance of considering chemical interactions in water quality management. In addition, considering the need of searching for sensitive organisms to represent the realities of different ecosystems to achieve a greater success in water resources management, *Macrothrix flabelligera* can be suggested as a potential test organism for tropical studies.

TU111  
Ultrastructural effects of chronic exposure to Pb and Cd in hepatocytes of *Macrothrix* sp.  
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Lead (Pb) and cadmium (Cd) are two non-essential trace metals (TMs) widely detected in the surroundings of the former Pb and zinc smelter of Metaleurop Nord (Nord-Paque-Cadiz, Spain). Combining qualitative and quantitative approaches for TEM and SEM. These ultrastructural parameters have been compared with TMs levels in liver of 64 wood mice live-trapped around the former smelter in 2011. The parameters quantified as biomarkers of hepatocyte damage for TEM and SEM are: localization, ultrastructure, and amounts of α-glycogen, ultrastructural damage and electrodensity matrix of mitochondria, subcellular localization of Pb and Cd, and immunolocalization of matrix metalloproteins (MMP2 and MMP9). Our results show an increase of all parameters related with cell damage and degeneration in relation to the gradient of pollution. Interestingly, α-glycogen accumulates in relation to Pb levels, whereas mitochondrial damage related to Cd levels, i.e. accumulated in α-glycogen and inclusions of Cd were observed in damaged mitochondria; and heavier localization of cytoplasmic MMPs was found in hepatocytes of mice from polluted sites. These findings indicate the necessity for an effort to study physiological effects of metal pollution at real world conditions in selected species that had previously demonstrated their suitability as bioindicators such as the wood mouse. Then, imagining of ultrastructural effects of chronic exposure to metals offers crucial biomarkers to understand toxic effects in natural populations exposed to anthropogenic stressors such as TMs.

TU112  
Comparison of Cadmium accumulation and subcellular partitioning between two marine fish species.  
G. Le Croizier, LEMAR UMR CNRS/BIOIRL/INREMER  
Kinetics of accumulation and elimination in liver and muscle of two marine fish species, *Dicentrarchus labrax*, and *Solea senegalensis*, dietary exposed to 3.5 ppm Cd, were investigated. During 60 days, fish were fed with a prepared diet containing Cd, after which a 30 day depuration period was carried out. No significant impact of Cd on growth occurred; however, significant accumulation of Cd occurred in contaminated fish liver and muscle for both species when compared to unexposed controls, with higher levels of accumulation observed in *D. labrax*. Concerning Cd depuration, 30 days seems to be a too short period to observe elimination in liver and muscle. Quantification of metallothioneins (MT) in liver by electrophotometric method carried out. Results show that basal liver Cd in *D. labrax* liver are higher than in *S. senegalensis*, although exposure dose seems to be too low to induce MT synthesis. Subcellular partitioning of Cd in liver was also investigated, and results show two very different patterns of metal intracellular repartition in these marine species: *D. labrax* accumulates more Cd in detoxified fractions (about 50%) than *S. senegalensis* which accumulates Cd in sensitive fractions (about 90%). Our results show a differential accumulation and repartition of Cd between two marine fish species, and underline variability of interspecific sensitivity in metal contamination.

TU113  
Bioaccumulation of manganese in an endangered carnivorous marsupial. How does manganese exposure from mining operations affect the health of northern quoll (*Dasyurus hallucatus*) on Groote Island?  
A.B. Amir Abdul Nazir, School of Biological Sciences; F.A. van Hippel, University of Alaska Anchorage / Department of Biological Science; R. Wilson, University of Queensland / School of Biological Sciences  
Manganese (Mn) is an element vital for normal cellular processes in all living organisms but deleterious in elevated concentrations. Over-accumulation of Mn in the brain has been reported to potentially cause a condition known as ‘manganism’, a neurodegenerative disorder characterized by a range of motor disabilities, psychiatric and cognitive symptoms. Little is known about how prolonged exposure to lower, atmospheric levels of Mn affects human and whether such exposure impacts natural wildlife populations. To address this knowledge gap, we explored the effect of environmental Mn exposure derived from a large Mn mine on the health of a population of northern quolls (*Dasyurus hallucatus*) on Groote Island, Australia, this island has a major Mn mining operation since 1967. In our study, we measured how proximity to the mine was associated with the level of Mn in the environment and the level of Mn accumulated in the hair and organs of the northern quoll. In addition, we are examining how the level of exposure of northern quolls to environmental Mn is associated with (i) motor control deficits, and (ii) altered cortisol level; both of which would indicate long-term health problems from Mn over-exposure. As northern quolls are both terrestrial and semi-arboreal, and thus must consistently traverse variable terrain in complex environments to hunt for prey and find mates, locomotion capabilities are direct measures of fitness and thus relate to the viability of the species on the island. Measuring cortisol level also has health implications for wildlife and humans since elevated cortisol levels are associated with many deleterious endpoints, such as reduced immune response and various disease states. The proximity to the mine was associated with the level of Mn accumulated in the hair and some organs in northern quolls. As Mn levels in hair are shown to be predictive of levels in these organs, so hair is a valid tissue to use for low impact studies (e.g. endangered species). Mn level also had a significant reverse correlation with cortisol level in breeding season, suggesting compromised immunity and reproduction for affected quolls. We demonstrate that ongoing research and monitoring will aid in understanding the costs associated with prolonged, low-level Mn exposure to wildlife health.

TU114  
Assessment of the toxicity of metal mixtures in microalgae *Microcystis sp.*.  
A. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia Laboratorio Alejandro Villalobos; J. Morales Torres, Universidad Autonoma Metropolitana Iztapalapa / CIENCIAS DE LA SALUD  
In this study, an evaluation the effects of metals mixtures: Cd + Cr, Cd + Cu, Cd + Hg, Cd + Mn, Cd + Ni, Cd + Pb, Cd + V, Cd + Zn and Cd + Cu + Hg + Mn + Ni + Pb + V + Zn in the freshwater alga *Microcystis aeruginosa* were performed with duration of 72 hours to determine the EC₅₀. Subsequently tests with duration of 8 days in which the microalgae were exposed to two subletal concentrations (EC₁₀ and EC₅₀) were performed to evaluate five biomarkers: Population growth, concentration of chlorophyll, carotene production, proportion of phenols and cyanophycin production. The toxicity of metals based on EC₅₀ values were (from highest to lowest toxicity): Cu > Cr > Hg > Cd > Zn > Ni > Mn > Pb > V > B. In bioassays with mixtures of metals was observed that the most harmful combinations was Cd + Cu and the mixture of all metals, agree toxic units calculated. The type of interaction observed in these mixtures was potentiation, with a value of magnification of the effect of 2.3 and 2.5 respectively. In bioassays with mixtures Cd + Cu and Cd + Ni, was observed a decrease in chlorophyll levels. In tests with all mixtures it was detected increased levels of carotenoids and phenols. The highest levels of lipid peroxidation were obtained in bioassays with Cd + Cu + Mn + Cd + Zn mixtures. Because EC₅₀ values for Cu, Cr, Ni and Zn metal are lower than those established by the NOM 001-SEMARANT for diseases in aquatic systems on Groote Island. Located in the Warm Temperate Envinroment, Australia, this island has a major Mn mining operation since 1967. In our study, we measured how proximity to the mine was associated with the level of Mn in the environment and the level of Mn accumulated in the hair and organs of the northern quoll. In addition, we are examining how the level of exposure of northern quolls to environmental Mn is associated with (i) motor control deficits, and (ii) altered cortisol level; both of which would indicate long-term health problems from Mn over-exposure. As northern quolls are both terrestrial and semi-arboreal, and thus must consistently traverse variable terrain in complex environments to hunt for prey and find mates, locomotion capabilities are direct measures of fitness and thus relate to the viability of the species on the island. Measuring cortisol level also has health implications for wildlife and humans since elevated cortisol levels are associated with many deleterious endpoints, such as reduced immune response and various disease states. The proximity to the mine was associated with the level of Mn accumulated in the hair and some organs in northern quolls. As Mn levels in hair are shown to be predictive of levels in these organs, so hair is a valid tissue to use for low impact studies (e.g. endangered species). Mn level also had a significant reverse correlation with cortisol level in breeding season, suggesting compromised immunity and reproduction for affected quolls. We demonstrate that ongoing research and monitoring will aid in understanding the costs associated with prolonged, low-level Mn exposure to wildlife health.

218  SETAC Europe 26th Annual Meeting Abstract Book
action of the discharges and different tensions to prevent irreversible deterioration of the populations in the medium and long term.

Amphipods as models to investigate toxicity of environmental contaminants at the land-sea interface (P)

TU115 Development of assays to determine anxiety-like behaviours in crustaceans. S.A. Kohler, University of Portsmouth / Biological Science; M.C. Bossus, Y.Z. Gravez, M.O. Parto, University of Portsmouth; A. Ford, University of Portsmouth / Biological Sciences

Concerns over psychoptics drugs found in the aquatic environment have necessitated the development of behavioural assays to determine the effects and risk posed by these compounds. Biogenic amines such as serotonin, dopamine and epinephrine are known to control complex behaviours in crustaceans including aggression, movement, and locomotion. However, it is often difficult to differentiate these without carefully designed experiments. For example, negative phototaxis can be interpreted as scototaxis and positive phototaxis can be interpreted as anxiety-like behaviours. Some recent studies have suggested that invertebrates might be highly sensitive to antidepressants, such as the Selective Serotonin Reuptake Inhibitors (SSRIs), the Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) and Serotonin Antagonist and Re-uptake Inhibitors (SARI)s. These drugs alter neurotransmitters in the synaptic cleft by inhibiting its re-uptake into the presynaptic cell, which result in increased stimulation but are also known to affect multiple receptor targets. This ongoing study is currently analysing the effects of a variety of antidepressants on the behaviour of Palaemonetes pugilator. Specimens of the amphipod, Echiurigosmarinus marinus were exposed to the most prescribed SSRIs (citalopram, sertraline and fluoxetine), SARI (trazodone) and SNRI ( duloxetine) at environmentally relevant concentrations from 0.001 to 1 μg/L during acute (1 hour and 1 day) and chronic (8 day) exposures. The amphipods were kept under the DarkVision system with EthoVision software for behavioural analysis during 12min alternating dark/light cycles. Results show that light had a significant effect on velocity (P<0.001) indicating escape-related behaviour. Antidepressant concentrations had a significant effect (P<0.01) on velocity for Duloxetine (1hr, 1day & 8days); Sertraline (1hr & 1day) and Fluoxetine (1day); no significant effects were observed for citalopram and trazodone (P>0.05). Analyses are ongoing to further differentiate scototaxis, phototaxis, anxiety-like and escape response behaviours. Preliminary results indicate duloxetine does not influence use of space in a 6-wall plate system at any of the exposure concentrations. However, we are currently investigating whether this result may be due to experimental design and test chamber space available to the test organism. These results may have implications for future study design of these types of experiments.

TU116 Frequency of intersex phenotypes of Gammarus pulex (Amphipoda) upstream and downstream from effluent of sewage treatment works. A. Love, Sparsholt College / School of Biological Sciences; A. Ford, University of Portsmouth / Biological Sciences; N. Crooks, University of Brighton / School of Pharmacy and Biomolecular Sciences

There is unequivocal evidence from around the world that sewage treatment effluent has the capacity to cause reproductive endocrine disruption in many fish species. However, the capacity of these effluents to cause reproductive aberrations in invertebrates communities is less clear. This study aimed to determine the sex ratios, intersex frequency and fecundity of Gammarus pulex around two sewage treatment works (SW) of similar output discharging into two chalk rivers (the Test and the Anton) in the UK. G. pulex were collected at approximately bi-monthly intervals from several points upstream, downstream, and at the point of effluent discharge. Gammarus were measured and categorised as male, female, male intersex and female intersex and embryos were counted from ovaries from females. Greater incidences of intersex were observed downstream of the WTP although only significantly (p < 0.05) on the river Test. Though the overall incidences of intersex were quite low, they increased by two to three times at and below the effluent discharge point (~1% upstream and ~3% downstream respectively) at both sites. The proportion of males and females that were intersex also increased significantly (p < 0.05) around the discharge point (~3.1% and 4.1% respectively) compared to upstream (~0.7% and 2.1% respectively). Unlike other similar studies, there was no difference in the male/female ratio seen between the sample sites, however, there was a significant difference in the sex ratios seen between the rivers. The Anton had a much greater difference in the percentage of males versus females (36.7% and 60.2% respectively, compared to 48.8 and 48.9% in the Test). Fecundity varied between sample points but at both rivers it was significantly higher at or near the discharge point (~100 mg-1 female BW) compared to the upstream site (~8.0 mg-1). In other studies, STW effluent has been associated with increases in the fecundity and frequency of female Gammarus as well as changes in ovariian development. These changes have been associated with elevated oestrogenic compounds. To date there have been no reports of elevated intersex frequency in amphipods associated with STW effluent; however, amphipod intersex frequency has been shown to increase around sites impacted by industrial pollution. Suggested causal mechanisms include later feminising the animals directly, or indirectly through immunosuppression and associated increases in feminising parasites.


In animals, the embryo stage could be more susceptible to a toxic exposure than later stages. Nowadays, several invertebrates such as fish and frog embryos, are extensively used. Nevertheless, for invertebrates such studies remained rare. The toxicity of environmental compounds in early life stages is of importance because embryo toxicity has been shown directly implicated in population decline. The aim of the present study is to develop an ecotoxicological assay in embryos of Gammarus fossarum, it is an amphipod widely distributed in European freshwater systems. As it contributes to the degradation of the organic matter and it serves as food for many macroinvertebrates, fishes and amphibians, it plays a major role in the food web. After fertilization, embryos were gently removed from the females and directly exposed to increasing cadmium (Cd) concentrations (0 μg/L, 2 μg/L and 4 μg/L) until they hatched. Cadmium is a non-essential metal widely detected in freshwater ecosystems. Gammarus spp. are particularly sensitive to this metal. Daily, the mortality was recorded. After hatching, we measured in the neonates: i) the mass; ii) the locomotor activity and iii) the total phenoloxidase activity (both phenolphoxidase and phenoloxidase activities). In arthropods, phenoloxidase is an enzyme involved in the sclerotization of the exoskeleton, the melanization and the immunological response. By comparison with the control group, we observed a slight – but not significant – increase of the mortality during the hatching period in the group exposed to 4 μg/L. The mass of the neonates was not significantly altered by Cd exposure, whereas the locomotor activity was significantly reduced in the group exposed to 4 μg/L. Surprisingly, with increasing Cd concentrations, the phenoloxidase (an inactive precursor of phenoloxidases) activity was increased whereas the phenoloxidase activity was decreased. These results suggested that embryo of Gammarus fossarum might be used as a sensitive life stage in ecotoxicological studies.

TU118 UV irradiation and leaching reduce adverse effects of imidacloprid contaminated leaves for the shredder Gammarus fossarum D. Engler, Institute for Environmental Sciences / Institute for Environmental Sciences; C. Neubaier, University of Koblenz-Landau / Institute for Environmental Sciences; J. Zubrod, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment Setac Europe 26th Annual Meeting Abstract Book

Imidacloprid (IMI) is a widely used systemic neonicotinoid pesticide which enters surface waters along with treated plants. The effects of IMI in the aquatic environment are well documented; however, few studies have investigated the effects on species other than fish such as invertebrates. We have exposed Gammarus fossarum to neonicotinoid concentrations. To assess alterations in the toxicity of the photolabile and water soluble neonicotinoid imidacloprid within leaves we used the key leaf-shredding macroinvertebrate Gammarus fossarum (Amphipoda) as test species, while its feeding behaviour and consumption rate is an ecotoxicological response variable. In two independent experiments, Gammarus was exposed to leaves from imidacloprid-treated (0.15 g active ingredient per cm3 trunk diameter at breast height; soil applied) black alder (Alnus glutinosa) trees which were either a) leached in water (for 1, 3 and 7 days) or b) irradiated with UV light (for 24 h at UV-A: 41 W/m2 and UV-B: 0.15 W/m2; in addition to 1 day leaching in water) prior to use in the experiment. Contaminated leaves without prior UV or leaching treatment as well as leaves from untreated trees served as positive and negative control, respectively. Both experiments revealed a by ~70% reduced feeding rate of Gammarus when exposed to contaminated leaves without prior treatment compared to the uncontaminated negative control. In contrast, treatment of leaves with UV-B irradiation (24 h) and leaching (7 days) completely reduced the adverse effects on gammarids’ feeding. Therefore, although consumption of neonicotinoid-contaminated plant material adversely affects leaf-shredding organisms and, hence, leaf litter breakdown, results of this study suggest a mitigating effect of UV light exposure and dwelling time in water.

TU119
Development of a SRM-based multiplexed quantitation of protein biomarkers in *Gammarus fossarum*

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Biomarkers are recognized as relevant tools for diagnostic and hazard assessment of aquatic systems. However, few are used in invertebrate species studies. It mainly results from the lack of genomic and/or proteomic data, preventing the development of specific and direct method for the quantification of biomarkers. Recent advances in novel proteomics approaches offer a promising forward strategy to discover proteins in non-model organisms. This approach was applied in the amphipod *Gammarus fossarum*, generating a large proteome dataset and offering the opportunity to develop direct and specific analytical mass spectrometry methods. Selected reaction monitoring (SRM) is a liquid chromatography mass spectrometry based technique for the quantification of a specific protein of interest. SRM has the ability to detect low amounts (to the picograms per milliliter range) of a targeted analyte, with tryptic peptides as multi-indicators of proteins. Absolute quantification can be performed by the addition of an isotopically internal standard. Targeted proteomics can be performed to quantify simultaneously multiple peptides per protein from the same biological sample. Multiplexing analyses by mass spectrometry is in this case straightforward and offers innovative perspectives in ecotoxicology. Using less biological material, resource investments in terms of organism and experimental maintenance are reduced and yield indicators on each biological sample. Herein, we described a highly-multiplexed MRM-based assay for the determination of protein biomarkers in *Gammarus fossarum*. The assay uses 71 stable isotope-labelled peptide standards for the quantification of 40 putative biomarkers of candidate proteins related to essential physiological functions including reproductive cycle or defence mechanism. A good linearity was observed for the spiked peptides in extracted individual and, for the protocol of concept, it was determined that the assay worked between 0 and 20 % and between 80 and 120% respectively. The assay presented in this study is easy to use, robust and sensitive. High-throughput capabilities will be established to quantify protein biomarkers in *Gammarus fossarum*. Validation of assay was performed with protein changes assessment throughout contrasted physiological process (sex, reproductive status).

**TU120**

**Dietary uptake of systemic fungicide contaminated plant material affects energy processing and growth of a leaf-shredder**

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Leaf-litter containing microinvertebrates can be negatively affected by fungicides if their or their food are exposed to these substances via the water phase. Systemic fungicides, on the other hand, may result in an additional effect pathway; these substances penetrate plants and remain active within the vegetation, rendering possible the transport of contaminated plant parts into aquatic systems during leaf fall or storm events. In a previous study, we showed that the shredder *Gammarus fossarum* preferred microbially conditioned leaves treated with systemic fungicides over control leaves. This may be related to a higher production of carbohydrates but lower investments in self-defense of plants and consequently in the development of a microbial community more palatable for *Gammarus*. Therefore, we tested if this putatively higher quality of leaf material treated with systemic fungicides would affect ingestion rates and physiological processes when such leaves are fed over the long-term. We investigated *Althus glutinosus* trees (n=3) with fungicide-free water, a mixture of four systemic fungicides at recommended field rates (axoxyazin 200 g/ha, cyprodinil 400 g/ha, quinoxyfen 100 g/ha, and tebuconazole 200 g/ha; FR), and at rates 10-times above the field levels (FR10). The fungicides were applied twice, with a six-week interval. Leaves were picked from the trees six weeks after the last application and were microbially conditioned for 12 days before fed to *G. fossarum* in a 24 days lasting bioassay with a food and medium renewal every 8 days. Gammarids’ leaf consumption, feces production and final body mass were measured. While there were no significant differences for feces production, gammarids fed fungicide-treated leaves showed a higher leaf consumption (FR by −30%: FR10 by −12%), while this difference was only significant for FR. Also final body mass tended to be higher in the fungicide treated (FR by −5%: FR10 by −12%) but was significant only for FR10. Our data suggest that systemic fungicides can affect shredders’ energy processing and physiology if applied at field levels. This effect may be explained by the usually higher frequency of fungicide applications (up to 20 per season) than used during this study, further experimentation is recommended to test the effects of more realistic application patterns and their potential consequences for ecosystem structure and function.

**TU121**

**Are waterborne and diet-related effects of fungicides transferable among leaf shredders?**

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Leaf litter breakdown is of prime importance for the energy budgets of detritus-based stream ecologists. Therefore, an important question to answer is how toxicants are transferred to shredding invertebrates. To this end, the present study investigated the effects of a mixture of five current-use organic fungicides (MOF) and copper (Cu) on larvae of the calidrisid Chaeotoperix villosa. We assessed the fungicides’ effects on *C. villosa*’s feeding activity (waterborne path) as well as food choice when offered leaf material microbially colonized in the presence or absence of fungicides (diet-related path). *C. villosa* was less sensitive towards waterborne toxicity than *G. fossarum* with EC20-values being higher by a factor of three and six for MOF and Cu, respectively. In contrast, for food-choice, significant preferences where detected for *C. villosa* at exactly the same concentrations as for *G. fossarum*; a significant preference for unexposed over MOF-exposed leaves was observed at 600 and 3000 µg/L, which is probably explained by a repellent effect. In contrast, the larvae preferred leaves exposed to 250 and 500 µg Cu/L over unexposed leaves, which is most likely explained by Cu-induced shifts in the leaf-associated fungal community. Waterborne toxicity thus appears to be of comparably little relevance for the assessed caddisfly since its low sensitivity (compared to *Gammarus*) results in effect concentrations much higher than field levels. In contrast, diet-related effects seem highly transferable among shredder species. Assuming also a similar relationship between effect threshold concentrations for food-choice reactions and physiological fitness effects due to long-term feeding on fungicide-affected leaf material (factor of ~10) as observed for *Gammarus*, effects via this pathway may also be detected at field-relevant concentrations for *C. villosa*. Currently, we experimentally test this assumption, while affirmative results would further support the importance of this pathway for shredders.

**TU122**

**Corophium volutator: a model marine regulatory test species for chronic sediment testing.**

M. Fox, Cefas / Environment and Animal Health

The amphipod, *Corophium volutator* is a model test species for marine and estuarine sediment toxicity testing. They are ecologically important species of the estuarine habitat involved in sediment bioturbation and as a food source to a number of birds and fish including the Redshank, Dunlin and Flounder. *C. volutator* are already being used as a test species in the 10 day sediment rework/ecotoxicity test (OSPAR PARCOM Procotol 1995 Part A) as part of the offshore chemical notification scheme (OCNS). There is a growing need in regulation of chemicals for test guidance on chronic marine sediment testing. The American Society for Testing of Material (ASTM) have guidance documents for chronic amphipod testing using amphipods found within United States coastal waters however there is a lack of such guidance for a European equivalent species. The European Food Safety Authority (EFSA) have produced a technical guidance document to assess the safety of feed additives for the environment, this includes feed additives for aquatic animals. The guidance for feed additives in aquatic animal feeds focuses on chronic marine sediment testing and suggests using the ASTM methods. However, the ASTM methods are for North American specific test organisms, which are different to those species available in the UK and Europe and are not environmentally relevant to European waters as native species. The poster will contain methodology and data from a series of Good Laboratory Practice (GLP) 28 day chronic tests which assess the survival and growth (net weight and length) of *C. volutator*. The tests conducted have been submitted and accepted for use in regulatory dosiers. Further work is being conducted to assess the potential of an extended 75 day test to incorporate a reproduction protocol.

**TU123**

**Population Dynamics of Parhyale hawaiensis (Crustacea: Amphipoda) in laboratory culture: Comparative endpoints as a tool to measure ecotoxicological stress.**

B. Barstln, G.L. Ferreira, SCHOOL OF TECHNOLOGY UNICAMP / LEAL
Laboratory of Ecotoxicology and Environmental Microbiology School of Technology; L. Allegreti, LEAL Laboratory of Ecotoxicology and Environmental Microbiology; G. Umbuzeiro, SCHOOL OF TECHNOLOGY –UNICAMP / LEAL; M. Flynn, LEAL Laboratory of Ecotoxicology and Environmental Microbiology

An important step in ecotoxicology studies is to find an appropriate model organism that can help both acute and chronic toxicities with reproductive standardized and comparative results on individual and population levels. The objective of this work is to describe the life cycle, fitness impairment and population dynamics of a laboratory culture of Parhyale hawaiensis population throughout the construction of age-specific life tables for obtaining population rates related to reproductive success. Attending on this manner the suggestion of refinement of specific parameters such as, intrinsic growth rate (r), reproductive potential (R0) and generation time (T) obtained from a field population. Demographic parameters were calculated from the construction of an age-specific life table through a cohort of a P. hawaiensis laboratory culture and compared to logistic growth models built for the same species considering data derived from the construction of a time specific life table from a field population. Differences in generation length are apparent between lab and field populations. The intrinsic rate of increase (r) are higher in lab population (cohort) because they live more and attained larger size with a higher fecundity translated in a higher net reproduction rate (R0). The net reproductive rate R0, which is the average number of female offspring per female per generation, does not provide a reasonably good comparison of absolute fitness differences between field and lab populations because the average generation time T is not the same in both situations [4]. Therefore, the measure of fitness most often used, the so-called DimuXL fitness, which is a measure of the total number of offspring produced by an animal over its lifetime, is not applicable. In the laboratory environment, where conditions were constant and population densities were low despite rapid population growth, the intrinsic rate of increase is probably the most reasonable single measure of absolute population fitness, even though it is calculated only for females and depends on the assumption of a constant age distribution.

TU124 Parhyale hawaiensis population parameters as ecotoxicological endpoints L. Allegreti, LEAL Laboratory of Ecotoxicology and Environmental Microbiology; B. Burstin, G.L. Ferreira, SCHOOL OF TECHNOLOGY –UNICAMP / LEAL; M. Flynn, LEAL Laboratory of Ecotoxicology and Environmental Microbiology

Population responses to environmental stress have been evaluated for effects on intrinsic growth rate based on evidence obtained by the established association between declining growth and increased stress and environmental carrying capacity, defined as the size of the population where the population growth is zero, crucial to the perception of toxic effect in natural environments. The objectives of this study include the description of the structure and dynamics of a natural population of P. hawaiensis through the construction of time-specific life table in order to obtain population vital parameters to be applied at endpoints for chronic toxicity tests known as LTRE (Life Table Responds Experiment). Such demographic statistics can be used in response to exposure to toxic agents, as well as serve as basis for evaluating the welfare of cultures kept in the Laboratory of Ecotoxicology and Environmental Microbiology School of Technology – UNICAMP / LEAL. Used the data of 12 monthly time-specific life tables the parameters mean reproductive potential (R0), generation time (T) and intrinsic growth rate (r) were calculated in order to model the logistic growth curves. The model was built considering the fecundity obtained through field data as well as lab culture. Values of R0, T and r are presented as the mean values for the 12 monthly samples as 1.56 (+/- 0.15), 3.75 (+/- 0.35) and 0.09 (+/- 0.05) respectively. The model indicates that considering field fecundity and an initial population of 10 individuals, the final population would reach carrying capacity (K) in approximately 35 generations and for lab fecundity the appreciation of a cohort derived from a P. hawaiensis intrinsic growth rate (r), the reproductive potential (R0) and the generation time (T) is recommended as more potentially effective endpoints for ecotoxicological chronic test with the suggestion that they are refined by experiments with laboratory cultures at controlled conditions. Such basic information on the test species clipping must be up to paramount importance if population parameters are to be effectively used as an endpoint in ecotoxicology.

TU125 Assessing estuarine quality: A cost-effective in situ assay with amphipods M. Díaz, E. Robledo, Instituto de Investigaciones de Recursos Cinegeticos / SaBio; A. Hoyo, Instituto de Investigacion en Recursos Cinegeticos / SaBio; M. Acuña, Instituto de Investigacion en Recursos Cinegeticos / SaBio; J. Martín, MARE; R. Ribeiro, Universidade de Coimbra / Centro de Ecologia Funcional; J.C. Marques, IMAR-Institute of Marine Research, Department of Life Sciences, University of Coimbra / Department of Life Sciences

In situ assays based on feeding depression can be powerful ecotoxicological tools that can link physiological organism-level responses to population and/or community-level effects. Amphipods are traditional target species for toxicity tests due to their high sensitivity to contaminants, availability in the field and ease of handling. However, cost-effective in situ assays based on feeding depression are not yet available for amphipods that inhabit estuarine ecosystems. The aim of this work was to assess a short-term in situ assay based on postexposure feeding rates on easily quantifiable food items with an estuarine amphipod. Experiments were carried out under laboratory conditions using juvenile Echinogammarus marinus as the target individual. When 60 Artemia franciscana nauplii (as prey) were provided per individual for a period of 30 minutes in dark conditions, feeding rates could be evaluated and temporarily contribute to environmental factors. Based on this study, biomarker analysis for monitoring and assessment of hazardous substances using mussel should in this sea area be avoided during the spring and early summer period. Furthermore, routine examination of the CI is recommended as this provides a good basis for assessing the general health status of the mussels that is often linked with the background levels of many biomarkers.
Hymeniacidon perlevis in assessing biological effects of harbour seawaters subjected to metallic and organic contaminants

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Since these last years, different approaches were settled to assess seawater quality in the aim to improve the good chemical status of coastal waters demanded by EU-WFD. One approach is to provide biological model (bioindicator) as environmental sentinel of harmful chemical introduction. The marine model organism which is often retained as integrative matrix of contaminants is the marine mussel (Mytilus edulis), the management of the French benthic bioindicators has been performed since 1970's years. This species presents criteria retained for bioindicators such as abundance, sedentary, tolerance to physico-chemical variations, and can integrate chronic contaminations of “water masses” over a large period. Moreover, this organism presents some limits as integrative model linked to its reproductive cycle which impacts the specific period of the year. For this reason, bioindicators such as sponges has been developed. The sponges are sessile species without organs (in opposition to bivalve molluscs) whose morphology is adapted to maximize the efficiency of water flow. After a specific study to the identification and the distribution of the sponges populations present along the coasts of Normandy, one species (Hymeniacidon perlevis) showed promising results in accumulation of different chemical compounds such as metallic and organic contaminants (PCB and HAP). To demonstrate the interest of using this species as bioindicator, natural and local samples of H. perlevis and M. edulis were transferred in same time by caging in different sites of one harbor in Normandy. Organisms were then exposed to different antrhopic sources of contamination in order to compare their acute cytotoxic performance. This study was performed on this sponge set up in mesocosms to assess copper bioconcentration factor. The aim was to evaluate the in-situ harbor seawater concentration of this element strongly accumulated in sponge tissues. Results will be presented in this work and discussed in the perspectives of the Water Framework Directive.

TU132 Biological-chemical parameters' coupling for the monitoring of trace metal contaminants in food webs: the case study of a land-to-sea continuum in the North-East Atlantic

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Biomonitoring of chemical contaminants is positioned at the biological-chemical interface, requiring skills and knowledge in various domains – analytical chemistry, biochemistry, biochemistry, ecology and physiology of organisms – in order to be useful to fulfil its role: describe levels of chemicals, interpret them, and assess their potential effects. Biomonitoring can therefore provide a global picture of contaminants in systems, even if general strategies must be amended to fit site-specific constraints such as local physico-chemical forcing variables, occurrence of monitored species, etc. Here, we propose to combine both chemical (metal concentrations, carbon (C) and nitrogen (N) stable isotopes measured in various species), and biological parameters (metallothionein (MT) concentrations, data on the physiology of organisms) to describe contaminants in food webs, taking the Loire estuary as a case study. Thus, ten species (including 7 fish, 2 crustacean and 1 bivalve species) representative of the food web were analysed for these parameters. C and N isotope results traced the different sources organic matter sustaining the different species, assessed their habitat and trophic level, and consequently were crucial in interpreting metal burdens. MT concentrations and metal distribution between soluble and insoluble fractions also revealed differential metal regulation and/or detoxification capacities among the species. For instance, among fish, the microphytobenthos-grazer (then low trophic level) mullet Liza ramada presented the highest metal concentrations probably due to sediments speciation, but exhibits a efficient mechanism of regulation and/or detoxification (high MT levels and metal insolubilisation). This integrative approach adds to quantitative monitoring of environmental contaminant because it provides a view of the effective bioavailability of chemicals, and of their transfer and bioaccumulation in food webs – a major component of ecosystems. This study therefore proposes that the combined use of such tools has considerable interest in monitoring programmes that aim at describing the temporal and spatial contaminant variability in ecosystems. Finally, in the perspective of the European Marine Strategy Framework Directive (MSFD) implementation, it represents a concrete example of biological-chemical parameters’ coupling to document both MSDF-descriptors D4 (Food-web) and D6 (Pollution), and to more broadly monitor contaminated in food webs.

TU133 Genotoxicity assessment in the spermatozoa of the common prawn Palinur s serrat us using the alkaline Comet assay

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Sperm quality is essential for reproduction success. Thus, there is an increasing concern to understand how the xenobiotic exposure affects the structure of spermatozoa. The measure of DNA integrity appears as an interesting and relevant marker to assess the sperm quality. Nowadays, majority of ecotoxicity studies was relatively narrow to molluscs and fishes despite the interest to investigate the different phyla. Crustaceans and particularly the marine and estuarine species are still rarely studied despite their ecological importance. The purpose of this study was to develop and optimize the alkaline comet assay in marine and estuarine Palamemonidae prawns to assess sperm quality. In first times, an optimization of procedures including the collection of spermatozoa, the maintenance in appropriate media as well as different steps (i.e. lysis, denaturation) of the comet assay was performed in Palaemon serratus. Then the sensitivity of cellular model was assessed using a variety of well-known genotoxicants with diverse modes of toxic action (i.e. UV, H2O2, MMS). Results attested the reliability and the sensitivity of the procedure developed. In the second times, DNA integrity of spermatozoa was measured in differentially impacted Palamemon sp. populations along the Bay and the Estuarie of Seine (i.e. the coastal species Palaemon serratus, and the estuarine species Palaemon longirostris) during the year 2015. The first results suggest important DNA damages in populations from the Seine estuary and a lower effect in populations away from the estuary. In conclusion, this work showed the pertinence and the potential of the alkaline comet assay on Palamemonidae spermatozoa in the monitoring of environmental genotoxicity.

TU134

Immune capability of humpback whale cell lines and application in immunotoxicity

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Humpback whales have an elevated sensitivity to accumulate persistent organic pollutants (POPs) and chemical pollution may impact animals’ health. Measuring the immunotoxicological impact of POPs on wild populations of humpback whales is challenging, however, toxic effects in humpback whales are still poorly understood. In this study we use commercial multiplexed ELISA microarrays as a screening tool to measure and quantify relevant proteins. Gene ontology and ingenuity pathway analysis are applied to identify protein targets involved in immune responses and disease processes. In the used approach over 160 proteins were detected and revealed a big overlap between humpback whale derived cell lines and dermal biopsies. Thus, we provide a minimally invasive method for the study of the whole cell sensitivity and cellular response to chemicals and other environmental stressors.

TU135

Multiple stress effects on marine planktonic microalgae

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In the present context of global warming, more knowledge on the combined effects induced by temperature changes and mixtures of relevant environmental contaminants is urgently needed. Therefore, the main objective of this study was to investigate the effects of temperature increase (20°C to 25°C) on the toxicity of one metal (cadmium) and one polycyclic aromatic hydrocarbon (benz[a]anthracene), alone and in mixture, to the marine planktonic algae Tautreniella chudzienia. Microalgal cultures were exposed for 96h to different concentrations (0.04 - 20 mg/l), of the two substances individually and in binary mixtures, at 20 and 25 °C. The effect criterion was the inhibition of culture growth. The increase of temperature by 5 °C significantly increased the toxicity of both contaminants. Toxicological interactions among the stressors were found. The findings of this study highlight the need of more research on the effects of temperature on the toxicity of chemical mixtures.

TU136

Eco-toxicological assessment of lipid absorbents and long-term monitoring of marine microorganism at lithium-recovery field site


The adsorbents which made by lithium manganese oxide for recovery of mineral resources from seawater were developed by KIGAM (Korea Institute of Geoscience and Mineral Resources) and the effectiveness of lithium recovery was verified. When the field application of large amount of adsorbents in ambient seawater (Okye Harbor, Gungnung, Korea), it should be considered on impact on marine environments by lithium and manganese. In this study, long-term and periodic monitoring has been carried out to detect changes of marine general factors (i.e. chlorophyll, standing crops of phytoplankton, and concentrations of trace metals) at lithium recovery pilot plant and eco-toxicological effects on marine environment by exposure of lithium absorbents. First, abundances and species of phytoplankton have been evaluated through monthly interval sampling from February 2013 to May 2015 since phytoplankton is important indicator of primary productivity of marine. Abundance and species diversity of phytoplankton went up to summer from winter during whole periods. Eco-toxicological assessment of lipid absorbents was performed with Microtox using bioluminescence bacteria Vibrio fischeri. The lithium adsorbents was soaked in sterilized seawater and aeration for 1, 3, 5, 7, 10 and 14 days intervals under controlled temperature. As the results, toxicities of adsorbents were found in more than 10 days exposure and maximum EC50 concentrations were 61.4%. At this time, concentrations of included metals such as As, Cd, Cr, Cu, Zn, As, Sn, and Pb were also higher. The study is in progress about eco-toxicological assessment as maximum allowable concentration with species sensitivity distribution (SSD) of monitoring factors. Acknowledgement This research was supported by the national research project titled “The Development of Technology for Extraction of Resources Dissolved in Seawater” of the Korea Institute of Geoscience and Mineral Resources (KIGAM) funded by the Ministry of Oceans and Fisheries (Project No. PKF061).

TU137

Toxicity of herbicides used to control the invasive weed Spartina anglica to for incongruently species inhabiting an Australian saltmarsh

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Spartina anglica is recognised as an invasive weed in Australia and as a threat to local flora and fauna. Control efforts include the use of the herbicide Fuislade Forte with the advantaj Hasten. Anderson Inlet, Victoria, Australia is subject to routine spraying of herbicides for Spartina control and little is known of the effects of these chemicals on local invertebrate species. Four species commonly found in Anderson Inlet, Lumnibrus variagatus, Aegla rhodomitra, Tecta rufilabris, and Allocheses compressa, were tested in the laboratory with Fuislade Forte and Hasten alone and as a mixture, using 96 hr acute toxicity tests to estimate median lethal concentrations (LC50). A. compressa was the most sensitive species in all tests, and T. rufilabris the most tolerant. The herbicide mixture was more toxic to the three marine species, A. australiensis, T. rufilabris, and A. compressa than Fuislade Forte alone, with toxicity increasing by 146%, 129%, and 181% respectively. However the mixture was less toxic than Fuislade Forte alone by at least 25% to the freshwater species, L. variagatus. Sub-lethal tests on T. rufilabris also revealed a higher toxicity of the mixture compared with the toxicity of Fuislade Forte. The laboratory results indicate that using Fuislade Forte with Hasten is more toxic to non-target marine invertebrates than using Fuislade Forte alone. However, consideration of dilution scenarios in the field suggests that rapid degradation and dilution from the incoming tide would reduce the concentrations to well below the LC50 values measured here, resulting in low risk of acute toxicity to aquatic invertebrates from these herbicides in field sites. Monitoring of field invertebrate communities for a period of one to six months after spraying of herbicides as Fuislade Forte alone or as a mixture, yielded little evidence of detrimental effects.

TU138

Organohalogen compounds in fish biota of Guanabara bay, Rio de Janeiro, Brazil

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Organohalogen compounds have been reported as an environmental concern issue, since last decades. Nevertheless, their global threats are still difficult to access due to most researches are focused on selected groups of contaminants while other groups remained unstudied. Persistent organic pollutants are regulated under the Stockholm convention since 2001 and constitute a diverse group of organic substances, which are toxic, persistent, bioaccumulative and tend to long-range transport. Urbanization and industrialization contributed to the contamination of hotspots and worldwide distribution of organohalogen pollutants. The most anthropogenically disturbed area along the brazilian shoreline is Guanabara bay, situated in Rio de Janeiro state. This estuary is bordered by 12000 industries and four cities, with a total population of about 11 million people. Despite the anthropogenic pressure, knowledge of the anthropogenic impact on wildlife, which makes it an important fishing point to the local market and
population. Focusing on expand the knowledge about organohalogen compounds, we tried to determine some target compounds classified as POPs and non-target compounds, in three fish species with high level consumption, by gas chromatography (GC) coupled to electron capture negative ionization (ECNI) with a quadrupole mass spectrometer (MS). The profile contamination of POPs was mainly composed by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) followed by legacy organochlorine pesticides (OCPs) and PBDEs both natural and anthropogenic compounds, according to the literature. Moreover, the presence of some organohalogen compounds less commonly analyzed as the heptachlorobipyrrole (Q1) and pentachloroanisole (PCA) were found in all samples. Regarding these non-target compounds, Q1 has been previously reported in Guanabara Bay by Rosenfelder et al., 2012, in a study which they also document the presence of a new DDT metabolite in the local biota. Pentachloroanisole is a common metabolite of pentachlorophenol (PCP) in fish, although it is not well documented. This product is only used in Brazil in wood protection since 1998, before that it was used as fungicide, algaecide and insecticide. As depicted by our results, legacy and emerging POPs can be easily found in the high polluted Guanabara Bay. This may represent a health risk hazard to fishermen and their families. We suggest that more research ought to be done in order to clarify this issue in the near future.

TU139 Acute and chronic toxicity of chlorine to selected marine species of the Arabian Gulf waters S. Aeed, ExxonMobil Research Qatar / Environment and Water Reuse Program; N. Deb, ExxonMobil Research Qatar / Environmental; S. Bacha Ben Said, Qatar University / Environmental Science Center; F. Warren, ExxonMobil Research Qatar / Environment and Water Reuse Program Chlorine is extensively used as a powerful oxidizing agent in the countries surrounding the Arabian Gulf for water treatments and biofouling control. The usage has been increasing significantly as demand for water grows considerably both in industry and domestic use. This is the fact that it is a well-tested technology, has had a history of long-term worldwide industrial use and is of acceptable cost. In seawater, chlorine produces a mixture of hypohalous acid and hypochlorite ion. These rapidly react with the bromide ion to form a mixture of hypobromous acid and hypobromite ion. The acute oxidants formed by chlorination are therefore short lived and are not persistent in seawater, but can be quite toxic. Further complicating the environmental concern of chlorination is the production of numerous, and more persistent, compounds formed by complex reactions between chlorine/bromine and the organic constituents of seawater, collectively described as chlorination by-products (CBPs). Many CBPs are persistent and may be toxic to marine organisms subjected to long-term exposures. In order to evaluate the risks of chlorine exposure to Arabian Gulf marine organisms, the aims of this study were to 1) develop protocols for acute and chronic toxicity tests involving native species at different trophic levels, 2) correlate sensitivity to other marine organisms used as indicator species in risk assessments, 3) draw conclusion from the results and explore ways that this could be used for informing environmental management activities. Species were collected from different locations around the coastal areas of Qatar. They were then cultured in the laboratory using conditions of the Arabian Gulf. Species used in the tests include phytoplankton (Synechococcus spp.), zooplankton (Euplotes sp., Enteromorpha, Enteripna acutifrons), pearl oysters (Pinctada radiata), sea urchins (Diadema setosum) and kelp (Dictyota dichotoma). Different tests were either administered as a continuous flow through or via daily renewal. Sensitivity varied across the trophic levels for each species and was found to be in line with other test organisms that are used in established toxicity procedures. All five species used were found to be useful for certain types of toxicity testing. For example, Pinctada radiata and Diadema setosum were excellent in testing for successful fertilization and early development, while local Aphanthis dispar embryos were useful for following sub-lethal effects such as malformation and hatchability. Embryos of oysters and urchins were found to be the most sensitive to chlorine. Effect of chlorine was only observed near or after hatching has occurred as fungicide, algaecide and insecticide. As depicted by our results, legacy and emerging POPs can be easily found in the high polluted Guanabara Bay. This may represent a health risk hazard to fishermen and their families. We suggest that more research ought to be done in order to clarify this issue in the near future.

TU141 QUASIMEME development exercises to improve world-wide reliability of data in the marine environment S. Cram, Alterra Wageningen UR / ERA team; I. Harsman, Alterra, Wageningen-UR / CALM; W. V. Vark, Wageningen University / Soil Quality An integrated biological-chemical monitoring and assessment of chemical pollution in the marine environment is not only a matter of using chemical analytical methods, biological effects methods such as biomarkers and a modelling tool for interpretation. It needs reliable data and common approaches. Results from different countries including their laboratories are often involved in the necessary investigations. Results from these laboratories should be comparable and used in one single assessment model. Standardization and proficiency testing is always a solution to improve comparability of laboratories. Especially if methods are still in development, standardization is not yet an option. Exchange of information by involved scientists and technicians as promoted in the QUASIMEME development exercises has shown to be an effective tool to improve results. QUASIMEME stands for Quality Assurance of Information for Marine Environmental Monitoring. At the heart of the programme is a holistic learn-by-doing spiral. The laboratory performance studies provide the basis of external quality assurance for institutes that make regular measurements in the marine environment. For routine parameters a performance study can be organized on regular basis. For new parameters a more fit to purpose approach is necessary as used in the QUASIMEME development exercises. These improvement programmes may be initiated through a workshop and with a series of Development Exercises to provide detailed tuition, information and test materials tailored to the specific needs of the problem. The workshops to exchange information and experiences are essential. Successful exercises has been organized for perfluorinated compounds, chlorinated paraffins, marine biotoxins, organotin compounds, chlorophyll and passive sampling using seawater, sediments and aquatic organisms. Because a large number of marine laboratories are already member of QUASIMEME, it will not be too difficult to extend the approach to other and new quality parameters in the marine environment.

TU142 Development and application of oxidative stress biomarkers and models in ecotoxicology and environmental monitoring (P)

TU140 The IPOC project: Interactions between POLLution and Climate changes, Development of improved monitoring strategy M. AUFFRET, IUEM / LEMAR UMR; M. FOURNIER, Université du Québec À Rimouski / ESMERQAR Anthropogenic pressure (habitat destruction, chemical contamination, eutrophication) is responsible to induce deleterious effects on aquatic ecosystems, especially coastal waters whose production is of major importance for humans. Ongoing climate changes are expected to generate in a next future unexperienced, stressful environmental conditions which will probably deeply affect biota in targeted habitat as rivers and estuaries. These natural systems are linked as a continuum through water transfers. Coastal, intertidal habitats are naturally highly variable, both spatially and temporally. Such environmental conditions make them highly exposed to synergistic effects of pollution and temperature stress. At the basis of the trophic chain, benthic invertebrates, especially filter-feeders, control ecosystem productivity by locally producing a huge amount of biomass and should therefore be considered as key-stone populations at risk. The project IPOC (2013-2016) is a joined, international project born by French and Canadian laboratories and companies which aims to improve already broad range of habitats, from Arctic to Austral regions, for observations in natural populations of mussels (Dressena spp and Mytilus spp.). IPOC scientific rationale relies on a better understanding of the sensitivity of these sentinel species in the continuum freshwater-to-coastal areas and thus their vulnerability to environmental stress. The approach combining observation and experiment will allow to: 1. Assess the diagnostic capacity of a selection of molecular, cellular and physiological biomarkers for assessing biological effects of the combined action of major anthropogenic stressors in aquatic environments. 3. Provide the scientific community and environmental managers with an improved "tool-kit", based on original, up-to-date scientific knowledge, for long-term monitoring of aquatic environments, according to recommendations from international organizations. 2. Foresee the capacity of aquatic populations at risk to cope with environments altered by the global change and further, possible alterations of local ecosystem services Keywords: Aquatic ecotoxicology, anthropogenic pressure, bivalve mollusks, biomonitoring The research project IPOC has been funded by the ANR and NSERC Agencies (joined project France-Canada) for the 2013-2016 period.

TU141 Integrated chemical and toxicological profiling of surface water samples from Swedish agricultural areas L. Hedvall-Hedvall, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; C. von Brömssen, A. Rosennami, T. Le Godec, Swedish University of Agricultural Sciences; J. Kreuger, Swedish University of Agricultural Science / Centre for Chemical Pesticides; A. Oskarsson, SLU Dept Biomed Sci Vet Public Health / Dept of Biomed Sci and Vet Public Health Pesticides applied on agricultural land can contaminate ground- and surface water and adversely affect ecosystems and drinking water quality. As part of a Swedish program on environmental monitoring of pesticides, we have collected 161 surface water samples from six small catchment areas representing four large
agricultural regions and two rivers, during the years of 2013-2014. The water samples have been analyzed for residues of 131 pesticides, using GC-MS and LC-MS/MS. Of the analyzed pesticides, 87 were detected in the water samples. The water samples were also used for toxicological profiling in vitro, using cellbased bioassays. Oxidative stress response is a sensitive indicator of toxicity, revealing the nature of chemicals. We investigated the oxidative stress response exerted by the water samples, by measuring the NRF2 activity. Furthermore, we measured the aryl hydrocarbon receptor (AHR) activity, indicating induction of metabolism of xenobiotics and cytotoxicity by MTS test. The water samples from the pesticide monitoring program were extracted, concentrated and analyzed in both water and sediment in vitro and in vivo. No cytotoxicity was observed. A large number of the water samples exerted significant induction of the NRF2 activity and the AHR activity. The effect was particularly strong in the AHR assay, where 123 of the water samples activated the receptor >2 fold compared to the control. Oxidative stress (NRF2 activity >1.5 fold compared to control) was exerted by 288 of the water samples. The results did not indicate a correlation between AHR and NRF2 activities. A correlation was found between NRF2 activity and season. By stepwise regression a statistically significant positive correlation was found between NRF2 activity and concentration of five pesticides: diuron, lindane, metachlor, rimsulfuron and tertbutylazine. These effects remained when area and season were included in the model. In conclusion, we found that a large number of the water samples contained substances that exert oxidative stress and activates AHR in vitro. No correlation between the two parameters was found but a positive correlation between NRF2 and some of the pesticides monitored in the program. The study demonstrates that these bioassays are valuable tools to monitor water quality and evaluate environmental effects of pesticide residues.

TU143

Nrf2 as an oxidative stress biomarker of drinking water from source to tap - association with AHR activity and chemical pollutants

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Drinking water contains a complex mixture of known and unknown chemicals, where the individual compounds may be present in concentrations too low to cause adverse effects, but added together may result in combination effects. Oxidative stress response is a sensitive indicator of toxicity, responding to a wide variety of chemicals. The objective of this study was to evaluate the influence of different steps of water treatment on oxidative stress response by measuring Nrf2 activity. In addition chemical pollutants, cytotoxicity and AHR activity were analyzed in the water samples. Water was sampled from five sites before the intake (including up- and downstream a waste water treatment plant (WWTP), after a sand filter, after a granulated active carbon filter, at the outlet of the drinking water treatment plant (DTWP), and from a nanofilter pilot plant. The samples were concentrated by solid phase extraction with two different adsorbents, HL5 and ENV. Chemical analysis was performed by LC/TOF-MS for 124 target chemicals, mainly medical drugs, pesticides and households chemicals. In all samples a final concentration factor of 50, Nrf2 and AHR activities were detected. Raw water samples had in general higher activities than samples from inside or taken after the DTWP. No evident decrease in activity from a specific treatment step in the DTWP was detected, except in the nanofilter pilot plant, where activities of Nrf2 and AHR decreased after a filter of old granulated active carbon. An association between Nrf2 and AHR activities was found in many of the water samples. Preliminary results did not indicate any genotoxic activity in the water samples, as measured by Comet assay. No cytotoxic effects were revealed in the water samples. The total concentration of target chemicals followed another pattern and decreased considerably from downstram the WWTP to the outlet of DTWP. No nrf2 activity was detected in a sample spiked with all the target chemicals, indicating that the activities in the water samples were not caused by the target chemicals and that other, yet not identified compounds, are responsible for the observed activities. The study demonstrates that these bioassays are valuable tools to monitor water quality and evaluate effects of water treatment.

TU144

Fungicide prochloraz induces oxidative stress and DNA damage in vitro

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Prochloraz is widely used in horticulture and agriculture, e.g. as a post-harvest anti-mold treatment. Prochloraz is a known endocrine disruptor causing developmental toxicity with multiple mechanisms of action. However, data are scarce concerning its effects on the genotoxic and antioxidant systems. Since high formation of reactive oxygen species (ROS), is a common mechanism for different toxic endpoints, e.g. genotoxicity, the aim of this study was to investigate if prochloraz can induce oxidative stress and/or DNA damage in human cells. We used a cell culture based in vitro model to study oxidative stress response by prochloraz, as measured by the activity of the nuclear factor erythroid 2-related factor 2 (Nrf2), a key molecule in oxidative defense mechanisms. We found that prochloraz is able to induce oxidative stress in cultured human adrenocortical H295R and hepatoma HepG2 cells at non-toxic concentrations. Further, we used Comet assay to investigate the DNA damaging potential of prochloraz, and found that prochloraz at non-toxic concentrations is able to induce DNA damage in HepG2 cells. These are novel findings, contradicting previous studies in the field of prochloraz and genotoxicity. This study reports a new mechanism by which prochloraz may exert toxicity. Our findings suggest that prochloraz might have genotoxic properties.

TU145

BEHAVIORAL, OXIDATIVE STRESS AND GENOTOXICITY EFFECTS IN THE POLYCHAETE NEREIS DIVERSICOLOR TO THE CYTOXIC DRUG CISPLATIN

T.G. Fonseca, T.L. Rocha, University of Algarve / CIMA; M.J. Bebianno, University of Algarve / CIMA Centre for Marine and Environmental Research

An antitumor drug cisplatin (Cis) is a powerful inducer of cell replication and transcription through interaction with DNA, inducing potential mutagenic, cytotoxic, genotoxic and carcinogenic effects. Concerns about their fate and long-term effects in aquatic invertebrates are arising, due to the continue discharge in water bodies by municipal and hospital effluents. The aquatic sediment represents an important reservoir for those compounds that can pose a risk to the benthic biota. Guidelines for sediment quality assessment do not include trace organohalogen compounds as contaminants of concern, and little attention has been paid to the potenti effects of cytotoxic drugs to non-target organisms. Cisplatin (cis-diammine-dichloroplatinum II) is an alkylating agent globally administered, which environmental levels range from ng Pt.L-1 to µg Pt.L-1. The present study aimed to assess the potential effects of the antitumor drug cisplatin on the ecotoxicological behaviors, oxidative stress and genotoxicity in the environmental relevant concentration and worst-case scenarios addressed in water bodies. Polychaetes were exposed to cisplatin at 0.1, 10 and 100 ng Pt.L-1, in systems containing sediment and water (1:4), over 14 days. The multimobiarmer approach involved a behavioural assessment and biochemical analysis, including neurotoxicity (AChE), antioxidant enzymes (SOD, CAT, GPx, GST), and oxidative damage (LPO). Genotoxicity was performed through comet assay. Results showed neurotoxicity in animals exposed to the 100 ng Pt.L-1 concentration, by the significant inhibition of AChE (p<0.05). Likewise, the activity of antioxidant system was significantly impaired by depletion of SOD, CAT and GST levels, and overexpression of GPx, at the highest concentration of the drug, which lead to high levels of lipid peroxidation products. DNA damage was not detected in polychaetes as a consequence of intra and interstrain cross-links in DNA, associated to the cisplatin mode of action. The multimobiarmer approach confirmed the sensitivity of the polychaete N. diversicolor to a potent pharmaceutical of cytotoxic activity, addressing that low concentrations combined with chronic exposure may represent a hazard for representative benthic species of coastal waters.

TU146

Towards an early molecular response in periphytic diatoms to reveal impacts of multiple contaminants: quantitative gene expression

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This poster aims to highlight the potentialities of the gene expression tools in diatoms to reveal the different impacts of various contaminants which may be found in the natural aquatic systems. Through three independent exposure experiments of a periphytic diatom species to pesticides (diuron), to metal (cadium) and to metallic nanoparticles (AuNP), the use of gene expression has shown great efficiency to provide rapid responses and the metabolic functions affected. Diatoms are unicellular algae responsible for a large proportion of vegetal primary production in upstream watercourses and have therefore an essential role at the basis of the freshwater trophic web. They are excellent ecological indicators at the species level, sensitive to a number of environmental variables (light, temperature, nutrients) and mechanical disturbances of pollution (eutrophication, organic and metallic pollution). Thus laboratory experiments have been performed to expose different diatoms species: Eolimna minima, Achnanthes minutissimata, Nitzschia palea, Gomphonema parvulum and Planothidium lanceolatum cultivated in lab to various contaminants during 48 hours exposure period. A quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) (total density) to follow their growth in the experimental conditions in parallel to molecular biology analyses. The expression level of target genes involved in the mitochondrial metabolism (cox1, md5, 125), response to oxidative stress (sod Cu/Zn, sod Mn, cat), detoxification (phytochelatin synthase) and photosynthetic processes (psa A, d1), has been determined by real time quantitative PCR. Genetic responses evidenced the presence of a stress response in diatoms exposed to the combined effect of contaminants on the mitochondrial metabolism, oxidative stress response and the chloroplast
Neutralizing Agents for Livestock Biological Hazardous Factors, Project

Atrazine on aquatic species using a typical freshwater flea

and 7. In conclude, the recombinant yeast showed a high ability of toxic detection in pure toxic chemicals and mine water samples. The results understanding about how these toxics effect the aquatic organisms, especially at Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)” Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Toxic Detection of Mine Water by Recombinant Saccharomyces Cerevisiae for the studied species: Eolimna minima and Nitzschia palea appeared to be more tolerant. The results showed that the development of molecular tools and more probably the biomarkers is an asset to assess organisms’ contamination and water quality.

Neutralizing Agents for Livestock Biological Hazardous Factors, Project

Atrazine on aquatic species using a typical freshwater flea

and 7. In conclude, the recombinant yeast showed a high ability of toxic detection in pure toxic chemicals and mine water samples. The results understanding about how these toxics effect the aquatic organisms, especially at Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)” Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Proteomic Analysis of Daphnia magna Exposed to Lead (II) Acetate Trihydroxyl and Atrazine for Screening Potential Biomarker

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Heavy metals and pesticides are main factors causing water pollution via industrial and agricultural waste. However, it still exists a limitation of well understanding about how these toxics effect the aquatic organisms, especially at molecular levels. In this study, acute toxicity tests were performed according to EPA protocol (2002) to assess the impacts of Lead (II) acetate trihydroxyl and Atrazine on aquatic species using a typical freshwater Daphnia magna. Besides, proteomic analysis was performed to identify proteins involved in the stress responses of D. magna to these toxic chemicals. The lethal concentrations LC20 of each chemical, which were determined by previous acute test, were used for a 24 h exposure to the 21 days daphnids before isolating the total protein. The proteomic profile was examined with a pH range from 3 to 10 using 2-DE method and then analyzed with Progenesis software to explore the differentially expressed proteins (DEPs) compared with control organisms. The results showed that there were some up- and down- regulated proteins in D. magna responding to these toxic chemicals. The DEPs are helpful to understand the molecular responses of D. magna to Lead (II) acetate trihydroxyl and Atrazine and can be used as novel biomarker candidates to detect these heavy metal and pesticide. This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)” Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Development of Triclosan Detecting Biomarker Using Daphnia magna

S. Bang, J. Min, Chonbuk National University / Division of Chemical Engineering; H. Shin, Chonbuk National University / Graduate School of Semiconductor and Chemical Engineering; J. Park, Division of Chemical Engineering; Y. Kim, Chungbuk National University / Department of Microbiology

Triclosan has been used as an antibacterial and antifungal agent found in consumer products including toothpaste, soaps, toys and cosmetics. However, it is toxic to aquatic / land organisms, human and environmental. Therefore, there is a need for the development of novel biomarker to monitor the toxicity of the triclosan. In this study, acute toxicity tests were performed according to EPA protocol to assess the impacts of triclosan on the typical freshwater Daphnia magna. The lethal concentration for 24 h for LC50 of D. magna was determined to be 300µg/L. Additionally, the proteomic profile of treated D. magna LC20 was also analysed using two-dimensional(2D) electrophoresis technique. The comparison in protein expression pattern between control and TCS-treated organisms was then carried out using Progenesis software to explore the differentially expressed proteins(DEPs). After that, MALDI-TOF analysis was also conducted to identify the proteins dots of interest selected from 2D gels. The changed protein spots due to triclosan treatment can be used as new biomarkers to detect triclosan as being mentioned above. From MALDI-TOF results, certain promoters that can express certain DEPs were inserted upstream to the green fluorescent protein of plasmid vector. Then plasmid transfection was carried out on Hela cell to confirm the working ability of these promoters. The changed protein spots due to triclosan treatment can be used as novel biomarkers to detect triclosan as being mentioned above. This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No:Pj01051502)” Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Interactive effects of climate change and contaminants: environmental risks and human health implications (P)

Methylmercury (MeHg) and climate change: neurophysiologic and behavioral responses of Senegalese sole Solea senegalensis

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Methylmercury (MeHg) is an extremely hazardous pollutant. Consequences of MeHg exposure on biota in a climate change context are still unknown. Acetylcholinesterase (AChE) hydrolyzes acetylcholine, a prominent neurotransmitter in all vertebrates, both in the sympathetic and parasympathetic nervous systems. Lateralization produces faster and more efficient responses to external stimuli. Solea senegalensis use the bottom as camouflage, thus disruption in both choosing ability and lateralization may prove ecologically disastrous. The brain is divided in morphologically different neural substructures, each playing different roles in neurotransmission and MeHg accumulates at different rates on each substructure. We assessed the effects of joint exposure to MeHg contamination and climate change variables in S. senegalensis behavior processes, as well as acetylcholinesterase activity and different brain regions weight (forebrain, optic tectum, cerebellum and brainstem). During 28 days, S. senegalensis specimens were maintained under three factor crossed treatments: MeHg contaminated feed, pH (ambient CO2: 8.0, high CO2: 7.6) and temperature (ambient: 19°C, high: 23°C). Behavior was assessed by calculating absolute and relative lateralization using a detour test, and by habitat preference, measuring time spent between two habitats: simple and complex (all tests, n=10). Neural substructures were separated, weighed individually, and AChE was assessed in each region. Relative weight for each was plotted and a linear regression from the control was calculated. MeHg intake correlated with increased time spent in complex habitat, where fish could not camouflage or hide efficiently. In addition, MeHg reduced lateralization in a fish brain, leading to complete loss of lateralization at populational level. Acidification also led to increased time spent in complex habitat, only in non-contaminated treatments. All four brain regions registered differences in relative weight under different stressors. Optic tectum relative weight decreased under all stressors, whereas other brain regions were increased. AChE data is being analyzed. Climate change variables were as important as MeHg in altering brain region relative size, but were downplayed at an ecological level. Disruption of important behavior processes along with deregulation of neural substructure functions, may lead to decreases in S. senegalensis ecological fitness.

Can bivalves recover from short-term exposure to environmental changes? The combined effect of salinity shifts and Arsenic contamination

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Arsenic (As) is one of the most toxic elements in the environment, especially when in its inorganic forms, commonly as priority hazardous substance in the world. Although many authors have investigated the levels of As in sediments and water, and As bioavailability and bioaccumulation patterns in the marine environment, less have addressed the toxicity of this metalloid namely in marine bivalves. Besides As, salinity is also a stress factor for aquatic environments since it can change across spatial and temporal scales, species richness, abundance and spatial distribution but also individuals health status. In a foreseeable climate
changes, predicted for the next 100 years, will cause salinity shifts in estuarine and coastal areas. Beyond this long-term environment alteration, extreme weather events are becoming more frequent which can also lead to salinity fast changes. Salinity changes are also expected to change the sensitivity of aquatic organisms namely to metals and metalloids, due to alterations on their physiological and biochemical characteristics. Salinity changes can alter the metabolism of contaminants by changing their chemical speciation, solubility and adsorption, with consequences to their bioavailability. Thus, the current study assessed the biochemical alterations induced in the clam species _Ruditapes philippinarum_ after exposure to salinity shifts (14, 28 and 42 g L\(^{-1}\)) and As contamination (0 and 2 mg L\(^{-1}\)). The capacity of this species to recover (96th and 28 days) after exposure to both stressors, acting alone and in combination, was also evaluated. After exposure, regardless the salinity tested, clams contaminated with As showed higher concentrations than non-contaminated specimens. After recovery, As concentration in clams decreased, with contaminated and non-contaminated specimens presenting similar values. The results obtained further demonstrated that exposure to As (2 mg L\(^{-1}\)) at different salinities (salinities 14, 28 and 42 g L\(^{-1}\)) and salinity 42 g L\(^{-1}\) (As 0 mg L\(^{-1}\)) lead to an increase of lipid peroxidation and detoxification mechanisms in clams, compared with clams non-contaminated under salinities 14 and 28 g L\(^{-1}\). After recovery, at salinities 14 and 28 g L\(^{-1}\), clams previously exposed to As were capable to decrease their oxidative stress levels found in non-contaminated clams. Nevertheless, at salinity 42 g L\(^{-1}\) both contaminated and non-contaminated clams did not survive.

**TU154**

**Levels and trends of contaminants in human populations in the Arctic**

Y. Gibson, B. Adlard, Henry Cambeiro, K. Olafsdottir, University of Iceland; T.M. Sandiur, UiT The Arctic University of Norway

The Arctic Monitoring and Assessment Programme (AMAP) is one of six working groups (WG) established under the Arctic Council. AMAP is tasked with monitoring the levels of contaminants present in the Arctic environment and human populations. This work was reporting these results regularly. This presentation provides an overview of the human biomonitoring data from all eight Arctic countries reported in the 2015 Human Health Assessment Report. Levels of contaminants are declining in the monitored Arctic populations, but not consistently across the Arctic. Certain populations are experiencing more rapid declines than others, and certain populations have concentrations that are remaining stable or are still increasing. Most Arctic populations described in this chapter continue to experience elevated levels of these contaminants compared to other populations monitored worldwide, for example, mercury, where 7 to 85% of Inuit women 18 to 39 years of age in Arctic Canada and Greenland exceed the Canadian provisional blood guidance value of 8 µg l \(^{-1}\). Established for children and women of childbearing age. There are certain contaminants, like perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) which are still increasing in Arctic populations, and require more investigation to find the predominant and important sources of exposure. Most of these data have been collected over the last twenty years and are now all regularly reported. Concentrations of these contaminants must continue in the future to determine if levels of these contaminants and others, are changing in Arctic populations. This work continues to support international risk management efforts under the Stockholm Convention. This work also supports the objectives of Canada’s Chemicals Management Plan.

**TU153**

**The combined effect of salinity shifts and Arsenic contamination and climate change variables in Atlantic salmon and yellow perch**

S. Bang, Chonbuk National University / Division of Chemical Engineering; N. T. Lee, Chonbuk National University / Department of Atmospheric Sciences; J. H. Kim, Chonbuk National University / Division of Marine Environmental Science

The Arctic Monitoring and Assessment Programme (AMAP) is one of six working groups (WG) established under the Arctic Council. AMAP is tasked with monitoring the levels of contaminants present in the Arctic environment and human populations. This work was reporting these results regularly. This presentation provides an overview of the human biomonitoring data from all eight Arctic countries reported in the 2015 Human Health Assessment Report. Levels of contaminants are declining in the monitored Arctic populations, but not consistently across the Arctic. Certain populations are experiencing more rapid declines than others, and certain populations have concentrations that are remaining stable or are still increasing. Most Arctic populations described in this chapter continue to experience elevated levels of these contaminants compared to other populations monitored worldwide, for example, mercury, where 7 to 85% of Inuit women 18 to 39 years of age in Arctic Canada and Greenland exceed the Canadian provisional blood guidance value of 8 µg l \(^{-1}\). Established for children and women of childbearing age. There are certain contaminants, like perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) which are still increasing in Arctic populations, and require more investigation to find the predominant and important sources of exposure. Most of these data have been collected over the last twenty years and are now all regularly reported. Concentrations of these contaminants must continue in the future to determine if levels of these contaminants and others, are changing in Arctic populations. This work continues to support international risk management efforts under the Stockholm Convention. This work also supports the objectives of Canada’s Chemicals Management Plan.

**TU152**

**Transcriptomic response of the aquatic plant Elodea nuttallii to mercury and ultraviolet radiation**

N. Regier, University of Geneva / Institute Forel Earth and Environmental Sciences; V. Slaveykova, University of Geneva / Section of Earth and Environmental Sciences Institute Forel; G. Cosic, Geneva University / Institute Forel Earth and Environmental Sciences.

We set out to investigate the occurrence of enhanced UV radiation on the response of the aquatic plant _E. nuttallii_ to interacting stresses. In the present work we exposed _E. nuttallii_ to UV (0.55 Wm\(^{-2}\) UVa) for 6 h and to Hg for 24 h or to both for combined treatment. We analysed Hg content, oxidative stress response, effect on pigment content, as well as the transcriptome. UV radiation decreased Hg uptake in shoots as compared to plants exposed to Hg alone. Pigments tended to decrease in response to UV and Hg, and a cumulative effect of combined treatment was observed. Looking at oxidative stress enzymes, we observed an opposite effect of combined treatment: peroxidase activity was significantly decreased by UV and Hg treatments alone, whereas a combination of both abolished this effect. Results of RNA-Seq confirmed results obtained from analysis of pigments and stress response. At the transcriptome level, in terms of stress response, combined exposure had more severe effects than Hg or UV single exposures. Briefly, we found genes involved in sugar metabolism and oxidative stress to be up-regulated, and many photosynthesis-related genes to be down-regulated by combined Hg and UV exposure. In conclusion, we were able to show that UV exposure influences accumulation and tolerance to Hg.
The decline in pH and rise in CO₂ manifested through ocean acidification (OA) could potentially result in significant physiological effects on microbes, plankton, and larger organisms, and lead to alterations in biogeochemical cycles and ecosystem function. Concurrent with OA, a variety of other physical and chemical changes are projected for coastal regions that could affect organisms, including increases in the quantity of UV light, increases in sea surface temperature, reduction in salinity, and increases in emerging contaminants e.g. human and veterinary medicines. Phytoplankton and zooplankton comprise the basis of productive coastal and open ocean food webs and provide valuable ecosystem services that humans are socioeconomically dependent on (e.g. animals from fish larvae to baleen whales dependent on plankton for nutritional needs). The phytoplankton and copepod species (S. costatum, R. bulbica, and T. battagliai) that were investigated in terms of response to OA/multiple global change variables are considered vital/keystone to coastal Arctic and Norwegian ecosystems and are the focus of this paper. Two distinct but complimentary systems for the manipulation of phytoplankton were used: “micro-scale” and a more traditional “macro”, litre-scale system were developed. For the micro-scale system T. battagliai was used as a model organism. There has been documented resilience of copepods to acidification (due to proteins and chitin) and they have a greater

T. battagliai

µg/l), Ag EC20 (2.3 µg/l) and the mixture of (Cu EC10 (6.7 µg/l) +Ag EC10 (1.3 µg/l) was used as a model organism. There has been documented resilience of copepods to acidification (due to proteins and chitin) and they have a greater capacity to adapt to climate change. However, other factors such as changes in temperature have proven to affect various life stages. In addition exposure to high CO₂ levels can enhance the sensitivity of organisms to thermal extremes. Furthermore, changes in salinity may affect organisms as an independent stressor as well as by altering the bioavailability and in some instances increasing the toxicity of chemicals. The two systems were successfully developed for use in OA experiments, the micro-scale system showed great potential to investigate multiple stressors, but did not allow for sample collection for independent studies relative to OA, however, employing more sensitive endpoints like naupliar development and reproduction showed responses vary between life stages. Reproduction and development were greatly influenced by the modification of environmental parameters such as pH, salinity and temperature, which also influenced the toxicity of known toxans to T. battagliai.

TU157

Combined effect of metallic pollutants (Cu and Ag) and temperature on the early life stages of the Mediterranean mussel Mytilus galloprovincialis K. B. AMMAR, Gironde; M. Banni, Laboratory of Biochemical and Environmental Toxicology; J. CACHOT, EPUC University of Bordeaux

The present work aimed to assess the effect of metallic pollutants (Cu and Ag) exposure along with temperature rise, on early life stages of the Mediterranean mussel Mytilus galloprovincialis. For this, embryo-toxicity assay and RT-qPCR were used to evaluate developmental effect/s and gene expression modulation in D-larvae exposed to both thermal and chemical stress. At first, mussel embryos just fertilized were exposed to rising concentrations of copper (Cu) (0.5 – 500 µg/l) and silver (Ag) (0.1 – 100 µg/l) along with temperature gradient (18, 20, 22 or 24°C) in order to characterize their toxicity and determine the EC50 of each toxicant for the different tested temperatures. Then, increasing concentrations of a Cu-Ag mixture were applied in order to understand and assess the mixture effect at different temperatures. Embryotoxicity was measured after 48h (stage D-larvae) by considering both the percentage of abnormalities and development arrest in D-larvae. Eighteen target genes involved in antioxidant defense, DNA repair, apoptosis, proteolysis, transcription, thermal stress and metal transport were investigated in D-larvae. Then, these were exposed to Cu EC20 (9.6 µg/l), Ag EC20 (2.3 µg/l) and the mixture of (Cu EC10 (6.7 µg/l) + Ag EC10 (1.3 µg/l)) at 3 temperatures (18, 20 or 22°C). The results suggest that the best temperature for mussel larvae development is 18°C (∼10% malformations). The rate of malformations increases with increasing temperature to reach 100% at 24°C. Silver has been proved to be more toxic than copper with a half maximal effective concentration (EC50) at 18°C of 6.20 µg/l (5.09 – 6.72) and 18.14 µg/l (13.23 – 18.26) respectively. Temperature seems to increase the toxicity of these metals as proved with the EC50 obtained at 20°C irrespective for Cu and Ag 16.3 µg/l (12.01-16.42) and 5.7 µg/l (2.81-4.07). The higher toxicity of Ag/Cu mixture suggests slightly antagonistic effect of the two metallic ions with a synergy factor of 0.68 at 18 °C. Significant gene transcription modulation was observed for several genes involved in thermal stress (hsp 70), proteolysis (catepsin), DNA repair (p53) and anti-oxidant defense (cat). These changes in gene expression provide new insights to better understand sensitivity of mussels early life stages to combined effects of metallic pollution and temperature rise.

TU158

Did intertidal biota decline after the 2011 Great East Japan Earthquake and Tsunami and the Fukushima nuclear disaster? T. Shimizu1, National Institute for Environmental Studies / Center for Environmental Risk Research; H. Yoshii, National Institute of Radiological Sciences; S. Mizuno, Fukushima Prefectural Government; H. Shiraiishi, National Institute for Environmental Studies

In 2011, and 2013, in the intertidal zones of eastern Japan, we investigated the ecological effects of the severe accident at the Fukushima Daiichi Nuclear Power Plant that occurred in the 2011 Great East Japan Earthquake and Tsunami. The number of intertidal species decreased significantly with decreasing distance from the power plant, and no rock shell (Thais clavigera) specimens were collected near the plant, from Hirono to Futaba Beach (a distance of approximately 30 km) in 2012. The collection of rock shell specimens at many other sites hit by the tsunami suggests that the absence of rock shells around the plant in 2012 might have been caused by the nuclear accident in 2011. Quantitative surveys in 2013 showed that the number of species and population densities in the intertidal zones were much lower at sites near, or within several kilometers south of, the plant than at other sites and lower than in 1995, especially in the case of Arthropoda. There is no clear explanation for these findings, but it is evident that the intertidal biota around the power plant has been affected since the nuclear accident.

Combining exposure and effects models and data for landscape based risk assessment in a regulatory context (P)

TU159

Effect modelling as a bridge from the first tier to the landscape level: An example for mammals M. Wang, WSC Scientific GmbH / Dept Efate Modelling; M. Fouldoukalis, Dow Agrosciences / RSRA ERS; S. Norman, RidgewayEco; N.N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department

In the present study body burden modelling is used to predict potential effects of a pesticide application in mammals. The study is based on a substance with a rather worst-case first tier profile. For parameterisation of the body burden model purpose-designed studies were conducted and the model was validated using data from existing independent studies, covering different species. The validation demonstrated that the model provided robust but slightly conservative results. Body burden modelling was then used to consider realistic feeding behaviour obtained from the literature and measured residues in food items. Results indicated a low risk in all focal species. These results were in line with data from large-scale field studies from different crops, which indicated no effects on population abundance or reproduction. This risk assessment exemplarily demonstrates how effect modelling can help to extrapolate results from the lower tier, which only facilitates a limited view of the potential risk since not all factors determining the risk are considered, to a real field situation. The gained knowledge of all factors, including mode of action, individual behaviour or ecology allow a much better understanding of risk and to draw conclusions about the acceptability of a pesticide use with much more certainty.

TU160

Pesticide treatment regimes in landscape based risk assessment F. Streissl, EFSA / Pesticides Unit; J. Richardson, EFSA / AMU; C. Sinclair, D. Garthwaite, FERA Science Limited

In order to develop landscape based risk assessments it is essential to know all the different stressors, including pesticides, which can potentially impact populations of non-target organisms in the different agricultural landscapes of Europe. The environmental risk assessment evaluates the risk for one single substance at a time. This makes it difficult to apply the recovery principle in the risk assessment. In order to have a correct estimate of the risk to non-target organisms it is essential to link effects to real exposure to pesticides in agricultural landscapes. The European Food Safety Authority (EFSA) recently published a technical report on pesticide application data representative of different crops and different regulatory zones in Europe which can be used as a basis for a future landscape based risk assessment. A total of 394 farms and 2814 fields were surveyed covering the following Northern (Lithuania), Central (Belgium, Netherlands, Poland and United Kingdom) and Southern (Greece, Italy and Spain) zones. Data were collected for arable crops, orchards and grape vines. For 580 fields detailed information on the field margins and surrounding structures was collected and for these fields in some countries detailed historical pesticide usage data, for up to five years, was also collected. The number of products and substance classes applied and the number of spray applications within one growing season varied enormously among the different crops (e.g. 25 spray applications and 39 different formulations in apple orchards versus 5 spray applications and 14 formulations in sugar beet). This suggests that the exposure and hence risk for non-target organisms may vary to a large extent depending on the crops which are grown in different agricultural landscapes. Therefore the development of environmental scenarios including crop relevant treatment regimes are essential for a realistic landscape level risk assessment approach.

TU161

From the first tier to the landscape: How modelling helps to understand the risk M. Wang, WSC Scientific GmbH / Dept Efate Modelling; M. Fouldoukalis, Dow Agrosciences / RSRA ERS; S. Norman, RidgewayEco; N.N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department

In pesticide risk assessment first tier calculations are thought to provide an efficient screening method to detect substances that may need a more detailed evaluation. The first tier risk assessment is usually viewed as a method indicating the level of risk of a given toxic substance. Being based on toxicity and exposure
only, other factors that may potentially play an important role in determining the risk to individual animals, are not included. On the other end of the scale, data from large scale field studies may reveal information of the real risk on the landscape level and under real field conditions. Quite often, it is important to understand the results in the field from actual use to reach evidence-based decisions which, in some cases, might be difficult for risk assessors to interpret contradicting results. In such cases, effect modelling helps to understand results in the field or why results of the first tier and the landscape scale are contradictory, by making it possible to understand the mechanisms that determine the real risk in a real agricultural setting. In the present study, an example of a substance with a rather worst-case first tier profile is presented, for which information from the field scale indicates a low risk in birds. Body-burden modelling was conducted with fit-for-purpose laboratory studies to understand why no effects were detected on the landscape scale, while the first tier indicated a risk. This evaluation helped to determine the factors that mostly affected the risk and that were not covered by the first tier risk assessment. This example shows how effect modelling can provide a bridge between the first tier and the field scale, and how both regulators and industry can find the evidence and benefit from a better understanding of the mechanisms determining or preventing a risk.

TU162 Models’ output on how to combine spatiotemporal scales, biological organizational levels and interactions in environmental risk assessment: A short review.
E. Chiaideftou, Benaki Phytopathological Institute / Department of Pesticides Control Phytopharmacy Laboratory of Toxicological Control of Pesticides; K. Kornakoupolou, K. Machairis, Benaki Phytopathological Institute
This literature review chronicles the modelling approaches that have been developed for assessing potential risk in the context of environmental risk assessment for pesticides with the aim to reflect the up-to-date outcomes on how to combine spatiotemporal scales, biological organizational levels and interactions in regulatory risk assessment. For this purpose the literature review was carried out by using keywords in all fields of the “Expert search” option “Journals” of ScienceDirect with the formula “environmental risk assessment” and “exposure” and “effect” and “model” and “pesticide”. From 83 publications a thorough full text examination was conducted to include only the publications with consistent information. Therefore only publications that studied and proposed the development, combination and/or improvement of models to be used in environmental risk assessment were incorporated in this review’s database. Thus data from a corpus of 22 publications from 1997 to 2015 were considered in the results of the current review. Different biological organizational levels were included from individual to populations to communities to landscape. Most models have been initially developed for aquatic organisms and environments though a small minority for soil organisms. Although toxicokinetic parameters have already been mostly developed for pesticides, they were not included in the majority of the proposed models and approaches in the reviewed publications to link exposure with effect. Therefore a clear picture on their contribution to predict dynamic responses over space and time under different exposure regimes cannot be regarded. This applies even for the studies proposing a combination of individual- and population-based models. Population dynamics are considered in either effect or exposure models as well as their combinations. Biological interactions such as predation, competition and density dependence, interactions between different species, synergism, antagonism and toxicokinetics and toxicodynamics of individuals and their environments from which population dynamics emerge were studied in approximately half of the reviewed database.

TU163 Investigating an ecosystem services framework to advance chemical risk assessment and risk management.
S. Marshall, Unilever / SEAC; A.R. brown, University of Exeter; G. Whale, Shell Health / Shell Health; M. Jackson, University of Exeter / Biosciences; M. Hamer, Syngenta / Environmental Safety; A. Solga, Bayer CropScience AG; P. Kabouw, B. Bourne, R.W. Woods. EnvironmentMobility BW; E. Garthwaite, FERA Science Limited
These are most at risk from chemical exposure.

TU164 Using in vitro assay based critical input parameters to evaluating a fish bioaccumulation model for ionogenic organic substances (BIONIC) s. droge, Utrecht University / IRAS; Y. Chen, Cgbh; J. Hermens, Utrecht University / Institute for Risk Assessment Sciences; J.M. Armitage, University of Toronto - Scarborough / Physical and Environmental Sciences; J. Arnot, ARC Animal Research & Conservation Sciences
Recent Developments and Current Issues in Bioaccumulation Assessment (P)
This work presents the model for the prediction of bioaccumulation half-lives of ionogenic organic substances (BIONIC) for fish in the “in vitro” scenario. A comprehensive literature review was carried out for both fish and mammals. This approach allowed a comparison of the experiments carried out in vivo to those carried out in vitro. The model presented here estimates the half-lives from the in vitro experiments for fish using critical input parameters that are experimentally determined in vitro. A new approach was used to determine the best estimation of the concentration of the substance in the assay. This allows a more accurate estimation of the bioaccumulation half-life of the ionogenic organic substances (BIONIC) for fish in the “in vitro” scenario. This approach is compared to the in vivo experiments and the results are presented in the form of a table. The model presented here is an improvement over previous models because it uses critical input parameters that are experimentally determined in vitro. The model presented here is an improvement over previous models because it uses critical input parameters that are experimentally determined in vitro. The model presented here is an improvement over previous models because it uses critical input parameters that are experimentally determined in vitro.

E. Paspalis, Department of Theoretical and Applied Sciences; A. Sangion, P. Gramatica, University of Insubria / DISTA
Investigating an ecosystem services framework to advance chemical risk assessment and risk management.
S. Marshall, Unilever / SEAC; A.R. brown, University of Exeter; G. Whale, Shell Health / Shell Health; M. Jackson, University of Exeter / Biosciences; M. Hamer, Syngenta / Environmental Safety; A. Solga, Bayer CropScience AG; P. Kabouw, B. Bourne, R.W. Woods. EnvironmentMobility BW; E. Garthwaite, FERA Science Limited
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Environmental Quality Standards in marine water: study of bioaccumulation (BAF) and bioconcentration (BCF) factors

A. SIRE, IFREMER / RBE; I. Amouroux, IFREMER / RBE

The Water Framework Directive (WFD) establishes Environmental Quality Standards in marine water for 34 priority substances. Among these substances, 25 are classified as priority substances for the primary environmental quality standards and 23 as priority organic compounds. For these 25 substances, monitoring in water matrix is not inappropriate and an alternative matrix should be developed. Bivalve mussels, particularly mussels (Mytilus edulis, Mytilus galloprovincialis), are used by IFREMER as a quantitative biological indicator since 1979 in France, to assess the marine water quality. This study has been carried out in order to determine thresholds in mussels at least as protective as EQS in marine water laid down by the WFD. Three steps are defined: 1. Provide an overview of knowledge about the relations between the concentrations of contaminants in the marine water and mussels through bioaccumulation factor (BAF) and bioconcentration factor (BCF); 2. Compare BAF and BCF of the small mammal (Aporrectodea caliginosa) obtained with various methods for these substances; 3. Using experimental process, BCF calculated by QSAR and BAF for field data. This study points out that experimental BCF data are available for 3 substances (Chlorpyrifos, HCH, Pentachlorobenzene). BCF by QSAR can be calculated for 20 substances. The use of field data allows to evaluate 6 BAF: 4 for organic compounds and 2 for metals. These BCF or BAF value, thresholds in shellfish can be determined as an alternative to EQS in marine water.

TU167 A reference database for bioaccumulation of organic chemicals in aquatic and terrestrial invertebrates

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Recent advances on the bioaccumulation of organic compounds in fish have been made possible by the availability of high quality bioaccumulation and biotransformation databases. Despite the many bioaccumulation studies made for aquatic and terrestrial invertebrates over the last few decades, and the incorporation of invertebrates into standard bioaccumulation testing protocols, a comprehensive and quality bioaccumulation database of organic compounds in invertebrates has not been available. In order to meet this research need, we have commenced to review and compiling bioaccumulation metrics reported for both soil and benthic invertebrates. Standard bioaccumulation metrics (i.e., BCF, BAF, BSAF, etc.), kinetic rate constants for specific uptake and dissipation processes, details on exposure conditions and experimental setup have been gathered, classified, and homogenized from primary experimental literature. Target chemicals included both non-polar organic and ionogenic/ionizable organic compounds. Target invertebrate organisms included worms, bivalves, insects, amphipods, isopods, gastropods, decapods, and arthropods, etc. Secondary sources of information – existing bioaccumulation databases or technical summaries – that do not have adequate details and/or show of data points were used only as further sources of reference. To date, a total of over 4000 data entries have been gathered for worms, and approximately 2000 data entries have been collected for other non-worm invertebrates. Individual data entries were reviewed for their quality according to the experimental setup and protocol adopted as well as the data reported. This allows us to determine how a BCF or BAF can be determined. BCF and BAF values were compared with field bioconcentration (2 metals and 23 organic compounds). The completed version of this database and biomagnification of a plant protection product in the aquatic and terrestrial environment. The main objective of this presentation is to review what we have learned through direct experience with regards to the sample number requirements, the sampling techniques and the field study design during the preparation of this database. The study design was set up to investigate the spatial and temporal fate of the test item throughout terrestrial food chains for soil and crop and aquatic food chains within water and sediment. Large scale field sites (up to 24 ha) were selected according to worst-case conditions, especially in terms of aquatic exposure – e.g. adjacent water bodies. A combined sampling of terrestrial specimens (field soil, crop vegetation, earthworms, small mammals (e.g., common voles and common shrews)) was conducted over periods of up to six months to characterise the magnitude and duration of residues in the relevant terrestrial compartments. To achieve comparable data throughout different matrices and to control spatial variability, replicates with sub-samples were taken. The treated plot was set up for collecting soil characterisation, soil residue, earthworm residue and crop residue samples from co-located sampling areas. To assess the exposure of the aquatic system in the relevant aquatic compartments, pond water, run-off water, aquatic sediments, aquatic vegetation, macroinvertebrates and fish were sampled from well distributed sample areas. Sampling was performed from up to 30 replicates and with an abundant amount of samples per event to obtain statistically representative data. Further in assessing the potential for bioaccumulation and biomagnification, the following matrices were analyzed separately for residues: soil from soil cores, surface and deep burrowing earthworms, oilseed rape plants, oilseed rape leaves, oilseed rape litter, small mammals without gut and gut residue, aquatic macroinvertebrates. To be able to compare the residues of different matrices, soil and plant residues were normalized for organic carbon; residues of earthworms and small mammals were normalized to lipid content.

TU169 Results and interpretation of a field-scale bioaccumulation and biomagnification monitoring programme

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Bifenthrin was recently approved as an active substance, in accordance with Regulation (EC) No 1107/2009, with a confirmatory data requirement to submit: “a monitoring programme to assess the potential for bioaccumulation and biomagnification in the aquatic and terrestrial environment”. No guidelines are available for conducting monitoring programmes to assess the potential for bioaccumulation and biomagnification in the environment. Direct spray studies were conducted in North Germany to determine whether bioaccumulation or biomagnification occurred following realistic exposure of aquatic and terrestrial systems to bifenthrin. The investigations were designed to represent the full proposed GAP for bifenthrin in the EU, which includes application to early and mature crops, and to address worst-case scenarios in terms of exposure routes in both compartments. In the aquatic environment, residues on leaf litter (soil, earthworm, small omnivorous and invertebran mammal) and crop (soil, small herbivorous mammal) were sampled. For the aquatic environment, food chains based on water and sediment with aquatic macroinvertebrates and fish were sampled. The mitigation measures employed to ensure acute and chronic safety to aquatic organisms (20 m no-spray buffer and 90% drift reducing nozzles) ensured that bifenthrin residues in the aquatic systems were low, even following purposefully exaggerated irrigation. Detectable residues in fish surface mucus samples demonstrated the presence of bifenthrin in the aquatic system. Despite exposure, however, there was no evidence for bioaccumulation or biomagnification of bifenthrin in the exposed substances and residues in small mammals were low. Shrews had lower residue levels than earthworms. Other small mammals likely to consume earthworms also had residues below the levels in earthworms and crop, and significantly decreased over time. Residues in voles, which feed on vegetation, were also low and dissipated rapidly. These investigations, involving the application of a monitoring programme, the study to proposed GAP, demonstrated that bifenthrin does not bioaccumulate or biomagnify in aquatic food webs, nor does it biomagnify at the small mammal trophic level in terrestrial systems.
bioaccumulation of organic contaminants in soil and sediment. This will provide a solid starting point for understanding and modeling the fate of these substances. The completed version of this database will gather, classify, and archive data from primary experimental literature. Target areas include both non-polar organic and ionogenic/ionizable organic chemicals.

Calculated for 20 substances. The use of field data allows to evaluate 6 BAF: 4 for terrestrial food chains for soil and crop and aquatic food chains within water and terrestrial invertebrates. Individual data entries were reviewed for their quality and completeness. A C_SOG factor was calculated for bifenthrin in the homogeneous 10 cm soil profile. Collectively, the calculations suggest that the measured concentrations of bifenthrin for the homogenized 10 cm soil profile are not representative for calculating bioaccumulation factors (BAFs) and characterizing the bioaccumulation potential of bifenthrin in the field. In addition to interpreting the results, these extensive Everglades aquatic sediments, aquatic vegetation, macroinvertebrates and fish were sampled. The mitigation measures employed to ensure acute and chronic safety to the environment. In the terrestrial environment, appropriate food chains for soil and crop residues and plant residues were normalized for organic carbon; residues of earthworms and plant residues were normalized for organic carbon; residues of earthworms were low. Shrews had lower residue levels than earthworms. Other small mammals likely to consume earthworms also had residues below the levels in small herbivorous mammals.

A 9-day exposure period. Sandworms were held in cylindrical containers packed with gravel. The water level was controlled daily to mimic the tide. During the depuration period, the sandworms were fed commercial fish food. We used filtered (0.1 μm) natural seawater kept at about 17.1 °C. Sandworms and seawater were regularly sampled and analyzed for PFOS. Wet mass of the sandworms were monitored by weighing randomly selected individuals. The oxygen consumption rate of the sandworm was also measured. The PFOS depuration rate constant, respiratory uptake rate constant, and respiratory absorption efficiency for the sandworm were estimated by fitting the measured concentration to a first-order kinetic model. The PFOS concentrations at 80 ng mol−1 during the exposure period of the exposure treatment and were negligibly low in the control treatment and the depuration period of the exposure treatment. The PFOS concentrations in the sandworm samples from the exposure treatment increased during the exposure period and decreased afterwards. The estimated value of the PFOS depuration rate constant (0.050 d−1) was similar to those reported for a freshwater oligochaete and for the whole body of several fish species, but one order of magnitude lower than that for an oyster species. Although the value of the PFOS respiratory uptake rate constant (32 L kg−1 wet −1 d−1) was in the same range as for the whole body of several fish species and the oyster, the value of the respiratory absorption efficiency (0.13) was generally higher than those estimated for several fish species. These results suggested a more efficient respiratory uptake of PFOS by the sandworm than by fish.

Biaccumulation of Selenium through the food chain: water - Lemma minor - Pomacea paludosa T. Hoang, Loyola University Chicago / Institute of Environment and Sustainability; A. Ahmed, Loyola University Chicago Selenium (Se) has been used as a fungicide in sugar cane growth in South Florida for many decades. The long-term use of Se in these environments has led to an increase in Se concentration in soils. Comprehensive plans under the Comprehensive Restoration Plan, thousands of acres of agricultural lands will be flooded to create wetland habitats and water reservoirs. Flooding lands will result in Se desorption from soils to water and will affect aquatic organisms. Aquatic plants such as Lemma minor (duckweed), inhabitants of these systems, can accumulate Se which then may be transferred via the food web. A subjective aim of this study is to study how Se transfers through different levels of the food chain.
(water-duckweed – Pomacea paludosa (Florida apple snail). Under standard laboratory conditions, duckweed was exposed to three different concentrations of Se (100 µg/L, 500 µg/L, 1000 µg/L) for 2 weeks. Apple snails were fed with Se contaminated duckweed for 28 days. During the course of the study, apple snails and duckweed were collected at different time intervals for Se analysis. This study found that Se was bioaccumulated from both food and water by apple snails. Se concentrations in duckweed increased with Se treatment concentration and ranged from 1 mg/kg dw (control) to 431 mg/kg dw (T3). Concentration of Se in the snail soft tissue (food + viscera) increased with increasing Se duckweed concentration and ranged from 0.77 mg/kg dw (control, day 0) to 142 mg/kg dw (T3, day 21). In general, Se concentration in apple snails increased in the order of shell, food, and viscera. Se concentration in the snail soft tissue also increased with exposure time. Results of this study are in agreement with the results of other studies with copper and zinc and apple snails.

Challenges in environmental read-across and grouping of substances - when fate, bioaccumulation and ecotoxicological properties are similar enough? (P)

TU175
A comparison of the toxicity of hindered phenols and substituted phenyl amines to terrestrial plants and invertebrates in a sandy soil

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A research project was initiated by Environment Canada's Biological Assessment and Standardization Section (BASS) as part of the Canadian Federal Government's Chemicals Management Plan (CMP), to assess the toxicity and bioaccumulation potential of two groups of organic compounds to terrestrial organisms. The groups of organic compounds (hindered phenols and substituted phenyl amines) were selected based on the expectation of partitioning primarily to soil, varying availability in soil, and based on limited effect data in general. Two representative chemicals from each group were selected for toxicity testing with the intention of using the empirical data for read-across and to fill data gaps within the groups for subsequent risk assessment purposes. The hindered phenol group was represented by 3,6-di-t-butyl-phenol (CAS 128-39-2) and 2,2'-thiodithylene bis-[3,5-di-t-butyl-4-hydroxyphenylpropionate] (CAS 41484-35-9), while N,N-di-n-octyl-phenylamine (CAS 534-55-7) and N,N'-di-2-naphthyl-1,4-phenylenediamine (CAS 93-46-9) were selected to represent the substituted phenyl amines group. The inherent toxicity of the test substances was assessed through invertebrate (Folsomia candida and Eisetena andrei) reproduction and plant (Trifolium pratense and Elymus lanceolatus) definitive tests, using Environment Canada's standardized soil test methods. Effects on E. andrei reproduction were then evaluated in order to select a single chemical from each group for bioaccumulation tests. The generated data, with accompanying exposure characterization will be presented, with an evaluation of the read-across potential of these substances to environmental risk assessments.

TU176
How to group organotin compounds in view of their regulatory assessment under REACH?

N.M. Deleebecq, N. Scharpen, M. Mesiaen, S. Bergeret, ARCADIS / Environment; F. Van Broekhuijzen, A. Müller, National Institute for Public Health and the Environment RIVM

Organotin compounds represent a group of organometallic compounds which has attracted regulatory attention several times in the past. Due to a combination of known use patterns, expected/demonstrated fate and behaviour in the environment, and intrinsic hazards, this regulatory attention has not diminished – at the time of writing this abstract, the following activities have been identified under REACH: there are two restrictions (one on organotin compounds in general, and one substance-specific), four organotin substance have been included in the candidate list for inclusion in Annex XIV of REACH (authorisation list), four organotins are on the CoRAP list (Community Rolling Action Plan) for substance evaluation and harmonisation of data. This has focused the European and international guidance on various organotin compounds, and finally, a number of other evaluative activities have been identified in the PACT (Public Activities Coordination Tool), such as hazard evaluations (PBT/vPvB assessment, assessment of endocrine disrupting properties), risk management option analyses, etc. All these regulatory/evaluative actions are now being rolled up on a one-by-one basis. To come to a more efficient approach, it was considered necessary to investigate how organotin compounds may be grouped for further evaluation. Based on the currently available information in the +30 REACH registration dossiers for organotin compounds, an effort was made to map grouping and read across actions as proposed by industry. Specific attention was given to the information used to justify grouping and read across. Then, taking into account international guidance on grouping of chemicals in general and more specifically of organometallics (e.g. ECHA, OECD), as well as previous work on grouping of organotin compounds (e.g., OECD CoCAP), a framework has been proposed to evaluate stepwise whether or not an organotin compound can be included in a certain category of organotins for read across purposes for a specific (type of) endpoint. This framework indicates what information should minimally be available or be generated to allow this evaluation. Further, it is investigated to what extent information on uses can – on top of all other information – be applied to evaluate where risk management measures (if any) would be required to guarantee safe use of groups of organotin compounds. This project therefore contributes to reaching the objectives as put forward in the SVHC Roadmap 2020.

TU177
Towards the Environmental Read-across Assessment Framework


REACH is a Regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be caused by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals. Grouping of substances and read-across is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under the REACH Regulation. This approach uses relevant information from analogous ‘source’ substances to predict the properties of ‘target’ substances. During dossier evaluation and testing proposal examination (REACH Regulation Title VI), the justification for the grouping of substances and read-across provided by registrants is assessed by ECHA to check whether it fulfils the legal requirements. To allow transparency and consistency of the read-across assessment, ECHA published a hierarchical decision framework in 2015 and is now developing the Environmental Read-across Assessment Framework (ENV RAaAF). It aims at structuring and codifying expert judgement of complex scientific questions on the critical aspects of verifying the justification provided by the registrants. Here we present the ENV RAaAF which is divided into four sequential steps. First, the assessor performs an administrative check to make sure that a meaningful scientific assessment can be carried out. Then, the provided documentation will be assessed with regards to data quality and the hypothesis or rationale will be verified. All of the information provided for the target and source substances should be considered to enable a robust verification of the hazardous properties of the target substance when comparing them to the source. When concluding the assessment, it is kept in mind that the results of the study(ies) provided for the source substance(s) are also used for classification and labelling and/or risk assessment of the target substance. Therefore, it needs to be ensured that the hazardous properties of the target substance are not underestimated. Finally, the assessment and its outcome are documented. Application of the ENV RAaAF should result in a structured step-wise assessment, consistently performed between assessors, that recognises the strengths of the read-across and identifies possible shortcomings in documentation, scientific reasoning and/or supporting evidence.

TU178
Weight of evidence approach to assess the acute aquatic toxicity of GTL solvents relative to other hydrocarbon solvents

C. Hughes, Shell International Limited / Shell Health; C.V. Eadsforth, Shell International / Shell Health, S. Harris, Shell International Limited / Shell Health Hydrocarbon solvents are used in a wide range of industrial and consumer applications globally. These products consist of many different hydrocarbon constituents and are described as UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials). They are most commonly derived from the processing of crude oil, but other varieties, such as synthetic ‘isoparaffins’ and solvents derived by the Fischer–Tropsch, or ‘Gasi-to-Liquid’ (GTL) process, also exist. Although different solvents may share similar performance properties, their composition varies depending on the manufacturing process and they are regarded as different substances under REACH. In order to assess the relative aquatic toxicity of GTL solvents compared with other hydrocarbon solvents a range of screening methods have been used. These included testing of water-accommodated fractions (WAFs) using solid-phase micro-extraction (SPME) combined with gas chromatography (GC) analysis, MICROTOX® and DAPHNIX™ F™ assays, and toxicity predictions using the PETHETOX model. Results were compared with compositional information from the substances and departmental data from GLP compliant acute aquatic toxicity studies, conducted under OECD guidelines. The results of the screening tests suggested that acute toxicity of GTL Solvents is dependent on hydrocarbon chain length, similar to de aromatised and isoparaffinic hydrocarbon solvents. The white spirits were significantly more toxic based on screening data, which was expected based on the presence of aromatic compounds. On the basis of these results, a tiered screening methods applied to the two most sensitive approaches for detecting toxicity of the various products are SPME–OC and the DAPHNIX™ F™ test kit, whereas the MICROTOX™ assay is the least sensitive. The PETHETOX calculations predicted a similar relationship with chain length, but were generally more conservative than the experimental data. Overall, the use of a range of test methods and a weight of evidence approach, the acute aquatic toxicity of GTL solvents was
Environmental risk assessment of complex natural substances: needs for methodology adaptations

Litterstorf, S. ANDRE ; INERIS / Toxicological Ecotoxicological Assessment of chemical Substances

Natural complex substances (NCS) are under the scope of European regulations on chemicals (Biocides, PPP, REACH, CLP...). For example under REACH regulation NCS are considered as substances, either mono-, multiconstituent or UVCB. In most cases however, NCS can be considered as UVCB, hydrophobic and volatile substances. The objective of the study is to identify difficulties in environmental hazard and risk assessment of these substances and propose methodology adaptations for these chemicals. Two strategies are generally envisaged for their evaluation: one considering the NCS as a “substance” as a whole or taking its “constituent” as a basis of the assessment. The approach “substance” shows the advantage to consider the effects of all the constituents of the mixture without these needing to be totally identified. For convenience, certain required data have no meaning for a UVCB such as the solubility, the Kow, the bioaccumulation which are parameters intrinsically inappropriate to each of the constituents. Other data can be obtained by testing the substance directly. The “constituent” approach requires an important effort for the characterization of the NCS. Besides, the addition of the effects of each constituent is not necessarily equal to the effect of the mixture as such. The approach by constituent and the characterization of these allows the use of alternative methods as the approach QSAR, by congener or category and thus the read-across between constituents. Under REACH regulation, for the tonnage band greater than 10 T/yr, environmental exposure and risk have to be assessed. In this case, for environmental exposure, constituents have to be taken into account, the environment acting as a filter, the UVCB as such does not exist. It can be then decided to consider the predicted environmental concentration (PEC) of the most soluble of known constituents and to compare it with the predicted no effect concentration (PNEC) of the substance. This approach can be relatively conservative and can be refined in higher tier assessment. An example of this assessment is presented to illustrate the methodology.

Data Availability of REACH Registrations: Adaptation of Information Requirements for High Tonnage Chemicals


According to the REACH Regulation (EC) No. 1907/2006 chemicals produced, marketed or used within the European Union have to undergo a registration process, wherein the registrants have to provide information on hazards presented by the substances and assess potential risks. The standard information requirements defined in Annexes VII to X of the regulation may be waived or adapted by the registrants if adequate documentation and justification according to criteria defined in Annex X are provided. To evaluate the availability of ecotoxicological data in registration folders of high tonnage substances (1000 tpa or more) and their compliance with the REACH Regulation, the Federal Institute for Risk Assessment (BfR) in cooperation with the Federal Environment Agency (UBA) developed a systematic web-based screening scheme. In total, 1932 dossiers were checked for selected environmental endpoints such as biotic and abiotic degradation, bioaccumulation and ecotoxicity. A remarkable high rate, 43% to 82% depending on the endpoint, of the evaluated dossiers included waiving or adaptations from the standard information requirements such as read-across approaches. The assessment of the appropriateness of waiving and adaptations were part of a follow-up project. Herein, it was evaluated whether the given justifications were in accordance with the criteria set out in the respective REACH Annexes. Altogether 335 cases were evaluated for all environmental endpoints and categorized as “formally compliant” and “formally non-compliant” referring to the formal correctness, “obviously compliant” and “obviously non-compliant” indicating compliance with regards the content or “complex”. Thereby, 31 % of all cases were classified as “formally compliant” and 2 % as “obviously compliant”, whereas 12 % were assigned to “obviously non-compliant” and 19 % to “formally non-compliant”. However, 35 % were not concluded and remained as “complex”. Read-across approaches were most frequently used as an adaptation. The results for waiving/adaptation will be complemented by the PNEC and the PEC based assessment of the data gaps and will be discussed.

Environmental risk assessment of chemical mixtures: the steps ahead (P)

Addressing combined toxicity in the environmental risk assessment of inorganic UVCBs: some case studies

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There is a clear need to develop a systematic approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs (iUVCBs). Because the variability in composition is generally too arge, it is impossible to identify a set of samples that would be representative and conservative for the hazard identification of the iUVCB and hence experimental testing is not feasible. For this reason the iUVCB risk assessment is based on the assumption that the toxicity is driven by the toxicity of their constituents, resulting in parallel risk assessments of the constituents. In a conservative approach, each constituent of the substance is assessed based on its maximum concentration and its most severe chemical specification. Upon ECHA’s request, registrants of iUVCB intermediates committed to improve the the combined toxicity assessment part of their REACH registration dossiers. However, an appropriate standard approach to address the combined toxicity of inorganic constituents in a regulatory framework is still missing: most standard approaches yield indeed over-conservative results such as risk scenarios at natural background concentrations when several metals are combined. Therefore, a generic tiered approach has been recently developed for the environmental risk assessment of iUVCBs. This approach starts from the standard concentration addition evaluation based on summation of the PEC/PNEC ratios of the individual constituents and includes several options for refinement of this approach, e.g. taking into account bioavailability, using msPAF approaches or assessment per trophic levels. In addition, screening methods for evaluating the relative importance of the different constituents are included in the overall approach. This poster presents the results of this tiered approach for addressing the combined toxicity in a constituents-based environmental risk assessment for some representative iUVCBs.

Tu182 Toxicity and bioavailability of lubricants and their additives in the aquatic environment

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For the purpose of communicating environmental hazard via material safety data sheets and product labels, lubricants have to be classified according to their toxicity to the aquatic environment on a line with European Classification, Labelling and Packaging guidelines and GHS classification guidelines. The classification of mixtures should primarily be based upon the available test data on the mixtures as a whole or on similar mixtures. However, in those cases where these data are missing, the classification should be based on calculation (i.e. the GHS summation method) using those ingredients classified for the particular hazard. As experimental data on the aquatic toxicity of lubricants are often not available, hazard assessment of these products is regularly based upon the toxicity of the individual hazardous ingredients, the additives. Fully Formulated Lubricants (FFLs) were tested in screening tests with crustaceans (Daphnia magna), algae (Selenastrum capricornutum) and invertebrates (Vibrio fischeri). The test results were then compared with classifications derived from applying the summation method. It is suggested that the additives might not be bioavailable to the aquatic ecosystem due to their incorporation into the lubricant (OECD, 2004). An overestimation of the aquatic toxicity of the final product may be due to a reduction in the bioavailability of the highly toxic additives as a consequence of partitioning into the lubricant base oil. To test this hypothesis, toxicity data were generated (from water accommodated fractions (WAFs)) on the additive packages containing the toxic components on their own and in the presence of base oil as the FFLs.

Tu183 What do effect-based tools need to capture to support water monitoring?

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Environmental quality assessment of water resources is challenged with providing the basis for safeguarding the environment against adverse biological effects of anthropogenic chemical contamination from diffuse and point sources. While current regulatory efforts focus on monitoring and assessing a few legacy chemicals, due to progress in analytical techniques many more anthropogenic chemicals are and will be detected in our aquatic resources. Assessing this type of

SETAC Europe 26th Annual Meeting Abstract Book

233
exposure information based on available standard approaches from single chemical prospective risk assessment leads inevitably to indication of risk in most surface water bodies (Malaj et al. 2014, Stehle and Schulz 2015). However, exposure to neither individual chemicals nor mixtures does necessarily translate into adverse biological effects. As an alternative to generic assessment approaches effect-based monitoring approaches are suggested (Escher et al. 2014). To become a credible complement to chemical monitoring information we need, however, better understanding of the capabilities and gaps of available effect-based tools (EBT). In this work we therefore undertake to (i) compile organic contaminants detected in freshwater monitoring studies, (ii) provide a synopsis on the mode-of-action and available for the detected compounds to map against available EBTs coverage, and (iii) utilise a hazard identification approach to identify priority compounds for effect-based monitoring. From our work it emerges that chemical occurrence in European freshwaters seems to be highly variable in composition and relative abundances. Further, while we are substantially limited in our mode-of-action appreciation we can already identify major gaps in coverage of potential effect qualities when relying on established EBTs. Finally, we suggest a list of organic compounds that could serve as a reference list for EBT validation studies.

TU184

Attempt to apply the WHO/IPCS framework on assessment of combined exposure to multiple chemicals to regulatory environmental risk assessment of chemicals
K. Yamazaki, Env Health Dep., Ministry of the Environment / Environmental Health Department

Environmental risk assessment for regulatory objectives is in general conducted on a chemical-by-chemical basis. Under the limited resources, considering mixtures assessment for all of the chemicals under the regulatory environmental risk assessment will not be effective nor practical. High-priority chemicals groups should be identified efficiently. WHO/IPCS proposed a framework adopting tiered approach for assessment of combined exposure to multiple chemicals. We have been conducting attempts to apply this framework to consider multiple chemicals in the present practices on environmental risk assessment of regulatory objectives. Concentration of chemicals in the ambient environment (air and water), which can be obtained through monitoring programs by national and local governments as well as academic studies, can provide information on combined exposure to multiple chemicals. Concerning hazard to the environment, our tentative focus is on “conventional ecotoxicity” to typical aquatic organisms. In order to examine the applicability of the framework, case studies for certain groups of chemicals have been implemented. Target groups of chemicals have been identified considering structural similarity, modes of action and co-existence in the ambient water. Updated situation of the case studies will be presented at the Annual Meeting.

TU186

FRAM - The Centre for Future Risk Assessment and Management Strategies at the University of Gothenburg
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Chemicals are typically assessed and managed one after another for their environmental risks, based on the notion that the each chemical is the only toxic substance present in an otherwise pristine environment. It is becoming increasingly clear that this strategy is a major shortcoming of even modern regulatory frameworks such as REACH, as it is a precarious oversimplification of reality, in which dozens or even hundreds of chemicals are present at any given time and location in the environment. If failing to account for the presence of chemical cocktails in the environment is particularly problematic because scientific evidence shows that the toxicity of a mixture is not simply the sum of toxic effects of individual compounds, sources and activities erroneously implies an open, infinite ecosystem. This obviously wrong assumption is the Achilles heel of current chemical regulation. It is not sufficient to ensure that the actions of an individual company, authority or consumer are on an acceptable level - it is their joint impact that matters for ensuring sustainable chemical governance. To become a credible complement to chemical monitoring information we need, however, better understanding of the capabilities and gaps of available effect-based tools (EBT). In this work we therefore undertake to compile organic contaminants detected in freshwater monitoring studies, provide a synopsis on the mode-of-action and available for the detected compounds to map against available EBTs coverage, and utilise a hazard identification approach to identify priority compounds for effect-based monitoring. From our work it emerges that chemical occurrence in European freshwaters seems to be highly variable in composition and relative abundances. Further, while we are substantially limited in our mode-of-action appreciation we can already identify major gaps in coverage of potential effect qualities when relying on established EBTs. Finally, we suggest a list of organic compounds that could serve as a reference list for EBT validation studies.

TU187

Is the toxicity of pesticide mixtures occurring in agricultural influenced streams additive? An experimental approach with Daphnia magna
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A broad variety of pesticides are used for crop production and may consequently be exposed to a wide range of known and unknown chemicals. Target analyses are poorly suited to evaluate such exposure or associated risks. The objective of this study was to validate and test the application of bioanalytical screening approaches to investigate the combined effect of chemical mixtures on aquatic species. We applied a tiered bioanalytical screening approach with a battery of six in vitro bioassays. Baseline toxicity was tested with the bioluminescence inhibition assay (Microtox). Specific modes of action, AhR-CAFUX and BGL were applied to screen dioxin-like and estrogenic activities. For the reactive mode of action we used ARE32 assay targeting the oxidative stress response in the adaptive stress response pathway, NkB-bla assay as an indicator for immune response to infection and inflammation, and p53-bla assay as a response to DNA damage. First, we tested sediment samples from the three habitats to get insights into the bioavailability of the chemicals. Then we validated a QuEChERS extraction method on whole blood samples as a non-target approach for bioassays. We showed that there were no matrix interferences in any of the assays. Furthermore, 25-30 blood samples were analysed from each habitat to test if there are differences between them. Our results showed that sediments from the offshore site (Howicks) had consistently lower responses than the two coastal habitats. For turtle blood samples, three bioassays (Microtox, ARE32, AhR-CAFUX) were positive, with the agricultural habitat (Upstart Bay) showing significantly higher responses compared to the other two sites. Overall, this study demonstrates that cell-based bioassays provide a suitable non-target analytical tool to evaluate combined mixture effects of organic chemicals in whole blood samples. Combining such tools with information on sea turtle population health status could present a powerful tool to evaluate associations with complex chemical exposure in nearshore environments.
following an acute exposure. These data indicate that the pesticide mixtures show a lower toxicity than it would be expected from the pesticides’ individual acute toxicity. Also during chronic exposures, a significant decrease in reproduction was observed at a STU between 1 and 8, while higher STU partly resulted in substantial mortality (up to 100%). The study’s findings uncovered that mostly lower than additive or interactive effects occur for pesticide mixtures, suggesting the concept of concentration additivity is often protective for pesticides in agricultural stream.

TU188

Does pathogen infection affect the elimination of toxic compounds?
K.E. Pedersen, B.L. Fredensborg, University of Copenhagen / Department of Plant and Environmental Sciences; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences

The importance of combined exposure to pesticides and pathogens to organism health has gained increasing recognition, mainly to improve methods for controlling pests in agricultural settings and for reducing mortality in beneficial insects. However, very few studies have quantified the interactions or described the mechanisms behind observed interactions between chemical stress and pathogen load. One suggested mechanism of interaction is via enzymatic processes e.g. the cytochrome P450 (CYP). CYP are initially involved in the detoxification of chemicals, and often up-regulated in response to chemical stress. On the other hand CYPs have been reported to inhibit or interfere with the innate immune response towards infections. The inverse relationship of CYP in detoxification and immune defense indicates a trade-off between these systems, a trade-off that can result in altered chemical elimination kinetics and delayed chemotherapeutic effects. We aim to investigate the effect of pathogen infection on the elimination kinetics of sublethal doses of a chemical using the flour beetle Tenebrio molitor as model organism. Propiconazole, a widely used azole fungicide, which has relatively low toxicity towards invertebrates, was chosen as chemical stressor, whereas the tapeworm Hymenolepis diminuta, was chosen as pathogenic stressor. We hypothesized, that co-exposure to propiconazole and H. diminuta would result in 1) decreased CYP activity due to energy allocation towards immune defences and hence 2) decreased elimination rate of propiconazole. To investigate this, internal concentrations of radiolabeled propiconazole will be followed over time and CYP activity will be measured in vitro continuously before, during and after the chemical elimination phase in infected and non-infected beetles.

TU189

Spatial and temporal analysis of the risks posed by polycyclic aromatic hydrocarbon, polychlorinated biphenyl and metal contaminants in sediments in UK estuaries and coastal waters
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The environmental risks of 22 contaminants, comprising 6 metals, 10 PAHs and 6 PCB congeners occurring in UK estuaries and coastal waters were assessed as single substances. Sediment samples were taken within 12 nautical miles of the English and Welsh coasts between 1999 and 2011. The measured environmental concentrations were compared to quality standards including ERL, ERM and EAC, all of which have been established internationally. Out of a total of 38,031 individual samples analyzed, 42.6% and 7.7% exceeded the ERL/ EAC and ERM values, respectively. The highest Risk Characterisation Ratios (RCRs) for metals, PAHs and PCBs were observed for copper, fluorene and CB118 (2.3, 4.4, 5-pentachlorobiphenyl). In general, the highest concentrations of PAHs and PCBs were observed in 2011 in the Lower Medway indicating a potential risk to the aquatic environment. This study suggests that re-suspension of contaminants banned over 20 years ago is still an ongoing issue.

TU190

Lessons learned from testing PNECoral as reference value in a component based estimation of mixture effects of contaminants
E.S. Heimstad, NILU - Norwegian Institute for Air Research; D. Herzke, NILU - Norwegian Institute for Air Research / MILK

In the natural environment, living organisms are not only exposed to one single pollutant at a time, but to a variety of different contaminants. The exposure to a mixture of chemicals is foreseen, foremost through the use of pesticides, but also from water and the environment they live in. Component based-approaches are based on the concept that the effect of the mixture is a function of the effect of the individual compounds. The method of summing up PEC/PNEC or MEC/PNEC ratios, i.e. concentration addition, has been recommended as a justifiable mixture risk approximation in order to estimate in a first tier approach whether there is a potential risk for an exposed ecosystem (Buckingham, T., Fust, T., 2012). Within the European regulation, the risk for wildlife and predators due to oral intake from lower trophic levels of bioaccumulative contaminants, is estimated with the use of PNECoral. PNECoral values represent dietary predicted no effect concentrations, below which food concentrations are not expected to pose a risk to birds or mammals (ECHA 2008). Results from long-term laboratory studies are strongly preferred, such as NOECs for mortality, reproduction or growth. If a chronic NOEC for both birds and mammals is available, the lower of the resulting PNECs may be used as the secondary poisoning assessment to represent all predatory organisms (ECHA, 2008). The method of summing up MEC/PNECoral ratios was used in order to evaluate the potential risk for organisms in a study of urban ecosystems. In the study conducted in the urban area of Kumasi, where MEC was the median or 90 percentile concentrations of contaminants measured in terrestrial organisms such as earthworm and bird eggs. PNECoral values were adopted from previously assessed and reported values. We will present the results from our calculations, and further evaluate and discuss the applicability of this component based approach; i.e. concentration addition for mixture risk prediction for terrestrial urban ecosystems. Buckings, T., Fust, T., (2012).

TU191

Human health risk due to polycyclic aromatic hydrocarbon (PAHs) exposure in Kumasi, Ghana; and excretion of PAH metabolites in cattle urine from urban and rural sites
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Previous studies of polycyclic aromatic hydrocarbons (PAHs) in particulate matter (PM10), soils and livers of wild rats indicated that the city centre of Kumasi, Ghana was severely polluted with high cancer potency, and fuel, wood/grass combustion were the dominant sources. Cattle urine were therefore collected from Kumasi (urban) and Offinso (rural), Ghana, to determine concentrations of 15 PAH metabolites (OH-PAHs), and investigate exposure levels of cattle to PAHs from the different sites. From the results, geometric mean concentrations (adjusted by specific gravity), GM3, showed that 2-OHNaphthalene (2-OHNP) was the most abundant OH-PAH and significantly higher in Kokote (rural) than the other sites. The GM3 concentration (ng/mL) of \( \Sigma_{5-6} \) PAHs decreased in the order, Kokote (44 ± 10) > Offorikrom (7.8 ± 4.7) > Saboba (6.9 ± 3.8) > Santasi (6.6 ± 2.7) > and T. Estate (5.2 ± 1.5). Principal component shown high association of 2-OHNP, 2-3-OHPh, 2-OHPh and 3-OHPh in Kokote indicating high exposure of cattle to PAHS in this area. Studies have indicated that exposure of rats and mice to naphthalene caused nasal and bronchiolar tumours, respectively. Based on the above studies and because urine could be used as a biomarker of exposure, effect and susceptibility, we collected human urine from 3 hospitals in Kumasi, and also from KNUTS campus (where PAHs in PM10, soils and livers of wild rats were significantly low) to measure the concentrations of OH-PAHS and their association with oxidative stress. Results indicated that 2-OHNP (16 ± 28 ng/mL) was most abundant and highest in participants who visited Manhyia hospital (22 ± 40 ng/mL) as compared with the other two participants who visited Manhyia hospital (22 ± 40 ng/mL) and hospital (22 ± 40 ng/mL). Similarly, there was a significant decrease in naphthalene-containing-mothballs in water purification or insecticide. Highest level of 2-OHNP, (224 ng/mL), was found in urine of a female participant in Manhyia hospital, who reported symptoms of persistent cough, headache, dizziness, tachycardia, myalgia, nasal congestion and inflammation. 1-9, 4-OH-Phes, and 1-OHPh were most abundant from a ycess participant who visited Manhyia hospital. GM3 (ng/mL) of \( \Sigma_{5-6} \) PAHs, from participants in the hospitals decreased in the order, Manhyia (26 ± 41) > Tafo (17 ± 28) > Atonsu (16 ± 21). Although levels of OH-PAHs in urine collected from KNUTS were significantly lower than the hospitals, 2-OHNP was most abundant. Significant correlation between 2-OHNP in cattle and human urine could indicate a common source of naphthalene exposure.

TU192

Ecological risk of mixtures of radioactive and stable chemical compounds predicted by multi-subjective sensitivity distributions
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Nuclear power plants release both radionuclides and stable chemicals to the environment under normal operating conditions, notably in river ecosystems. However, within the chemical regulatory framework, the ecological impact of these radionuclides is still evaluated based on the separate risks engendered by single substances. Until now, no attempt has considered radionuclides and stable chemicals in a holistic environmental impact assessment. This study contributes to improving existing regulatory instruments with the aim to propose an integrated risk assessment approach taking into account radioactive and stable chemical compounds altogether. Species sensitivity distributions (SSD) are widely used to estimate the potentially affected fraction of species (PAF) resulting from exposure
to individual contaminant. This approach can be combined with mixture models in order to assess the multi-substance PAF (msPAF), a quantitative indicator of the ecological risk associated with mixtures. Such an integrated approach has rarely been applied to the case of mixtures that include radionuclides. Here, individual SSDs built for each radionuclide and stable chemical released to the Rhône River by 4 nuclear power plants were combined: the msPAFs associated with different release scenarios were derived using the concentration addition or response addition models according to the toxic mode of action of each substance. Due to the peculiarities of radionuclide ecotoxicology, the risk assessment is not straightforward and additional steps in the evaluation are needed. Particularly, chemical fate and distribution in the environment and their respective toxicities (by unit time due to ionising radiation from all radionuclides) and not directly to the radionuclide concentration in the exposure media. Those peculiarities also offer a unique opportunity to refine the ‘traditional’ approach (in which SSDs for each compound are derived before calculating msPAF using a mixture model) with a refined approach: exposed species are mixed and toxicity values for these species are calculated separately before deriving a single multi-substance SSD. The latter approach is considered to be more robust, but has only been tested theoretically yet because it requires toxicity data for the same species for each compound, which is possible in the radionuclide case. The poster will compare the different approaches, and discuss their feasibility and realism regarding mixtures that include radioactive compounds.

**TU192**

Prospective risk management for mixtures of agricultural chemicals in surface water


In March of 2015, a SETAC Pellston workshop was held to help inform decision making around aquatic mixture risk assessments of chemicals using exposure scenarios and decision trees. The efforts were broadly grouped into three areas of chemical origin: agriculture, domestic, and urban influences (a separate integration group was charged with looking at overarching issues). The agricultural land use combined effect measures with exposure scenarios of chemical mixtures for field and catchment-scale using procedures that are recognized and used in regulatory schemes in the U.S., Europe and other parts of the world. Chemicals modeled were those used in crop protection and livestock production, and were considered to occur as mixtures (in time and space). Two types of scenarios were defined including a single unit that could represent a variety of typical chemical input locations (e.g., feed lot, agricultural field, pasture, aquaculture, biosolids applications, etc). The second scenario was multi-unit, combining several different uses within a single catchment/watershed. These assessments considered inputs from spray drift, surface runoff and erosion, and/or tile drainage systems on a daily basis over an extended period of time (e.g., from one to 30 years). Case studies included a single unit scenario modeled as a wheat field in Eastern UK, consisting of crop protection applications of 13 substances over the course of the year. This scenario used standard FOCUS soil, water, and air models but required additional information. Another type of a multi-unit catchment scenario consisted of a combination of corn fields, pasture, and feedlot inputs based in part on the US EPA Iowa corn scenario used in pesticide registration evaluations. Manure from treated cattle containing two different pharmaceutical substances (a parasiteic and a macrolide antibiotic) was applied to corn fields as fertilizer, and also originated from pastured cattle. Twelve different active substances for crop protection were modeled on the corn field. These applications ranged from a pre-plant herbicide to a late-year fungicide application. A risk assessment decision tree looked at estimated environmental concentrations of all active substances on a daily basis, with subsequent mixture risk indices and associated methodologies developed under a separate expert working group within the same Pellston workshop. A summary of the framework, methodologies and results will be presented.

**TU193**

Assessing the combined effects of chemical mixtures using novel alternative tools

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Exposure to mixtures of chemicals is of increasing concern for human health and the environment. It is practically unfeasible to test all possible mixtures experimentally. Therefore, smart strategies are needed to assess the potential harms from mixtures so that negative effects are managed as early as possible. Several approaches for addressing mixtures have been reviewed and on expert experiences in the assessment of combined effects been conducted. The current state of the art for the application of alternative tools was evaluated, focusing on the adverse outcome pathway (AOP) concept, in vitro methods, omics techniques, in silico approaches, toxicokinetic and dynamic energy budget (DEB) modelling, and on integrated approaches to testing and assessment ([IATA]. Results: i) Novel approaches to the evaluation of the use of novel tools in the risk assessment of mixtures were split between those applying them (often more in a research context) and those that generally think these tools are valuable but their use is currently limited because of lack of guidance, lack of data, or lack of expertise. The literature review showed how these tools allow derivation of risk for both individual mixture components or whole mixtures, leading to a better understanding of the underlying mechanisms of their individual and combined effects. Conclusion: Using the above-mentioned tools in smart combination and an integrated way, different aspects regarding the hazard from combined exposure to multiple chemicals can be put into a holistic context. This may allow for an improved prediction of mixture effects. In order to benefit from these tools in the hazard assessment of mixtures, more guidance on their use is needed to facilitate a more widespread application and integration into regulatory assessments.

**Fate and Effects of Metals: Regulatory and Risk Assessment Perspective (P)**

**TU194**

Establishing a shared methodology to develop reliable dose-response relationships for trivalent chromium

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Risk assessment (RA) strongly depends on available ecotoxicological data and require adequate quantification of hazard (exposure, response relationships) and exposure (i.e., concentrations likely to be encountered in the environment). In the case of chromium, RA must consider the simultaneous environmental occurrence of the two main redox forms, namely Cr(III) and Cr(VI), that have markedly different environmental behaviour and reactivity toward biological surfaces. Current consensus is that Cr(VI) is much more toxic than Cr(III), so that risk assessment and management have been particularly focused on this redox form. The concern over Cr(VI) is fully justified. However, Cr(III) ecotoxicity may be largely underestimated due to the lack of appropriate consideration of Cr chemistry in standardized (and not standardized) media used in hazard assessment and in the study of Cr(III) effects on the physiology and biochemistry of living organisms. The chemistry of Cr(III) in (standardized) test media is rarely considered in detail. The main issue is that soluble Cr(III) concentrations will be controlled by the possible formation of insoluble Cr(III) hydroxides in the test medium. This is of particular concern at circumneutral pH, but can occur also at slightly acidic pH depending on the added Cr(III) concentrations and medium composition. The formation of insoluble hydroxides lowers the actual solubility of added Cr(III) and may cause a marked decrease of the Cr concentration in the test solution over test time. When these factors are not examined, exposure-responses relationship established for Cr(III) in different studies will not necessarily provide adequate information on the actual exposure concentrations. The appropriate way to establish correct exposure-response relationship for Cr(III), especially under circumneutral conditions, is to use protocols originally developed for sparingly soluble substances. Applying the recommendations of such guidelines to Cr(III) requires repeated measurements of total and filterable concentration over the entire test duration, calculation of exposure concentration as time-weighted mean or geometric mean, expression of Cr(III) toxicity in terms of (modelled) free toxic species instead of total (filterable) concentrations, and provision of apparently trivial details such as aging of the spiked exposure medium that, as for the ecotoxicity of nanomaterials, can affect Cr(III) behaviour during ecotoxicological tests.

**TU195**

The classification of Attapulgic clay under CLP/GHS

D. Heijerick, ARCHE; E. Drossos, G. Kakandes, Geoehellas GEOHELлас minerals and processes attapulgite clay (“fullers earth”) from near-surface, lateritic deposits in northern Greece. The material is produced in various particle sizes for use as absorbents in the food and feed industry. In edible oil applications, the material is able to remove oil contaminants, including dangerous 3-MCPD. In feed applications, the material absorbs mycotoxin, reducing the need for antibiotics in animal feed. While the present material has a long history of safe use, analyses indicate the existence of constituents that could trigger the CLP classification, such as cobalt (0.02%), chromium (0.13%), and nickel (0.3%) (three common laterite components), and crystalline silica (common in all types of clays). In order to determine the material’s status under CLP/GHS rules, the material was examined mineralogically and chemically (weight-of-evidence approach), and subjected to MeClas tier analysis. Potential human health hazards related to the presence of Cr and crystalline silica were rule out using mineralogical data on respirable silica content (SWERP method)
and on the valence state of Cr in the minerals present. The mineralogical data further demonstrate that Ni exists as an isomorphous substitution in the clay structure, ruling out the Car.Cat.1 classification associated with other forms of Ni. To address potential environmental hazards, a speciation-based assessment was first done assuming that 100% of the metals are bioavailable (McChais-Tier-1). The result was an Aq.Clonic 3 classification due to the combined effect of Co, Cr, Cd, and Ni. Because 100% bioavailability is not possible with the natural clay, a refinement was conducted using data from a 24h T/Dp test (pH6, 100 mg/L) on the finest fraction of the attapulgite clay. These data indicate low release factors of <0.1% for Co and Cr, and <1% for Ni (percent of total metal concentrations), and thus the need for these catalysts to be treated as low-release compounds (linear extrapolation; worst-case assumption). The extrapolated data give values of 2.53%, 0.59% and 23.16%, respectively. Inclusion of these release rates in the calculations (McChais-Tier-2) removed the provisional Aq.Clonic 3 classification. Based on evaluation of the hazard endpoints, it was concluded that the attapulgite clay in all its forms should not be classified for any human health or environmental hazard under CLP or GHS.

TU197 Water Framework Directive - Overview of Water Quality Standards and indirect assessment for mollusks

D. Heierick, ARCHE; N. Van Roey, SADACI; S. Carey, IMO

Environmental Quality Standards (EQSs) are tools used for assessing the chemical status of waterbodies. In summary, the maximum acceptable concentration and/or annual average concentration are established for priority substances. When these threshold concentrations are met in a waterbody, the chemical status of the water is considered as “good.” EQSs aim to protect sensitive ecosystems from possible adverse effects of chemicals as well as human health via drinking water or ingestion of food originating from aquatic environments. Several different types of receptors therefore need to be considered, i.e., the pelagic and benthic communities in freshwater, brackish or saltwater ecosystems, the top predators of these receptors, and human health. Five relevant EQSs were considered for the evaluation of molybdenum: quality standard for the protection of the water column (QStab-wat), protection of sediment organisms (QStab-sed), protection of predators (mammals, birds) from secondary poisoning (QStab-secpo), protection of men from secondary poisoning due to the consumption of contaminated fish (QStab-wat), and protection of men from poisoning due to consumption of contaminated drinking water (QStab-drink). The final QStab-wat for molybdenum in the freshwater and marine environment were 12.7 mg Mo/L and 2.28 mg Mo/L, respectively, and reflect the PNEC-values that were included in the REACH registration dossier for Mo-compounds. No formal drinking water guideline value has been adopted, but a health-based value has been put forward by WHO (2011) and is used as a starting point for the calculated QStab-drink. A 82 µg Mo/L, taking into account a removal efficiency of 15%. With regard to the QStab-secpo for molybdenum, a value of 207 mg/kg was derived. This QStab-secpo must then be translated to a concentration in the water column according to the formula “QStab-secpo = QStab-drink/ BAf.” As the BAf for essential elements depends on the exposure concentration, there is no straightforward option for solving this equation. However, bioaccumulation data for the most sensitive organism in the aquatic environment (Regoli et al, 2012) demonstrated that molybdenum was properly regulated in aquatic organisms when exposed to concentration levels that equal the QStab-secpo, with internal concentrations well below the critical concentration of 207 mg/kg. Therefore it was not required to derive a QStab-sed and/or QStab-drink.

TU198 Application of descriptive and multivariate analysis for obtaining the pollution markers of untreated wastewater in Novi Sad, Serbia

S. Pap, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; M. Vojnovic MIloradov, Faculty of Technical Sciences University of Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; M. Brboric, V. Bednarec, D. Miškovanovic, D. Ubjan, University of Novi Sad, Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; I. Mihajlovic, Faculty of Technical Sciences University of Novi Sad; M. Djogo, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health University Novi Sad, Faculty of Technical Sciences (a mixture of municipal and industrial wastewater and surface runoff) are a complex, multivariate environmental systems, in which a number of physical, chemical and biological processes occur simultaneously. For the selection of an appropriate treatment process and the design of the stable conditions for optimum performance of the wastewater treatment plants (WWTPs), it’s necessary to have a detailed information about the sources, composition and levels of pollutants in the wastewater. This study examined the relationship between these processes through analyzed parameters of untreated wastewater in Novi Sad, Serbia. The samples of wastewater were taken from one of the most burdened discharges in the city of Novi Sad during 2012-2014. Experimental data were analyzed by basic statistical methods for determination of mean, median values, standard deviations, minimal and maximal value of the measured parameters. Then association of cluster analysis (CA) with the principal component analysis/factor analysis (PCA/CA) was applied in order to establish a model link between parameters and sampling seasons, as well as to detect and diagnose abnormal events during monitoring periods, which helps in the design of the wastewater treatment plant in the future. Hierarchical CA grouped the eleven monitored seasons into three clusters based on their similarities corresponding to seasons of low polluted period (LP-P), moderate polluted period (MP-P) and high polluted period (HP-P). For the three different groups using PCA/FA three factor model was constructed and resulted in 70.08%, 67.54% and 76.99% of the total variance in the wastewater quality datasets of LP-P, MP-P and HP-P, respectively. PCA/FA indicates that in each period beside organic pollution primarily heavy metals are responsible for wastewater quality variation (point source: industry). These heavy metal factors are interpreted as metal pollution from the industrial effluents. Each factor has strong positive and negative loadings on Pb, Tcr, Zn, Cd and Fe concentrations. Large data base obtained within this study could give relevant information about the physico-chemical status of wastewater in order to establish the best treatment process of wastewater discharge. Acknowledgement: This study has been financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009 and TR34014).

TU199 Antimony fate modelling approach for regional nuclear risk assessment

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Antimony (Sb) is a highly toxic ubiquitous oxynan that has poorly been studied in the aquatic environment. In the aquatic environment, Sb forms inorganic complexes following a biogeochemical cycle and the processes taking place (behaviour, transformation and transport), especially in aquatic systems. In addition, it is a refractory fission product in nuclear power plants (NPP), released and detected after the Chernobyl (ChNPP, Ukraine 26th April 1986) and Fukushima Dai-ichi (FDNPP, Japan 11th March 2011) accidents besides the nuclear power plants. The Sb transfer to aquatic environments is influenced by the discharge point and the presence of discharge sites and time lapses. The aim of this work is to determine the environmental fate and potential dispersion of radioisotopes before accidental NPP events in the Gironde Estuary (France), the largest estuary in southwest Europe subjected to two NPPs (i.e., upstream and within the salinity gradient area). Measurements on stable Sb show a non-conservative behaviour along the salinity gradient with enhanced addition processes taking place during low freshwater discharges. Dissolved concentrations (SbD) range from ~0.12 µg l−1 at S=0 to ~0.4 µg l−1 at S=27, higher than available measurements from the Atlantic coast off Nantes (0.21 ± 0.01 µg l−1). Estimated net fluxes (Boyel’s method) range from ~20 to ~420 kg day−1, compared to average gross fluxes of ~2.5 and ~14 kg day−1 registered from 2004-2014 at the estuary upstream limit (i.e., La Rèole). Variations in particular concentrations (SbD) during different discharges along the estuary are less evident. Distribution coefficients indicate that high suspended particulate matter (SPM) favours Sb, up to ~90% of the total Sb with SPM ~1000 mg l−1 (corresponding to the maximum turbidity zone, MTZ). Estimations on radionuclide generation after an accident indicate critical times for radioactivity exposure (i.e., γ and β radiation) within the first 4 days (tidal scale) and 1 month after the accident, with environmental persistence of several years for the latter case (seasonal implications for the 125Sb isotope: half-life of 2.76 y). This study shows that freshwater discharge and the resulting position and dynamics of the MTZ will play a major role in transboundary Sb transport and release. Further studies of the coastal and within the Gironde Estuary, including the risk of upstream transport of radioactive particles to Bordeaux during summer drought.

TU200 Application of improved scientific approaches in support of metal risk assessments

K. Oorts, ARCHE; J. Schoeters, Rio Tinto

Several aspects must be accounted for in order to perform a scientifically-sound risk assessment of metals in soil, such as e.g. essentiality and variability of soil properties, influencing the bioavailability and toxicity of metals. During the last 2 decades, a lot of research has been performed in these fields and numerous chronic metal toxicity data were generated for various terrestrial species and microbial functions in different soil types. From these data sets, models were developed for correction for differences in bioavailabilty among soils or between lab and field conditions exposure times. For many metals, the large amount of chronic toxicity data for different species and functions allow the application of the species sensitivity distribution approach (SSD) to derive soil quality standards. This work was mainly triggered by the European legislation on chemical management (REACH and the former Existing Substances Regulation) and the data were therefore primarily used to derive predicted no effect concentrations (PNECs) for prospective risk assessment. The data can however also be used for other purposes. For example, different combinations of effect level (ECx) and protection level (hazardous concentration within the SSD, HCx) can be considered for the derivation of soil quality standards. The use of the SSD approach also allows predicting the potentially affected fraction (PAF) of terrestrial organisms affected by a specific metal concentration in a specific soil. In contrast to the use of ecological quality standards for metals in soil for different protection purposes.
(e.g. prospective risk assessment or remediation) and in different jurisdictions, while still making maximal use of the wealth of data and models already available, a tool is being developed that allows using the data and models originally developed for the European prospective risk assessments towards a more flexible derivation of soil toxicity thresholds. This can be done by allowing selection of the different effects level (e.g. EC₅₀, EC₅₅, EC₆₅, or EC₁₀₀) as a basis for the assessment, the protection level required, the group of organisms to be protected, bioavailability models to be included etc. The advantages of this approach are harmonization of toxicity datasets and bioavailability corrections used and the enhanced transparency in the derivation of ecological quality standards for metals in soil for different goals and different scenarios.

TU201 Implementation of soil bioavailability concepts in the determination of German precautionary values

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According to the German Federal Soil Protection Act (1998), soil precautionary values are defined as “soil values which, if exceeded, shall normally mean there is reason that concern for a harmful soil change exists, taking geogenic or wide-spread, settlement-related polluted contaminations into account”. In the German Soil Protection Ordinance (1999), such values are given for seven metals. Recently, more values have been proposed for arsenic and thallium. All of them were defined basing on concentrations (“aquagran”): the pathway “soil – soil organisms” it is well known that there is a poor correlation between total metal concentrations and their effects on microbes, plants and invertebrates. There is also a general agreement that a realistic risk assessment of metals should be preferentially based on concentrations of their biologically available fraction but it is not clear which definition should be used for regulatory purposes and hence to measure it. In any case the implementation of bioavailability should account for several aspects affecting metal toxicity to soil organisms, such as the differences between laboratory and field conditions (including ageing processes) or the variations in the properties of natural soils (i.e. pH, clay content, CEC etc.) – not to mention the form of the metal (i.e. water-soluble salts vs. metal oxides etc.). Corrections for ageing and varying soil properties have recently been used in the prospective risk assessment of metals in the European Union (e.g. in REACH dossiers). Referring to these experiences the German Federal Environmental Agency has launched a project in order to develop a transparent concept to derive soil precautionary values considering ecotoxicological effects of bioavailable fractions of metals. The work is divided in four steps, starting with a literature review on existing concepts on the derivation of soil values for metals. Based on the results of this compilation, a specific testing and assessment concept will be formulated as a basis for the third step, i.e. practical testing. The latter will focus on a limited number of metals, soils, ecotoxicological test methods and extraction methods. The concept will be tested for a cationic metal (copper) and an anoxic (either arsenic or molybdenium). In the final (fourth) step all available information will be used to propose a new concept for including bioavailability into the derivation of future precautionary values.

TU202 Bioaccessibility and Health Risk Assessment of Potentially Toxic Elements in urban soils of Lagos, Nigeria

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Total or pseudo-total extraction over estimates the risk of human exposure to PTE since only a fraction is bioavailable or bioaccessible. Bioaccessibility extraction tests simulate the human gastro-intestinal tract especially of children, the target group most susceptible to accidental or deliberate ingestion of non-food substances. The protocol estimates the actual fraction that is soluble and available for it is not clear which definition should be used for regulatory purposes and hence to measure it. In any case the implementation of bioavailability should account for several aspects affecting metal toxicity to soil organisms, such as the differences between laboratory and field conditions (including ageing processes) or the variations in the properties of natural soils (i.e. pH, clay content, CEC etc.) – not to mention the form of the metal (i.e. water-soluble salts vs. metal oxides etc.). Corrections for ageing and varying soil properties have recently been used in the prospective risk assessment of metals in the European Union (e.g. in REACH dossiers). Referring to these experiences the German Federal Environmental Agency has launched a project in order to develop a transparent concept to derive soil precautionary values considering ecotoxicological effects of bioavailable fractions of metals. The work is divided in four steps, starting with a literature review on existing concepts on the derivation of soil values for metals. Based on the results of this compilation, a specific testing and assessment concept will be formulated as a basis for the third step, i.e. practical testing. The latter will focus on a limited number of metals, soils, ecotoxicological test methods and extraction methods. The concept will be tested for a cationic metal (copper) and an anoxic (either arsenic or molybdenium). In the final (fourth) step all available information will be used to propose a new concept for including bioavailability into the derivation of future precautionary values.

TU203 Introduction speciation changes, human bioaccessibility and bioavailability in the risk evaluation of dietary inorganic arsenic intake: a Belgian case study

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Arsenic (As) is an ubiquitous element frequently present in food items. Human health risks related to its dietary intake are not linked to total As intake, but depend on the chemical form (speciation) in which As is taken up. Hence, normative legislation should preferably be specification-based in the case of As. To assess the risks associated to dietary As intake, the knowledge of the internal exposure to different As-species is necessary, and this can only be determined if speciation changes during food preparation and food digestion are known. The BIOTRaS project aimed at incorporating these changes and knowledge about As-species bioavailability into the regulatory framework. Re-estimation of the biogenically available As intake in Belgium. Different food items were analysed before and after preparation to determine the effect of preparation on As concentrations and speciation. The prepared food samples were subjected to a sequential in vitro digestion system (Unified Barge Method). To mimic colon digestion, the intestinal microflora of healthy adults was grown in a bioreactor system (a sulfitoreduction bioreactor). The bioaccessible fraction was collected by centrifuging the small intestine- or colon digests. Apparent permeability values of As and its species from the digested food matrices were obtained by means of the Caco-2 cell system. From these data, human intestinal absorption values were derived (bioavailability). To perform As in- and uptake calculations, different food consumption scenario’s were elaborated based on the Belgian Food consumption survey and literature data. To evaluate the risk of inorganic As (iAs) in- and uptake, margins-of-exposure were calculated. An increasing consumption of typical iAs sources (e.g. rice, hijiki seaweed) increases the intake of iAs, although cooking these food items in an excess water mitigates the increase. The calculated systemic uptake of iAs for a usual Belgian consumption pattern is calculated as 7 g/d. A high consumption of selected food items leads maximally to a doubling of the iAs intake. Ethnics groups with a rice-based diet have a 25- to 100-times higher uptake of iAs than the average Belgian population, depending on the consumed amount of rice and its preparation method. No health risks due to iAs intake or uptake are expected for the general Belgian population. A long-term ethnical rice-based diet is discouraged for people with an increased risk for lung cancer such as smokers.
and organic carbon. The water level (WL) in the containers was maintained at 20 cm below the soil surface for five weeks (1st High WL) and then at 45 cm during the following five weeks (1st Low WL), repeating this cycle once more along the study (2nd High WL, 2nd Low WL). The pH, Eh, and soluble metal concentrations (Cd, Cu, Fe, Mn, Pb, and Zn) were measured regularly at each depth for 18 weeks.

The contents of both treatments showed that the highest levels of As were found in the pot treated with lime, likely due to the pyrometallurgy techniques for recovering eutrophic wetlands polluted by metal mine wastes depends on the particular characteristics of the soil-water-plant system, the hydric/flooding regime, and the type of pollutant (nitrogen, phosphorus, and metals).

TU205 Impact analysis in the frame of Life Cycle Assessment, of the toxic emissions induced by industrial activities to agricultural soil in Fez area

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Contamination by trace metals in agricultural soils in the Fez region are linked to their irrigation using water from the Oued Fez. The latter is fed by sewage from surrounding industries and the city, which have a high pollution load in spite of minimization’s efforts. Several reports and studies in this region confirmed the state of high metallic pollution in Oued Fez’s waters and its confluence with the Oued Sebou. The effluents of the tannery and textile industries as well as those of the metal finishing industry, present the greatest risk because of the use of some metals in their processes. This work aims at interpreting the metals quantities which are emitted during the industrial activities life cycle, in terms of potential impacts on water, soil and human health. The Life Cycle Assessment (LCA) was adopted as multi-criteria methodology for identifying and quantifying the major contributions related to various local industries’ metal emissions. In particular, it is question of analyzing the anthropogenic and terrestrial ecotoxicity, and human toxicity. The characterization of these impacts takes into account the fate of trace metals emissions from their sources to the waters, sediments and agricultural soils through irrigation water. Their toxic effects on aquatic species and human health are also considered. The fate of metals emissions was determined by a geochemical study in the areas at different sites. The analysis of total concentrations and bioavailable fractions was conducted on the industrial effluents and the three environmental compartments: water, sediments and soils. The effect factor is based on the effect concentrations HCₚ, of the Impact2002 database and by specifying some parameters such as the partition coefficients of trace elements, the density and the moisture content of the soil. At this stage of analysis, it could be identified that the trace elements Cr, Ni, Cu and Zn are the major contributors to the terrestrial ecotoxicity. This is due to several causes combined or separated; high total concentrations in the soil, a greater mobility of bioavailable fractions and/or lower concentrations of toxic effect.

Higher tier tests in the risk assessment of plant protection products (P)

TU206 Reflections on bird and mammal risk assessment: past, present and future


The use of plant protection products on agricultural crops can result in exposure of birds and mammals to toxic chemicals. The risks from such exposures are assessed under the current guidance document, EFSA (2009). The risk assessment procedure is sequential, moving from worst-case screening steps through to increasingly more realistic, higher tier risk assessments. The current guidance document was designed to increase the realism of these theoretical risk assessments, in comparison to its predecessor (SANCO/4145/2000). Since its adoption over 5 years ago, many plant protection products have been registered successfully using EFSA (2009). However, there are still many cases where low risks cannot be demonstrated using the current scheme, even using extensive and robust higher tier refinements. The aim of this presentation is to discuss the implementation of the current scheme, including levels of conservatism in input parameters, and interpretation by regulatory authorities, together with proposals for how the guidance document could be improved when it is revised in the not too distant future. The content of this presentation forms the basis of an invited manuscript to be published as a ‘Focus’ article in Environmental Toxicology and Chemistry.

TU207 Honeybee brood studies under field conditions: is there a difference of the brood termination rate compared to semian field studies?

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Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk to honeybee larvae or honeybee brood. According to the new “EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)” (EFSA 2014), both, the Oomen bee brood feeding test (Oomen et al., 1992, EPPO Bulletin 22: 61-70) and the EPPO 170 field studies using the OECD GD 75 bee brood evaluation might be one option to get more reliable BTR data, which was envisaged already some years ago in 2009 (Becker et al. 2009, Julius-Kühn-Archiv 450: 53-92). Therefore the performance of EPPO 170 field studies using the OECD GD 75 bee brood evaluation might be more reliable BTR data, which was envisaged already some years ago in 2009 (Becker et al. 2009, Julius-Kühn-Archiv 423: 43-44), used for several years, and followed-up by Giffard & Haart (2015, Julius-Kühn-Archiv 450: 111-120). However, broader data sets supporting the EPPO GD 170 field studies/neutralization tests are needed for an overall evaluation. Thus, the current presentation summarises control BTRs gained under field conditions which have been performed since 2012 in Germany. They were conducted according to EPPO guideline 170 (4) (2010) with detailed brood evaluations according to OECD GD 75 and covered the assessment of one or two brood cycles during and after the exposure of the bees to flowering crops. In addition, studies covering the analysis of a 2nd brood cycle with free flying honeybees conceived as a 1st brood cycle derived from semi-field trials were also considered. Overall the evaluation covers the data of almost 50 control colonies. These data were compared to the updated findings on control BTRs from almost 70 semi-field bee brood studies (Becker et al. 2015, Julius-Kühn-Archiv 450: 53-92) now. Finally, the advantages and disadvantages of both test approaches are discussed.

TU208 Semi-field Study for the Honey Bee (Apis mellifera) using a Micro-Colony System

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Higher tier studies, which require the use of bee colonies and more complex test designs, are often difficult to evaluate because high levels of background mortality are seen in controls and yet the findings from these multi-disciplinary studies are used to assess the potential for risk to bee populations exposed to plant protection products. Currently, tests are frequently based the 2007 OECD Series on Testing and Assessment: Number 75 “Guidance document on the honey bee brood test under semi-field conditions,” with an initial 7 day direct exposure in a tunnel after the application of the test article, followed by a 19 day observation period outside of the tunnel. This only addresses the impact on eggs present at the time of application and not the later stages of development, which some test articles are known to have an impact on. Therefore, there is a need to develop a test that is sufficiently robust to provide reliable and reproducible data within a contained environment to allow the bee colonies to survive for a period of at least 22 days (time taken for development of a newly laid egg to emergence) so that all stages of development can be assessed. This study is designed to evaluate the survival of un-treated adult bees and associated brood under test conditions to verify that control colonies exhibit adequately low mortality rates in the absence of test article to justify the use of mini-colonies of ca. 2000 adults. In addition, both control internal frames and OECD divided frames, which allow the bees to specific areas for egg laying are being evaluated. The hives are provided with adequate food stores above a queen excluder to prevent her from using the food frames for egg-laying. This is in contrast to the conventional approach of providing the minimum amounts of stores to prevent their use in preference to incoming nectar and pollen which may (in a real study situation) contain the test substance, although bees in fact only use the stores when there is no incoming food. Individual hives are manipulated to ensure that all stages from eggs to old larvae are present at Time 0 (day of application). Timed assessments of all life stages allow comparison of the previously used Oomen method with the current OECD test and also the translation of this combined methodology into an international test. The selected hives are established in the enclosures (ca. 70 m² of flowering Phacelia) with the mortality of foragers being monitored during the pre-assessment period.

TU209 Minimum Detectable Doses as a criterion to assess the reliability of micro- and mesocosm studies

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The new European guidance document on tiered risk assessment for plant protection products for assessing risks to soil micro- and mesocosms in agro-ecosystem surface waters requires reporting the minimum detectable differences for tests on significant differences between population abundances in the controls and treatments in mesocosm studies in order to assess the reliability of the statistical analysis. If the study should be used to derive a regulatory acceptable concentration under the eco-toxicological thresholdoptimising the risk assessment analysis of, it is possible for a least 8 potentially sensitive populations. However, no clear guidance is given
on how to assess if the MDDs are sufficiently robust. Recently, Brock et al. (2015) proposed how the MDD could be calculated and suggested also a criterion on how many sampling dates the MDD should be below specific values to allow a reliable analysis of direct effects on a taxon. Here we will present experience with the MDD concept in the re-evaluation of older mesocosm studies and the use of MDDs in studies recently conducted. We will focus on host and which potentially sensitive taxa fulfilled the criterion proposed by Brock et al. (2015) and how this relates to different test systems, level of taxonomic identification, sampling methods, sampling frequency and mode of action of the test item. We will discuss the practicality and relevance of the MDD criterion for different taxa and the potential consequences for the role of mesocosm studies in the aquatic risk assessment in the future.

TU210 Tiered testing for sediment organisms: towards calibration of the EFSA proposal

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The EFSA Scientific Opinion (SO) on the effect assessment for pesticides on sediment organisms in edge-of-field surface water proposed a tiered effect assessment approach for sediment organisms. The aim of this study was to assess whether the tiered approach is suitable for sediment organisms. The global data requirements for sediment testing were reviewed and, example molecules covering different modes of action (insecticide, fungicide and herbicide) were selected. Datasets were compiled for each EFSA tier, i.e. laboratory, modified laboratory studies and screening level studies as well as full mesocosm studies. All data were extracted from the regulatory (EFSA conclusions, DARs, RARs and US-EPA documents) and the open literature. Based on the laboratory data Regulatory Acceptable Concentrations (RACs) in sediment were determined. From the higher tier data, information concerning sediment-associated organisms (especially root macrophytes) was extracted. A comparative analysis of this data is being presented to validate/calibrate the assessment factor used to trigger additional testing (tiers 2 and 3) or conclude on acceptable risk.

TU211 Do stable isotope signatures and elemental stoichiometry mirror toxicant-induced changes in predation success?

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Contaminant-induced changes in food-web structures are difficult to detect using traditional methods employed in multi-species studies. Stable isotope signatures and elemental stoichiometry may help to overcome this problem as both can provide information about the food source utilization by consumers. To assess the suitability of these tools, we conducted an experiment with the omnivore amphipod Gammarus fossarum, who had the choice to feed on black alder leaves or to prey on mayfly nymphs (Baetis sp.). As a model stressor, we applied thiacepoxide at 0.75 μg/L, which is known to increase the predation success of Gammarus on Baetis. To assess all factors potentially affecting gammarids’ stable isotope signals and elemental composition, a factorial approach was employed (insecticide x Baetis x leaves). The consumption of Baetis and leaves was recorded over two weeks in microcosms (n=5-10) and stable isotope signals and elemental stoichiometry, for N and C, were finally analyzed in gammarids and both food sources (n=5). As expected, thiacepoxide exposure increased the predation success of Gammarus. This pattern was, however, not reflected by elemental stoichiometry, neither using C, N, nor the C:N ratio. Moreover, N content differed significantly between unexposed and exposed gammarids fed only Baetis. As these treatments did not differ regarding predation success, this observation-associate organisms (typically emergent insects and rooted macrophytes) was extracted. A comparative analysis of this data is being presented to validate/calibrate the assessment factor used to trigger additional testing (tiers 2 and 3) or conclude on acceptable risk.

TU212 Can we combine data from avian reproduction studies with different test species to improve risk assessments?

A. Lawrence, Cambridge Environmental Assessments / Regulatory Ecotoxicology; S. Taylor, Cambridge Environmental Assessments

When conducting risk assessments for birds and mammals potentially exposed to plant protection products, there may be more than one study available for each endpoint. In between years, the current regulatory framework (EFSA, 2009) states that it is possible to combine acute data on different species using a geometric mean approach, and that the intended level of protection is preserved. The guidance states that for reproductive studies, the geometric mean approach is currently not supported. When more than one reproductive study is available on the same species, the guidance states that it may be possible to combine or merge the studies as if they were one study. In order to do this, various criteria must be met, including: studies conducted to a similar protocol or guideline, same key endpoints assessed, similar dose responses present in each study, same test species used, same protocol used, similar number of animals used, same endpoints assessed, and some toxicological outcomes used. We used two avian reproduction studies are available, many of the above criteria are fulfilled, because the protocols are the same. Studies may be available for different test species, however, typically mallard duck and bobwhite quail. This poster will describe how it may be considered acceptable to combine the reproductive toxicity data for two avian test species when the endpoints are derived from very similar studies, to improve accuracy of endpoints used in risk assessment. This may be especially relevant when EC10 values cannot be reliably calculated, and dose spacing is wide, leading to large differences between NOEL and LOEL.

TU214 MDDs in micro-arthropod field testing

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Micro-arthropod field tests can be carried out as part of the risk assessment of plant protection products according to Römbeck et al. (2009). This document provides information on experimental design, but do not give indications about the available statistical power of the test or about the effect size that can be detectable in such a field study. Recently, it has been suggested (EFSA, 2013 and Brock et al., 2015) to use the minimum detectable difference (MDD) to evaluate the statistical power of aquatic test systems and validate the usage of the study for risk assessment purposes. In order to provide a better understanding of the micro-arthropod field test we carried out a retrospective MDD analysis of a series of field studies for the order Colembola. In the publications requesting MDDs, it is stated to calculate MDDs only for species. Although in reality only the lowest tax level that can be identified is used. The reason for the identification above species level is restraints either in time, knowledge or money. We tried to use ecological groups for Colembolans in the MDD calculation besides the taxonomic groups. The impact of grouping on the statistical power in terms of MDD will be discussed.


Oil and Gas Extraction: Ecological Effects and Science-Based Management (P)

TU216

Effect-driven analysis for sequentially fractionated Iranian Heavy crude oil on zebrafish embryo model

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Crude oil is a complex mixture of chemicals. Therefore, identification of key toxic components in crude oil that cause toxic effects in biological receptors is a crucial, but daunting challenge. In this study, we investigated whether fractionation of oil by distillation is an effective method for investigating crude oil’s toxicity via the effect-driven analysis. To this end, Iranian Heavy crude oil was fractionized into 34 fractions by true boiling point. Each fraction was separated at ten boiling point intervals. Zebrafish embryo assay test was employed for observing different toxicological outcomes (mortality, hatchability, time to hatch, and malformation rate), and was used to determine the toxicity of the distilled fractions of crude oil. There were no differences in time to hatch and embryo survival compared to control group, following the exposure to different fractions. However, malformation survival rates were significantly different among different fractions. Oil fractions with bulky aromatic compounds such as dibenzothiophene and phenanthrene, and their alkylated compounds, caused greater adverse effects such as severe yolk sac edema and heart edema. Our results show that identification of toxicological effects of different components of oil enable better understanding of adverse effects by specific crude oil components. (HS and JL contributed equally to this project). This work was supported by Korean Ministry of Ocean and Fisheries Project PM56951.

TU217

Assessing the Environmental Fate and Toxicity of Surfactants Used for Chemical Enhanced Oil Recovery (eEOR)

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A host of chemical enhanced oil recovery (eEOR) technologies are currently being evaluated to extend the life and maximise production of oil from new and existing reservoirs. Shell Chemcials currently produces two general classes of surfactant for enhanced oil recovery: alcohol alkyl sulfates (ENORDET-J- and A-series) and internal olefin sulfonates (ENORDET O-series). These products are currently being deployed in pilot projects around the world to demonstrate the potential of surfactant-based eEOR technology. To better understand their environmental fate and toxicological impacts, we have been conducting a range of studies on selected products from within the ENORDET surfactant portfolio. The objective of this poster is to provide an overview of the experimental environmental fate and effects data currently available for ENORDET O-Series surfactants and how this compares with analogous classes of anionic surfactants (e.g. detergent range surfactants). The challenges faced with generating scientifically robust and relevant environmental test data for ENORDET surfactants along with potential opportunities for future research and testing will also be discussed.

TU218

Oil recovery from oil sludges obtained from different sources using surfactants in an oil sludge washing process

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Oil sludges are mixtures composed mainly of crude oil, water and sediments. These are hazardous wastes generated in the petroleum extraction and refining process, and therefore need to be treated to have no impact on the environment. Treatment of oil sludges to date has been focused on physicochemical remediation and bioremediation rather than oil recovery by oil sludge washing (OSW). OSW with surfactants has been recently applied for the extraction of oil for reuse. Due to the unique nature of each sludge, this study aims to compare the oil recovery among a number of oil sludges from different sources by assessing the following parameters: surfactant type, concentration, and surfactant to oil sludge (S/O) ratio. Oil sludge samples were obtained from oil/water separation, oil drilling, following the removal of chemical additives by heating and centrifugation, and crude oil contaminated with sediment and water. Four synthetic surfactants [Sodium dodecyl sulphate, SDS (anionic); Tween 80 (T80); Triton X-100 (TX100); and Triton X-114 (TX114) (non-ionic), and one biosurfactant (rhamnolipid, RL)] were used in this study. Surfactant absolute concentrations in terms of critical micelle concentration (CMC) were established. Cyclohexane was used as co-solvent to aid in the separation of oil in the OSW. A previous study found that no significant differences between toluene and cyclohexane were obtained. A crude oil recovery, so cyclohexane was used as an alternative solvent due to its lower hazard. Recovery of oil was measured gravimetrically. OSW parameters effects on the oil recovery rate (ORR, %) were matrix dependent (p < 0.01). RL and SDS had higher oil recovery rates compared with other surfactants for all sludges. In general, high oil recovery rates were obtained at a high S/O ratio (5:1). There were no differences among surfactant concentrations (p > 0.50) but the highest oil recovery was obtained at 5 CMC. Rosased sludge was composed mostly of C12-C18 aliphatic fractions, and it is suitable to be reused as feedstock for heavy fuel oil production. In conclusion, RL can be used as a surfactant to recover oil from oil sludges since they have an equivalent capacity to synthetic surfactants along with a lower toxicity, additionally low concentrations can be used to reduce costs.

TU219

Adapting chemical risk assessment for water systems related to unconventional hydrocarbons

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Chemical risk assessment is typically done per compound with a focus on human and environmental effects. Assessing the chemical risk of unconventional drillings on water quality presents difficulties that require adaptions to this risk assessment process. The scope of this literature review is to identify and describe uncertainties and knowledge gaps of chemical risk assessment relative to unconventional drillings, and, where applicable, to make recommendations. An extensive literature search was aimed at answering the following questions: (1) How to assess a large number of chemicals, and how complete are the databases available for physico-chemical properties and toxicological information?; (2) How applicable are available models for environmental fate modeling related to unconventional gas and oil activities?; (3) How applicable are current water quality monitoring methods to unconventional drillings? More than 7500 different chemicals are used during unconventional drillings. Advanced mass spectrometric techniques, such as liquid chromatography coupled with hybrid linear ion trap (LTQ) FT Orbitrap mass spectrometry, complemented by effect-directed analysis is the best option for broad screening of such a large list of candidates. There is however a lack of comprehensive physico-chemical and toxicological information available for assessing the screened chemicals. Moreover, available environmental fate models do not consider changing chemical behavior under high temperature and high pressure conditions. In addition, there is a need for more field-based approaches for the development of groundwater models. Current water quality monitoring is typically done at the surface and in the shallow underground, and thus does not take into account the potential for deep underground leakages. Underground leak probabilities are therefore not known. Baseline data and long-term monitoring are generally also not ensured. The tools and information necessary for chemical risk assessment of unconventional drillings on water quality are not all available. There is a need for further research into physico-chemical and toxicological information, chemical behavior under downhole conditions, and more field-based groundwater models. Baseline, long-term and deep underground monitoring would allow for a more comprehensive assessment.

TU220

Development of a Generic Exposure Scenario (GES) under the EU REACH Regulation, for substances used in high-volume hydraulic fracturing operations in response to EC Recommendation (2014/70/EU)

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The European Commission Recommendation (2014/70/EU) on minimum principles for high-volume hydraulic fracturing includes, in Section 10, the requirement that substances should refer to “hydraulic fracturing” in their REACH registration dossier if they are to be used in such applications. In response to the recommendation a team of industry representatives was established that includes members from the International Association of Oil & Gas Producers (IOGP), the European Chemical Industry Council (Cefic) and the European Offshore Speciality Chemicals Association (EOSCA). The team have been working on developing a Specific Environmental Release Category (SpERC) that is based on realistic data and assumptions for hydraulic fracturing as an intended use. It can then be used in REACH dossiers as part of a Generic Exposure Scenario (GES) that describes the necessary operational conditions (OCs) and risk management measures (RMMs) which can be implemented to control the risks associated with substances used in hydraulic fracturing applications. The SpERC was developed in tandem with a background document, which provides extended information on the operational conditions, available monitoring data and assumptions that would be implicit in the final SpERC. This poster presents the SpERC that has been developed for use by industry as part of a Generic Exposure Scenario for substances used in hydraulic fracturing fluid additive products, and the conditions that are specified in the SpERC to ensure safe use. In addition,
some of the assumptions made and the details of these assumptions as presented in the background document are also reviewed.

**TU221**

Hot spots identification, sediment management and environmental risk assessment within a gas production field through a multidisciplinary approach and a novel expert decision support system

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Offshore production of oil and gas involves some of the most advanced and massive engineering projects. Such industrial activities may induce both occasional as well as long lasting environmental impacts in the aquatic ecosystem, often acting in combination with other environmental stressors. Nowadays, complex and advanced multidisciplinary monitoring approaches combining chemical, ecotoxicological and ecological data are available worldwide. These collect large dataset of different information, which need to be correctly inferred and integrated to provide a comprehensive knowledge of the environmental health status. Indeed, a management focused on impacts of a single stressor is inefficient and often ineffective because co-occurring human activities lead to multiple simultaneous impacts on communities and individual species. Thus, a quantitative assessment of the spatial patterns of all human uses of the marine aquatic environment and their cumulative effects is needed for implementing the ecosystem-based management, the ecosystem services conservation and the sustainable exploitation of natural resources processes. In this case study, we apply methods developed to map cumulative impacts in sediments collected during the key steps of the set up and the production phases of a gas platform located in the Adriatic sea. Sampling sites are classified by means of integrating chemical data with ecotoxicological and ecological parameters. Furthermore, a sediment risk index is computed from combining chemical and ecotoxicological data. At sites identified as moderately contaminated, sub lethal stress index are integrated with chemical data into a biological vulnerability index. In the meantime, potential risk for human health is assessed in selected stations by integrating genotoxicity biomarkers. Finally, geostatistical tools are applied to show the space and time related risk index distribution.

**Development, standardization and implementation of LCA and integration with economics for transportation infrastructure and operations (P)**

**TU222**

Noise impact category implementation in an LCA software tool to assess road restorations


In several difficulties have arisen in the inclusion of road traffic noise as an impact category within the LCA method. There are many reasons for this marginal treatment of noise in LCA. Main reasons are related to the special characteristics of noise as a pollutant, such as the site-dependency of impacts, the human perception issues or the non-linearity of impacts and limited availability of data, which have hindered its inclusion within this method. Nevertheless, some specific studies within the LCA framework aimed to better accommodate the assessment of its impacts have appeared so far. Most of them have been focused not just in traffic noise but also in noise from any other source, from Müller-Wenk (in the 90s) to more recent authors, such as Franco, Cucurachi, Moliner or Althaus (in the last 10 years). This study presents how the endpoint methods of Moliner and Franco,considering the human health impact of noise could be implemented in LCA software tool, named Air.e LCA®. This proposal has been developed under the framework of a new Spanish R+D+I project whose main aim is to research and develop new materials and techniques to improve the road restoration activities. The fact of including the noise impact assessment will contribute to compute the benefits to human health of developing and using new sound-dampening, sound-reduction or anti-noise materials into road surface.

**TU223**

Environmental assessment of EAF steel slag use in road

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Electric arc furnace (EAF) slag is a non-metallic by-product of steel production by electric furnaces process. About 12 million tons of steel slag are produced annually in Europe. Considered as waste for a long time, this industrial by-product is increasingly used in civil engineering applications as its valorization allows a number of benefits such as the conservation of natural resources, the reduction of waste storage volumes, the decrease of material consumption and the transportation cost and transport demand and the promotion of local economy. The environmental assessment of EAF slag recycling using life cycle assessment (LCA) in road is investigated in this paper. Generally, in the LCA framework, the environmental assessment of recycling value don't takes into account the phases of life cycle corresponding to stockpiling and use, as they are considered to have no impact. Only waste processing is counted. Thus, we propose a method to assess recycling of EAF slag in road considering the phases of production of EAF salg (for recycling purposes), stockpiling (lixiviatiion test), transport (30 km) and use in road (uncovered 10-15 cm thick road layer) and to compare this scenario to natural sand in order to assess the environmental impacts. Various impacts obtained for each step of the EAF slag life cycle and discuss the interest of various solutions considering both toxicity and eco toxicity indicators. Furthermore, the calculation for use phase based on experimental data (results from percolation test) is compared to its calculation based on Ecoinvent data which take into account the total content (x-ray fluorescence) and a transfert factor (from Ecoinvent data base) for short term, in order to seek if the results are in the same range or not.

**TU224**

Life-cycle GHG emissions of alternatives for collecting and transporting municipal solid waste: The case of a Spanish community


Currently, the communities and municipalities are increasingly aware of managing a clean and sustainable transport in their mobility services. Thus, it is usual (and almost mandatory) to include aspects for improving sustainability in tenders for construction and execution of public services. More specifically, in the case of collecting and transporting municipal solid waste several key aspects are important to be considered in order to reduce emissions, energy consumption and other environmental issues. In this context, both the use of alternative fuels and the introduction of electric vehicles are positively valued in order to achieve environmental improvements and energy savings. This study presents an assessment of different proposals for introducing the use of alternative fuels (such as liquefied petroleum gas, natural gas and biofuels instead of conventional diesel) in the vehicle fleet of a Spanish small urban community to collect and transport municipal solid waste. The possible use of hybrid and electric vehicles has also been considered. The results show an important decrease in GHG emissions when natural gas is used as fuel. Nevertheless, this measure entails an increase of other harmful gases, such as CO or VOCs, when compared to other alternatives. Finally, in order to complete the environmental assessment of the whole activities regarding the management of municipal waste, the quantification of annual GHG emissions of the processes of washing containers and pneumatic collection of waste have also been included. These activities could be responsible for the 10 to 40% of total GHG emissions of the waste management in the community.

**TU225**

External costs of electrification of road transport: approach and setting-dependency

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The use of electric vehicles in comparison to internal combustion vehicles is a typical example for burden shifting from the use phase to upstream processes, i.e. fuel supply in the form of electricity. Within the collaborative EC project ScElec-TRA (2012-2015), funded within the frame of the Electro mobility+ Initiative, the following steps in the assessment chain were addressed: 1) definition of scenarios to enhance electromobility uptake in Europe in the mid to long term; 2) modelling their impact on the power and transport sector with the aim of estimating the TIMES PETRMod model; 3) identifying the most important means of power production and different transport modes; 3) assessing the environmental implications particularly in terms of emissions and abiotic resource uses following LCA principles, and 4) monetary valuation mainly of impacts due to classical air pollutant emissions (external costs). The study undertook to establish a set of new regional methodological tools to enhance the characterisation of human health impacts in LCIA including weighting that aim at being setting-dependent, as suggested by the expert panel of the Basel workshop (Fantke et al., 2015). To this end, pre-calculated external cost factors have been obtained from the literature. For power generation, a distinction is made between different fuel types and energy production technologies (coal, gas). For transport-related emissions, external cost factors specific to countries and/or setting (e.g. population density) are used to the extent available. While relying on LCI data and energy system analysis results from project partners, external costs for the different scenarios were quantified. Comparing the overall UDFs obtained this way with those derived generically for the EU27 shows a variability of about a factor of 0.2 up to 2. To this end, a range of input-output (MRIO) data from Exiobase. This in turn is connected to coal mining and processing/maintenance road projects, works and products. The proposed approach to an interdisciplinary perspective. Life Cycle Assessment (LCA) is used to evaluate the environmental impacts of electric vehicles in terms of electricity use, transportation and stockpiling and use, as they are considered to have no impact, only waste processing is counted. Thus, we propose a method to assess recycling of EAF slag in road considering the phases of production of EAF salg (for recycling purposes), stockpiling (lixiviatiion test), transport (30 km) and use in road (uncovered 10-15 cm thick road layer) and to compare this scenario to natural sand in order to assess the environmental impacts. Various impacts obtained for each step of the EAF slag life cycle and discuss the interest of various solutions considering both toxicity and eco toxicity indicators. Furthermore, the calculation for use phase based on experimental data (results from percolation test) is compared to its calculation based on Ecoinvent data which take into account the total content (x-ray fluorescence) and a transfert factor (from Ecoinvent data base) for short term, in order to seek if the results are in the same range or not.
transport activities thus leads only to a negligible difference in the results. The relevance of using country-specific or generic UDF also depends on the scenario analysed.

TU226 Building a common base for LCA benchmarking of Li-Ion traction batteries J.F. Peters, KIT Karlsruhe Institute of Technology / Helmholtz Institute Ulm HIU; B. Simon, Université de Sherbrooke / Department of Civil Engineering; G. Rodriguez Garcia, Helmholtz Institute Ulm / Systems III; M. Weil, Karlsruhe Institute of Technology KIT / Institute for Technology Assessment and Systems Analysis ITAS

Although the environmental impacts of e-mobility are usually dominated by the use phase, the production of the battery also plays a significant role, especially when it comes to choosing the most adequate battery type for a given application. Numerous studies quantify the impact of the battery production process along the life cycle for different chemistries. Nevertheless, these studies use different impact assessment methods and different approaches for modelling key parameters like energy demand for cell manufacturing or electricity mixes. On the other hand, the outcomes of these studies are highly sensitive on these parameters, why a direct comparison of different studies is critical. A common basis for comparing the environmental performance of different alternative Li-ion battery chemistries is not given, but seems very desirable to support decisions of technology developers. Based on a review of all available LCA studies on Li-ion batteries, the discrepancies in the key parameters between these studies are identified and their impact on the outcomes of the studies pointed out. The existing primary inventory data (LCI) for the principle Li-Ion battery chemistries and then recomplied and average values used for these parameters. In this way, the environmental impacts associated with the production of different battery chemistries are assessed on a common base. This provides an improved comparability between studies and allows for a technology benchmarking of different Li-ion battery chemistries. Furthermore, a sensitivity analysis is done by varying the values of the key parameters within the identified range, pointing out the relevance of each of the parameters and the uncertainty in this regard. It can be observed that different assumptions made for these parameters can overturn the differences between battery chemistries completely. Especially the approach for modelling the cell manufacturing energy demand influences the results significantly. Thus, putting existing LCA studies on a common base is essential for battery technology benchmarking and avoids erroneous conclusions when comparing different Li-ion battery chemistries regarding the impacts associated with their production and with their use in electric vehicles.

TU227 Regionalized life cycle impacts of worldwide coal transportation from mines to power plants C. Oberschelp, ETH Zurich; S. Pfister, S. Hellweg, ETH Zurich / Institute of Environmental Engineering

Motivation Coal-fired power production is known to substantially contribute to global health problems and environmental damages. Driven by consumer demands, these are caused by pollutant emissions occurring along its entire supply chain including transportation. Due to growing world-wide energy consumption, this technology remains essential for reliable energy supply at competitive costs in most countries around the world. Thus incremental improvement of the coal supply chain is an increasing opportunity for mitigating harmful impacts beyond improvement of energy efficiencies of power plants. One key problem for allocation of resources to these improvements is insufficient assessment of the regional resolution of emissions and impacts. These differ substantially depending on the transporting vehicles and environmental conditions. As such, species-rich habitats or densely populated regions are often particularly sensitive to transportation emissions compared to remote locations. This aspect has not been covered sufficiently by current publications yet. The present work aims at closing this gap by combining various types of regionalized information and calculating region-specific impacts. Methods Power-plant-specific consumption data is matched with the corresponding transportation routes to identify hotspots of the coal supply chain. Impacts on humans and ecosystems are quantified with spatially-explicit characterization factors by regionalized life cycle assessment (LCA). The required data for power plant coal demands is calculated from the WEPPE database which is linked to international coal trade via multi-regional input-output (MIO) data from Exiobase. This in turn is connected to coal mining data with regional coal specifics and explicit mine locations to determine transportation profiles and distances for different types of coal. Results and conclusions The results highlight hot spots in global coal transportation and show where modernization of vehicle fleets and restructuring of material flows is most relevant. As such the results are a step towards a fully regionalized inventory of the global energy sector and represent a guiding parameter for expanding regionalized life cycle assessments to transportation systems.

TU228 Evaluation of innovative products and processes for road infrastructure projects towards the LCE4ROADS Certification System methodology. R. Fernandez, E. Guedella Bustamante, A. Alvarez de Castro, Acciona Infrastructure; C. Bartolome, IeCa; J. Melero Corell, B.A. LeeGwate, T.N.O

Although there are many initiatives in the market capable to assess sustainability aspects for roads, many lack of a holistic view of sustainability because some of the current approaches do not cover all life cycle phases or all sustainability pillars and focus mainly on environmental aspects. Some examples are Greeroads, Envision and Invest, in the US, and Cequal (UK), and the Dutch CO2 Performance Ladder in Europe. The purpose of this paper is to show the results achieved on the development of a new sustainability certification system for roads named “LCE4ROADS”, currently under way as part of the FP7 project “Life Cycle Engineering approach to develop a novel EU-harmonized sustainability certification system for cost-effective, safer and greener road infrastructures (GA No 605748)”. LCE4ROADS is based on a Life Cycle Engineering (LCE) approach: all the aspects of sustainability (Environmental, Economic, Social, and Technical) are considered with the final goal of creating a holistic and EU harmonized methodology that will base these sustainability aspects, study of new and rehabilitation/maintenance road projects, works and products. The proposed certification system relies on current EN and ISO standards and considers previous developments from other research projects like MIRAVEC, EVITA, COST 354 among others. Key aspects at European level such as resilience to Climate Change and the implementation on TEN-T corridors are also considered within the certification system. This paper aims also to show the first results of using the LCE4ROADS Methodology for evaluating different innovative products and processes for road infrastructure projects. This is line with one of the main purposes of LCE4ROADS certification system which is to provide support and guidance to relevant industry stakeholders on the selection and implementation of technologies that could provide the opportunity to improve sustainability of roads.

TU229 Life cycle assessment and cost benefit analysis for carbon capture and storage A. edahiro, N. Isubo, Tokyo City University

Recently, a variety of mitigation and adaptation measures on climate change have attracted attention. In particular, CCS are growing interests from year to year. According to the roadmap shown by the International Energy Agency (IEA), many of mitigation procedures have been evaluated compare their priority. Many studies, evaluating environmental performance for CCS using LCA has been performed. However, most of studies focus on only GHG emission. On the other hand, there are few studies which apply the comparison and verification including the other types of environmental impact and cost analysis. Considering the above point, in this study, it was conducted an exhaustive evaluation including environmental influences such as SOx and NOx. Further, we examined the usefulness of mitigation measures and the relationship of trade-off of between environmental impacts by calculating the total cost results including the external costs.

TU230 Environmental impact assessment of rail freight intermodality. Energy related emissions A.L. Merchán, University of Liege / Chemical Engineering PEPs; S. Belboon, University of Liège – Chemical Engineering / Chemical Engineering PEPs; A. Leonard, University of Liege / Dpt of Chemical Engineering PEPs

BRAIN-TRAINS is a project supported by the Belgian Federal Government that deals with the possible development of rail freight intermodality in Belgium, approaching the problem from an interdisciplinary perspective. Life Cycle Assessment (LCA) methodology was used to analyse the sustainability impact of rail freight intermodality for three different Belgian scenarios by 2030. This objective is directly linked to the goal of increasing the rail market share by 2030, stated by European Commission’s White Paper on transport (2011). The LCA rail freight system is divided in three sub-systems: rail transport operation, rail infrastructure and rail equipment (locomotives and wagons). The life cycle phases of construction, maintenance and disposal of rail infrastructure and manufacturing, maintenance and disposal of rail equipment are analysed. The sub-system rail transport operation includes direct and indirect processes that are connected with the train activity. As direct processes, we consider the specific energy consumption per travelled km of each type of locomotive and wagon. The life cycle phases expressed per tkm (both depending on the train traction, thus considering diesel and electricity traction separately), the direct emissions to air related to the diesel combustion in locomotives per tkm and to soil from braking and lining (iron abrasion of railroad tracks, wheels, brakes and overhead contact lines) and the people exposed to noise due to rail freight transport activity. Indirect processes include the upstream emissions from the different materials used for the construction and distribution of the diesel and electricity used in rail transport would also be

SETAC Europe 26th Annual Meeting Abstract Book 243
determined. Appropriate emission factors will be calculated to determine the direct and indirect emissions. The transport emissions related to the energy consumption during the rail transport activity will be determined considering the predicted transport volumes in a time horizon 2030.

TU231
The model for optimal transport selection based on multi-criteria decision making and life cycle assessment
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Considerable amounts of resources are used by transportation vehicles throughout their life cycle and environmental burdens are created during the production and operation to the end of life stage. When considering transport selection problem, nowadays environmentally conscious companies are paying special attention to environmental burdens where life cycle analysis (LCA) presents valuable assessment tool. Selection of optimal transport alternative for both - employees and goods, that considers the life cycle environmental burdens is a multi-criteria decision making (MCDM) problem. This paper proposes the model for optimal transport selection based on MCDM where the environmental aspects are considered through the life cycle perspective. An illustrative example has been provided for the verification of the proposed model. The transportation problem evaluated here is of a company that needs small packages to be delivered from point A to point B in urban environments. Proposed MCDM & LCA model considers environmental, economic, social and technical aspects for selection of optimal transport expressed through defined criteria. Environmental criteria is expressed through LCA of delivery vehicle considering the following processes: production, operation, maintenance and end of life. Economic criteria is presented through the travelling cost, as social criteria the personal subjective judgement is included, while in technical the delivery time criteria (expressed through the period of time needed for package delivery) is the most important one. The considered alternatives include: transport by foot, bicycle, scooter, car, and public transportation (bus and tram). The aim of the proposed MCDM & LCA model is to assist companies which activities are occasionally connected to in-time delivery business, in their environmental impact decrease.

TU232
Systematic LCA method in automotive sector
A. BOUTEILLE, Renault; P. Osset, Solinnen; M. Pelissard, Renault; D. Bauchot, Solinnen SAS; J. Beaulieu, S. Morel, Renault; C. Beauchard, Solinnen SAS

Life Cycle Analysis (LCA) is often viewed by companies as the most relevant tool to open the dialogue with stakeholders (Schmidt 2010). But on the other hand, it is perceived as a time-consuming, difficult to implement, with short-lasting relevance of its results and, above all, costly approach. The requirement of critical review for comparative LCA to communicate results to the public, often leading to additional delays and costs, can also discourage to adopt this approach. In parallel companies are facing more and more demanding environmental regulations and standards such as the Euro regulation in the transport sector, as well as regulations that require robust communication on the environmental performance of marketed products. For example, the ongoing Product Environmental Footprint (PEF) experimentation should lead to new regulations on environmental footprint communication based on LCA approach, that are expected in 2018. In that context, large companies have integrated environmental criteria (regulatory requirements as well as internal objectives) in their core business quality approach leading to the definition of very detailed processes that are automated/computerized in order to be easily repeated for any product. Thus, the group Renault endeavours to carry on this environmental integration into the quality approach and to develop a systematic LCA method with the following objectives of (1) communicating on the environmental footprint of their product in an easy, fast and robust manner, (2) ensuring the quality and reliability of data (notably regarding geographical, technological and time representativeness), (3) being able to improve constantly the approach and the modelling. The development of the systematic LCA method in Renault started in 2011 when Renault renewed its calculation model during the study of an innovative product, the electric vehicle. This first step of the model was assessed by a particularly skilled panel which cumulate 50 years of experience (Morel 2014) and set the baseline for further studies. The second step of the model developed in 2014 aims at comparing any new vehicle with its former model. The current model enables to perform a comparative study in about 15 days, with a creation of new configuration in less than 1 hour the model can be automated and described the developed method. The oral presentation with two speakers, one of Renault and one of Solinnen present the method from each point of view.

TU233
Life cycle assessment of underground construction Tunnel case study
A. BOUTEILLE, Renault; P. Osset, Solinnen; M. Gomez, Instituto de Ingenieria UNAM; L. Güereca, Engineering Institute Universidad Nacional Autónoma de México / Environmental Engineering

During this last decade, the European Union has led to the definition of a list of critical raw materials (CRM) which are key enablers of new technologies and products. Energy efficiency, as it enables the recovery of several different products that may be used to recycle, is a key factor of CRM. Two main methods are used to map the CRM supplies. The European commission registered critical materials in a list for which it is necessary to calculate material balance of a substance at a geographical scale (including upstream and downstream, indirect activities). The focus, therefore, is mainly on CRM for metals, plastics, and glass in industries in the sector (Aurubis, KGHM and Outotec). Copper is a key enabler of many products, all directly linked to copper production. The keystones of the methodology of underground structures depending on theirs geometry, functionality and method of construction. It is a linear underground structure; that serves multimodal displacement (light vehicles, public transport, cycling and pedestrians). The evaluation is performed by environmental impacts which are obtained from life cycle assessment (LCA): energy consumption, global warming, photochemical ozone creation potentials, toxicity, and acidification. The results serve also to compare the performances of this structure to those of alternative surface solution, ensuring the same service. The question of impact’s allocation to users of this multimodal transport infrastructure is also envisaged, taking into account the multimodal displacement in our case study. The results of allocation allow demonstrating the importance of mode-phase of life cycle to evaluate the environmental profitability, and then evaluate other sustainable development indicators, that will bring to think about indicators that provide results to enhance the benefits of underground construction. This initial work fits with a thesis IFSTTAR-CETU PhD, supported by the national project “VILLE 10D-VILLE D’IDEES www.ville10d.jr” about the design and the planning of the underground spaces to develop sustainable cities.

Expanding LCA: looking at organizations and at new policies
TU234
EXPERIENCES IN THE APPLICATION OF ORGANIZATIONAL LIFE CYCLE ASSESSMENT IN MEXICO
M. Gomez, Instituto de Ingenieria UNAM; L. Güereca, Engineering Institute Universidad Nacional Autónoma de México / Environmental Engineering

The evaluation of the environmental performance has been a strategy for setting goals related to the continuous improvement in organizations, but these evaluations are usually partial, because normally considered a specific facility and a reduced period. The Organizational Life Cycle Assessment (O-LCA) permits the determination of environmental indicators scientifically stronger because it considers all products and/or services of an organization, including the inputs and outputs throughout the life cycle of all associated processes. Taking into account the above, this paper presents the experiences in the implementation of the O-LCA methodology in the Central Bank of Mexico. The scope of this study concerns the banknotes factory whose function is the production of all denominations of Mexican bills. The system boundary includes all departments that are part of the factory (direct activities), the steps related to the supply of raw materials (upstream activities) and the steps related to the distribution, use and end of life (down-stream activities). For the definition of the reporting unit it took into account the production in 2013 with all related information. The assessment of environmental impacts was conducted with ReCiPe method and Eco Invent 3.1 database, using Umberto 7.1 software. The impact categories analyzed were: agricultural land occupation, climate change, freshwater eutrophication, human toxicity, ozone layer depletion, photo-oxidation formation, acidification and water use. The results show that for all the categories analyzed, activities upstream and down-stream together are generating the highest environmental impacts. The analysis of the direct activities shows that the departments of printing, distribution and administrative services presents the highest environmental impacts. It can be concluded that transportation, in direct and indirect activities, generates significant environmental burdens. These values are contained in a square matrix showing the monetary exchanges of all stages of life cycle. The analysis of the direct activities shows that the departments of printing, distribution and administrative services presents the highest environmental impacts. It can be concluded that these departments with the highest environmental impacts were considered and not the organization as a whole, due to the rigorous level of detail and the large amount of data required in this type of LCA.

TU235
The Organisation Environmental Footprint (OEF) pilot on copper production
L. Zampori, European Commission DG Joint Research Centre / Institute of Environment and Sustainability; R. Pant, European Commission / Institute for Environment and Sustainability; S. Sala, European Commission - Joint Research Centre / Sustainability Assessment Unit

In a global context of urban density, the underground space can be an opportunity for developing further cities with restricted spaces, and respecting at the same time the requirements of sustainability which lie strongly today to urban projects. The underground developing in urban zones provides an important space which is characterized by its ability to offer new spaces, separate functions, protect from negative impacts, in addition to many environmental and social benefits. In parallel, the construction and the exploitation of the underground facilities can significantly affect the environment, but can otherwise provide energy efficiency due to the thermal isolation or to the energy recovery. In return, these facilities can replace a significant financial investment, generally determined in the decision-making process. This article aim to is: I) propose a characterization methodology of underground structures depending on theirs geometry, functionality and method of construction. II) Propose a methodological approach for the evaluation of their environmental impacts. This approach is based on a case study. It is a linear underground structure; that serves multimodal displacement (light vehicles, public transport, cycling and pedestrians). This evaluation is performed by environmental impacts which are obtained from life cycle assessment (LCA): energy consumption, global warming, photochemical ozone creation potentials, toxicity, and acidification. The results serve also to compare the performances of this structure to those of alternative surface solution, ensuring the same service. The question of impact’s allocation to users of this multimodal transport infrastructure is also envisaged, taking into account the multimodal displacement in our case study. The results of allocation allow demonstrating the importance of mode-phase of life cycle to evaluate the environmental profitability, and then evaluate other sustainable development indicators, that will bring to think about indicators that provide results to enhance the benefits of underground construction. This initial work fits with a thesis IFSTTAR-CETU PhD, supported by the national project “VILLE 10D-VILLE D’IDEES www.ville10d.jr” about the design and the planning of the underground spaces to develop sustainable cities.
In the context of the “Building the Single Market for Green Products” initiative of the European Commission (COM 2013 196 final and related Recommendation 2013/179/EU), which proposes a set of actions to overcome confusion of consumers due to the stream of environmental information they receive, two Organisation Environmental Footprint (OEF) pilots are currently ongoing (cooper sectors are associated to their production. Smelter can process both primary and secondary raw materials, therefore they are both at the beginning and at the end of the life cycle of products. The product portfolio of a copper producing company covers many products, all directly linked to copper production: copper cathodes, anodes and blister, sulphuric acid, iron silicate, anode slime, NiSO₄, CuSO₄, silicon, gold, PGM concentrates, lead, Pb-Sn alloys, tin, crude selenium and tellurium. An organisational LCA, compared to a product LCA, considers the organizational activities as a whole: this approach allows to identify what are the most important environmental issues both in the sector and the organisations itself. It is useful for an organisation to rely on a robust measurement tool, such as the OEF, which can be implemented in other environmental management schemes (e.g. EMAS), to allow prioritisation of environmental management actions, both within the organisational boundaries (i.e. direct activities) and outside (i.e. upstream and downstream, indirect activities). The focus, therefore, is mainly on performance tracking and improvement. Results can be communicated depending on the target audience, and they can be used to provide information to investors, stakeholders and the public. The OEF model for copper production was approved in July 2015 by the Environmental Footprint-Steering Committee. The ongoing work within the pilot and the final OEFSS will show opportunities and limitations of the methodology.

TU236 Evaluation of Natural Capitals for organizations Considering Life Cycle of Perspectives
S. Suguri, N. Itsubo, Tokyo City University
In recent years, environmental evaluation of the organizations using the framework of LCA is now paid attention internationally. Although the interests in CSR and triple bottom line increase, the development of assessment method covering 3 aspects (environmental, economy and society) of organization is still limited. On the other hand, international organizations such as the World Bank and the United Nations discuss about framework of sustainability and indicators of subcategories. These outputs would be useful to apply for the development of LCA for organization considering all aspects of sustainability. In this study, we develop a research framework of organizational Life Cycle Assessment using Genuine Saving proposed by the World Bank. Genuine Saving is a method which evaluate increased or decreased of capitals. Genuine Saving can be obtained by the summation of savings, education payment the loss of natural capital including forest, minerals, and fossil fuels and environmental damages caused by global warming. We adopted Input-Output analysis and Life Cycle Impact Assessment to evaluate these above elements and aggregate them to consider the all attributes of sustainability. We discussed the feasibility to construct a framework of Organization LCA proposed by this research based on the case studies for Japanese companies.

TU237 RECO-INNO: An evaluation model approach for eco-innovation and its impact in the business strategy
F. Coca, I. Bosch-Frigola, Universidad San Jorge; M. Pino, San Jorge University / Facultad ciencias de la salud; S. Scarpellini, CIRCE
The scarcity of natural resources and the consequences of environmental challenges affect companies that are looking for more efficient processes and sustainable products. These factors strengthen the consolidation of the eco-industry and the implementation of Eco-innovation. Eco-innovation improves the technology upgrading, products and services linking the binomial firm-innovation and cost savings. It is related to business models based on a new strategy to incorporate sustainable development in business, reducing environmental impacts and saving raw materials through innovation initiatives. During this last decade, the profound changes led to the improvement of environmental and sustainability policy of European industry with new management models and the latest technological developments. With nearly 200 million Euros, it has established the objective of promoting eco-innovation within its Framework Program for Competitiveness and Innovation. The aim of this study is to define the company’s code of conduct and to evaluate sustainable companies through the development of models based on quantitative methods. Principal determinants of eco-innovation throughout their organizational life cycle are analyzed for integration of structural variables and they are applied to the appraisal of principal economic, social and environmental outputs in business. In the second stage, the thirty-six companies has been analyzed. Data have been obtained through a questionnaire in which variables have been identified to reflect the decision about eco-innovation and its principal determinants (technological options, investment, structural and environmental variables). The keystones of the business strategy related to eco-innovation have been analyzed as well with principal business results and the company’s environmental management factors. The results gained reflect an in-depth analysis of the situation of level of penetration in Spain of eco-innovation among companies, in order to overcome principal barriers.

TU238 How sustainable are WEEE? A review of LCA applied to waste from electric and electronic equipment
G. Rodriguez Garcia, Helmholz Institute Ulm / Systems III; J.F. Peters, KIT Karlsruhe Institute of Technology / Helmholtz Institute Ulm HIU; M. Weil, ECT Oekotoxikologie GmbH / Institute for Technology Assessment and System Analysis ITAS
The increased use of electric and electronic equipment (EEE) has turned their waste into a pollution problem worldwide. As a complex environmental burden, over the years the management of waste from electric and electronic equipment (WEEE) has been the object of a number of Life Cycle Assessment (LCA) studies. For this presentation, we reviewed 47 LCA publications evaluating end-of-life alternatives for WEEE. Our initial point of interest were recycling technologies, but was soon expanded to include the whole waste management: collection and transport, alternative treatments—landfill and incineration—as well as waste prevention strategies. The reviewed publications focus more on products than on processes, being more interested in the impacts of a given waste flow than on the impacts of the treatment technology. Most of them take into account the whole waste management—usually considered as transport and treatment, but studies evaluating only recycling technologies are also common. Publications where recycling is confronted with other treatments are less abundant and there is even fewer taking into account waste prevention strategies, either on their own or as an alternative to treatment. We only took into account studies that covered the whole life cycle if they had a particular focus on waste management. Those that did, concluded that waste management was not the most polluting stage. The environmental importance of waste management—namely of recycling—was due to the potential recovery of valuable materials—metals, plastics, and glass in particular—could avoid the extraction and processing of new materials. However, recycling is not necessarily the greenest alternative. Long-distance transport can make landfilling more attractive and there are several waste prevention schemes that, at least in the short term, could be more beneficial to the environment than recycling. Because of that, when conducting an LCA on WEEE management strategies, we should take into account more scenarios than an alternative recycling technology.

TU239 State of the art on coupling input-output table with material flow analysis in order to evaluate environmental impact of metals in an economic system
A. Thevenot, Université de Bordeaux; P. Loubet, Université Bordeaux; G. Sonnemann, Université de Bordeaux / The Life Cycle Group CyVi
In a context of increasing global demand of metals (e.g., for building and high technologies), European countries, especially France, widely depend on foreign imports. This situation implies a real risk of physical or economic disruption of supplies. At the European level, an unregistered critical metal flows data concerning flows should be improved and reliable. However, it is complex to calculate material balance of a substance at a geographical scale (including import, export, waste, stocks, recycling). Improving the knowledge in material flows in an economic system would contribute to evaluate the potential of secondary raw material sources. Currently, two main methods are used to map the flows of metals: Input-Output Tables (IOT) which is a top-down approach and Material Flows Analysis (MFA) which is a bottom-up approach. The IOT represent the exchanges in monetary value allocated to different economic sectors. These values are contained in a square matrix showing the monetary exchanges of materials between the activity. After conversion from price flows to physical flows, we obtain physical input-output tables (PIOT). Nevertheless, the stocks and waste flows cannot be reliably calculated with these tables. On the other hand, the aim of MFA is to quantify the whole flows (inputs, outputs, stocks) of a substance (or a product) at a process scale with defined boundaries. However, we do not necessarily know the flow of products and consumption and generate inconsistencies. A reliable “mapping” of metal flows from production to final use could be obtained by coupling these two methods. Eventually, life cycle assessment (LCA) can provide environmental impact regarding MFA results in order to obtain a multi-criteria analysis, which depends not only on resources flows but also on environmental impacts of a product and a substance. In order to evaluate copper flows in France, we had an approach dealing with various data from statistic studies, custom services, professionals or scientific research. These data can be restricted for some materials. It is needed to reconcile the data from PIOT and MFA for specific materials. A methodological challenge is to develop an innovative approach for improving the coupling of these two methods and to reduce the uncertainties of initial data. From the resulting flow mapping, we will be able to calculate more effectively environmental impact of metals.
TU240  
Eco-design of packaging for a circular economy in the food canned industry  
J Laso; M. Margallo, R. Aldaco, J. Celaya, University of Cantabria; C. Gazulla, ESCL, A. Bala, Escola Superior de Comerç Internacional (ESCII); P. Fullana, Universitat Pompeu Fabra (UPF) / UNESCO Chair on Climate Change Escola Superior de Comerç Internacional ESCII; A. Iribien, University of Cantabria / Department of Chemical and Biomolecular Engineering  
Since the industrial revolution, waste has constantly grown. This is because our economies have been dominated by a one-way or linear model of production and consumption that privileges make-breakdown, in which goods are manufactured from raw materials, sold, used and then discarded or incinerated as waste. [1]. For this reason, in 2014, the European commission (EC) published a communication on the circular economy which stimulates innovation in recycling and reuse, limit landfill, reduce losses of recoverable materials and creates incentives for behavioural change, turning waste into a resource as part of “closing the loop” in circular economy systems [2]. In the packaging sector, the circular economy model is about more than just recycling. It handles all stages of product’s lifecycle, namely: its design, production, distribution and use, but also its recovery. Regarding the product design, eco-design takes the environment into account reducing the product’s negative impact on the environment throughout its lifecycle while maintaining the same level of quality [3]. In this context, this work aims to apply the concepts of circular economy and eco-design under a life cycle approach to the seafood sector and, in particular, to the canned anchovy industry. This sector generates a high amount of packaging wastes of several sizes (0.05, 0.5 and 1 kg) and materials (glass, plastic, cardboard, aluminum) [4]. The life cycle assessment (LCA) of the production of one can of anchovy from cradle to gate revealed that the manufacture of these packages has the highest environmental impacts and, thus, recycling these materials would improve the environmental performance of the system. For that reason, this work proposes a methodology that combines the circular economy and eco-design concepts to reduce the environmental impacts of food canned industry. [1]. Ellen MacArthur Foundation and McKinsey & Company. 2014. Towards the Circular Economy: Accelerating the scale-up across global supply chains. World Economic Forum. [2]. European Commission. 2014. Towards a circular economy: A zero waste programme for Europe. Brussels. 25 September, European Commission. [3]. Conseil National de l’emballage. 2014. Packaging and circular economy: a case study of the circular economy model. French Packaging Council.

TU241  
LCA of Rare Earth Magnet Recovery in the REMANENCE project. The role of LCA in the development of recovery technologies; allowing circular economy of WEEE.  
R. Villalba, Leitat Technological Center / Sustainability Unit; M. Escamilla, Leitat Technological Center / RD Safety Sustainability Division  
REMANENCE is an ambitious project designed to dramatically increase the amount of rare earth materials recovered and remanufactured from existing waste streams, which contribute to the technology development necessary to make possible circular economy across EU. Co financed by the Seventh Framework Programme, the project brings together European industry and academia across the supply chain to develop innovative technologies, business models and market innovation required to exploit this valuable resource and reduce dependence on primary sources. Within this context, new and innovative processes are being demonstrated for the recovery and recycling of rare earth (RE) containing neodymium iron boron magnets (NdFeB) from a range of Waste of Electrical and Electronic Equipment (WEEE), and resulting in the development of new recovery technology able to recover RE material in a form that could easily re-enter the primary magnet manufacturing production process, so providing large energy savings and material costs. REMANENCE will provide a secondary source of materials for the EU, large enough to supply the entire EU bonded magnet manufacturing industry and a significant proportion of the EU high virtue sintered magnet production (1550-2000Tpa). Amongst other environmental indicators, it is expected that the introduction of the RE magnetic recovered and recycled material into the manufacturing process will significantly reduce the energy cost and environmental impact of these replaced materials. Thus, the recovery and repurposing of RE magnetic materials is foreseen to reduce energy consumption by about 12% when compared to emissions from primary production (mining, separation, purification and manufacturing) of RE. The analysis of such environmental indicators is possible through the use of Life Cycle Assessment (LCA) methodology, which allows comparison between the environmental impacts of the recovery technologies developed in the project versus the conventional extraction of virgin material, two very different ways of obtaining the RE material necessary in the time and demand. The LCA results will demonstrate how the REMANENCE technology is contributing to close the loop and boost business related to WEEE.

TU242  
Recycling Rare Earth Elements  
G. Bailey, KU Leuven / Material Sciences; K. Van Acker, KU Leuven  
Many rare earth elements (REEs) are used in clean technologies such as wind turbines and in permanent magnets found in electric vehicles. Today, the recycling rate for rare earth elements is less than 1%. It is expected that in the coming years, demand for rare earths will grow as the European economy makes the transition toward high-tech and green products. However, as the virgin REEs market is dominated by China, the European REE prices have historically been highly volatile. The environmental impact of RE mining is large, the recovery of the REEs is extremely important for both economic and environmental reasons. As highlighted by the European Rare Earths Competency Network (ERECON), recycling of rare-earth magnets should receive top priority. The following study will provide some of the current state and characteristics discussed above for RE recovery in permanent magnets. Neodymium is the most common rare earth element found in permanent magnets and will be assessed to determine where the greatest environmental impacts, or hot spots, in the recycling routes occur. Different impact factors are calculated, enabling detection of potential new recycling routes. Since permanent magnets contain a high concentration of REEs, a reduction in the demand for virgin sources has the potential to shift Europe’s reliance on Chinese exports, as well as lowering the environmental impact generated.

TU243  
Consequential Life-Cycle-Assessment as policy-support tool to promote green concrete.  
A. Di Maria, KU Leuven / MTM; K. Van Acker, KU Leuven  
The manufacturing of Ordinary Portland Cement (OPC), normally used as a binder in concrete production, is responsible for 5% of global carbon emissions. Potential sustainable alternatives, namely “green concretes”, are currently studied in scientific literature. In green concretes, OPC is replaced with by-products coming from other industries. At present, however, the economic and environmental consequences of the introduction of green concretes into the market are not clear yet. This paper aims at filling this gap by using a Consequential Life Cycle Assessment (C-LCA) for the case of green concrete made from Stainless Steel Slag (SSS), with a focus on the Flemish market (in Belgium) for construction blocks. For the present study, a green concrete block was produced using SSS as OPC replacement. Today, SSS is considered as a hazardous waste, therefore stabilization is needed before its recycling as low-quality aggregate. Alternatively, SSS can be also chemically activated to produce a new binder for green concrete, hence foregoing the need of SSS stabilization. Therefore, the potential switch in the recycling of SSS, from low quality aggregates to green concrete, affects both the construction blocks and the low quality aggregates markets. In order to analyse the environmental consequences of these changes in both markets, a C-LCA was performed including all processes that are significantly affected by the changes, namely the production of traditional concrete, the production of natural/secondary low quality aggregates and their transports. In order to understand how mechanisms of revenue maximization and price equilibrium are affected by the changes, market information and economic models are also considered during the Life Cycle Inventory phase. Finally, the recycling of SSS as a binder for building materials will reduce the emission of CO2, NOx, thanks to making REEs and OPC replacement. At the final stage, in the final use of SSS causes an increase use of natural aggregates for low value applications, unless alternatives from other by-products are considered. The C-LCA approach indicates that replacing a material by a more sustainable option does not lead necessarily to a better overall sustainability. Policy makers should therefore consider interactions between different markets when analysing the effectiveness of policy-support actions, like the increase of the tax on the CO2 emissions or subsidies for the use of SSS as a binder.

TU244  
LCA for supporting a sustainable transition towards circular economy  
L. Rigamonti, Politecnico di Milano / Department of Civil and Environmental Engineering; A. Falbo, Politecnico di Milano; L. Zampori, European Commission DG Joint Research Centre / Institute of Environment and Sustainability; S. Sala, European Commission - Joint Research Centre / Sustainability Assessment Unit  
Circular economy is an economic model based on the idea of reducing, reusing and recovering of materials in an endless cycle while fostering the use of waste as resource. Within more circular economies, resources are used efficiently within the life cycle of a products and the waste generated along the supply chains are minimized and used as much as possible, directly of after transformation, as input for other products and systems. Supporting circular economy means improving the analysis of resource flows, with a particular implementation on mining industries, ensuring low-toxicity in products and waste, as well as fostering waste prevention and recovery. Moving toward more circular economy requires that different waste management options are systematically scrutinized to assess the environmental impacts and benefit associated to them. The present work aims at illustrating how a sensitivity analysis could support the impact assessment of a LCA to the interpretation of a LCA study applied to a waste management system that...
includes material recovery. The focus is on toxicity-related and resource-related potential impacts as they are considered among the most critical ones, which may affect the way the final benefit from material recovery is evaluated. Possible alternatives in terms of impact assessment assumptions and modelling are tested by performing a sensitivity analysis on a case study on electronic and electric waste. For the toxicity impact categories, a sensitivity analysis is performed focusing on the role of metal toxicity and long-term emissions in upstream processes. For resource related impact category, a sensitivity analysis has been performed adopting different sets of characterization factors based on existing models for minerals and metals as well as recently proposed sets accounting to critical raw materials. The application of LCA is crucial for assessing avoided impacts and uncovers potential impacts due to material recovery. However, contrasting results may stem from the application of different assumptions and models for characterization. A robust interpretation of the results should be based on systematic assessment of the differences highlighted by the sensitivity analysis, as guidance for delving into further analysis of the drivers of impacts and/or to steer towards innovation to reduce those impacts.

**TU245**

**Life cycle assessment of co-digestion on a wastewater treatment plant**


The increase of electricity self supply is a aim on municipal wastewater treatment plants (WWTP). A significant share of energy demands on WWTP is provided by biogas production and co-digestion of substrates. The biogas can be utilized in combined heat and power plants (CHP). Digestion of co-substrates is applied to increase the biogas yield and thereby energy self supply. However, an increased substrate input into the digester comes with a higher energy demand on the WWTP; moreover, an alternative usage of the co-substrate is hindered. Consequently, energy flows within the LCA model to assessing the trade-offs between an increase of energy self supply on the WWTP and additional energy/substance demands as well as the competing use of food residues as a substrate for electricity generation and for fertiliser substitution. Life Cycle Assessment (LCA) is used. A parameterised LCA model is built to assess different quantities of food residues fed into the digestion process on the WWTP. The model allows assessing plant-internal changes of energy and material demands/flows depending on different feeding amounts of co-substrates and the consequences coming from the substituted alternative treatment of food residues. Data for material and energy demands for this case study comes from a WWTP with a population equivalent of 240,000 in Germany. In the analysed case study, an additional amount of co-substrate (food residues) of about 15 weight percent is necessary to provide energy self-sufficiency on the WWTP. Due to the high energy content and the fast degradability of the organic matter of food residues, the increase in electricity self supply on the WWTP overcompensates additional energy and working material demands for treatment. Considering an alternative treatment of food residues, the total system-wide environmental impacts mostly depend on the CHP efficiencies on the WWTP / alternative biogas plant. Electricity self sufficiency on WWTP can be achieved by feeding a relatively low amount of substrates with a high energy content and using energy on-site. Depending on efficiencies of the digestion and the CHP plant on the WWTP and the feeding biogas production a comparatively easy environment impact can be beneficial from a life cycle perspective. It is therefore necessary to include different treatment options and process efficiencies to assess impacts across systems.

**TU246**

**Plastic waste treatment strategy in Switzerland: LCA approach based on parameterisation for robust decision support**

T. Kugi, F. Dinkel, N. Ferronato, Carbotech AG

In this study we looked at post consumer plastic waste treatment options such as mechanical recycling (open loop and closed loop if possible) and thermal utilisation (plastic is not allowed in landfills in Switzerland). This study aimed at the strategic question: How should plastic waste from households be collected and utilized in Switzerland to lower the environmental (and economic) impact? A more specific question was: Does it makes more sense to continue the separate collection of waste plastics like PET bottles or is there a viability of a co-digestion collecting a larger amount of plastic by doing a mix plastic collection like in other European countries. The main focus was on creating an utmost parameterized model using the SimaPro software v8.02. The variation of parameters such as transportation, sorting efficiencies, recovery rates, material quality, efficiency of energy recovery in the municipal waste incineration, electricity and heat mix substitution allowed for a holistic scenario analysis for the optimisation potential with regard to collection processes, recycling processes and thermal utilisation processes. The findings of the model analysis showed that the existing separate PET-collection is meaningful but the present system could be improved by expanded separate-mixed collection like PE-bottles and beverage cartons or PE-bottles and films. The actual Swiss recycling system represents a scenario which is rated as average in a sense of functionality but it does still leave room for improvement compared to the best options resulting in reduction potentials of about 20 % in the next 15 years. Interestingly, the mixture of all plastics (mixed collection) performs worst mainly due to the negative influence to the material quality and sorting efficiency. In summary, an improved recycling system for the whole of Switzerland would be ecologically worthwhile and would allow an improvement and expansion in the plastic recycling system. The model proved to be useful for evaluating different options regarding measures taken in the field of circular economy of plastics.

**Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy (P)**

**TU247**

**Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy (poster)**

A. Alia, INRA-Agrocomplex Ouest / UMR SAS

LCA theory and practice still feature a number of methodological challenges, some of which are not fully solved by the research community. When LCA is applied to agriculture (i.e. cradle to farm gate), as well as to agri-food, bioenergy and fibre systems (i.e. cradle to processing plant gate) and supply chains, specific challenges arise, regarding for instance, modelling of direct emissions, allocation strategies and their consequences, land use and land use change (LULUC) and their impacts on biodiversity and climate change, responsible sourcing of agricultural products, the role of LCA to assess production and utilisation of bioenergy; as well as the extrapolation of farm level assessment to describe agricultural regions. Analytical methodological challenges, models for direct emissions in particular have been continuously developed and expanded to address the transportation and fate of pesticides (see FATE track), LULUC, carbon sequestration, and specific soil and water emissions, among other topics. A key question regarding direct emission models would be whether a common methodological framework is possible and desirable. Regarding the use of LCA to compare competing agricultural strategies, including bioenergy and fibre production, both at the farm, regional and supply chain level (i.e. beyond regional) levels; certain methodological challenges hinder the validity of these comparisons. This session intends to advance discussion on methodological challenges to agricultural LCA and its solutions, with emphasis on convergence of methods towards a common framework acceptable for both academia and industry. It will allow LCA practitioners studying agri-food, bioenergy and fibre-based systems and supply chains, but also to modellers dealing with agricultural systems at the field, farm and regional levels. The presentation of case studies is acceptable when it contributes to introduce methodological developments towards overcoming current (and future) challenges of LCA of agricultural systems and supply chains, including bioenergy production systems.

**TU248**

**Challenges for improving iLUC negative effects of biofuel demand in Spain**


From a consequential approach, biofuel life cycle greenhouse gases (GHG) analyses could present great differences if the effects of the indirect Land Use Change (iLUC) are considered. The potential impacts related to the unintended consequence of liberating more carbon emissions due to land-use changes around the world induced by the expansion of croplands for biofuel production in response to the increased global demand. The Energy Systems Analysis unit of CIEMAT has carried out some previous studies in order to analyse the iLUC effects, in terms of CO₂ emissions, of biofuel consumption in Spain. Results showed a great interval of variation of figures for the majority of biofuel types, especially in sunflower and rapeseed biodiesel, with average emissions ranging from 20 to 60 kg CO₂ eq / MJ in biodiesel, and from 16 to 60 kg CO₂ eq / MJ in bioethanol. The main reason for these large intervals was due to the selection of the location of raw materials. This study is divided in two parts. First part presents an analysis of the main economic and biophysical key factors for reducing negative effects from iLUC, such as the high dependency of the elasticity of prices, the choices of types and geographical location of crops, or the consequent use of coproducts from biofuel production. Finally, an example of the implementation of some of these measures in previous studies of iLUC effects of biofuel demand in Spain is presented.

**TU249**

**Environmental Impact of Food Losses from Agriculture to Consumption in Switzerland**

C. R. Beretta, Institute of Environmental Engineering ETH Zurich / IU; C. Oberschelp, ETH Zurich, S. Hellweg, ETH Zurich / Institute of Environmental Engineering

Twenty to thirty percent of the environmental impact of consumption are caused by food consumption (Tukker et al., 2006). A key element to make our food system more efficient and sustainable is the reduction of food losses across the entire food value chain (Bastl and Johnson, 2009). However, for the
implementation of measures against food losses is it important to know which losses are environmentally most relevant. We quantified the amount of food losses at the various levels of the Swiss food value chain (agricultural production, postharvest handling and trade, processing, food service industry, retail, and households) in terms of mass and energy (Beretta et al., 2012). About one third of the food available at the internal consumption level is lost in terms of mass and energy. However, the environmental impact of food losses do not only depend on the amount of food loss, but also on the type of food, the degree of processing, the level in the food chain on which the losses occur, and the method of treatment (incineration, composting, anaerobic digestion, feeding). Therefore, we quantified the environmental impact of the food losses at the various stages of the food value chain in Switzerland. Based on the mass and energy flow analysis, twenty-three food categories are modelled separately, representing the whole food basket. For the impact assessment the categories change climate, ecological scarcity 2013, water, and land use are considered. The results show that the food losses at the end of the food value chain are the dominant environmental impacts. Firstly, they are quantitatively relevant, representing roughly half of the overall food losses; and secondly, they cause higher impacts per kg of product because of the accumulation of the impacts of the previous stages of the food chain. The net environmental credits from the treatment of food losses are between 5 and 10% of the impacts of the supply chain allocated to the losses. Therefore, avoiding food waste is much more effective than optimizing the method of treatment. About half of the environmental impacts from total avoidable food losses can potentially be avoided by solely addressing households. Furthermore, the highest environmental benefits from avoiding one kg of food loss of average composition can be achieved in households and food service institutions. This can help public and private decision makers prioritize their strategies for preventing food losses.

TU250
Country-based and spatially-explicit Land Use Change matrix
M. De Rosa, Aarhus University / Agroecology; M. Vestergaard Odgaard, Department of Geosciences, Aarhus University; M. H. van der Werf, INRA / UMR SMART; H. van der Werf, INRA, UMR SMART / Environment et Agronomie European daily production is facing great changes. Removal of milk quotas, increased farm size and increased demand for dairy products are the drivers for these changes that are expected to affect the volume of raw milk produced, the structure of the milk production sector and the environmental impacts of the milk produced. Following the Common Agricultural Policy Health Check in 2008, impacts of quotas removal on prices and milk supply in the European Union (EU) and/or its Members States (MS) have been widely studied. However, supply and environmental impacts of this policy change through its effects on milk production systems and markets, and its effects on individual farms and farmers have been less studied, and, to our knowledge, never through a Consequential Life Cycle Assessment (CLCA) approach. We combined LCA and economic modelling to assess environmental impacts of public policies in the French dairy sector through CLCA. MATSIM-LUCA, a partial equilibrium model, was used to project agricultural markets to 2030. The impact of a change in policy, representing different policy and demand contexts, were simulated: i) SRef, which is the baseline scenario, representing the agricultural markets in 2030; ii) SRefL, representing the agricultural markets in 2030 if dairy quotas had still been in force; iii) S1 Ec, corresponding to SRef with reduced growth of world demand for milk as compared to OECD forecasts; iv) S1 Ec, where SRef is implemented with increased growth of world demand. For each scenario, MATSIM-LUCA gives results, for the various regions considered (including France and EU) for: i) the share of dairy production systems; ii) the quantity of milk produced, consumed and traded, the prices for milk and agricultural goods; iii) the areas for crop and grasslands. These outputs are used to feed a LCA model. CLCA, through scenario comparison, is then performed. A range of impacts are examined, including land use change and intensification of crop production.

TU251
Modelling the environmental effects of selected agricultural management strategies with regional statistically-based LCA
A. Béroud, INRA, UMR SMART; M. Corson, INRA, UMR SMART; H. van der Werf, INRA, UMR SMART / Environment et Agronomie. Despite the farm being considered by many as the most suitable level of decision making and strategic management in agriculture, there is an increasing interest in evaluating agricultural management strategies at the regional level. Recent initiatives attempted to aggregate and generalise farm-level life cycle inventory (LCI) data and LCA impact assessment (LCA) results to describe the environmental performance of agricultural regions. We propose a regional statistics-based approach for constructing virtual representative farms (VRFs), representing dominant farm types for a given region, as a tool for comparing alternative regional agricultural strategies in contexts of insufficient farm (e.g. LCA) data. The proposed method has been applied to the construction of a LCT for the Brittany region, in France. The environmental impacts of different agricultural management strategies were estimated at the regional level, by modelling the strategies as changes in VRF-based LCIs, calculating LCIA, and extrapolating their means to the total land use in the region. Based on this assessment, performed using a regional life cycle assessment (LCA) framework, we have analysed the relative environmental impacts of each management strategy on the region. By adding a concise, relevant economic assessment, a strategy comparison matrix was built to allow farmers and other decision makers to understand the implications of implementing each strategy. The method aims to approximate regional environmental impacts from agriculture, including farm-level changes in management practices propagated to the regional scale. This work is currently under development.

TU252
Combining Life Cycle Assessment and economic modelling to assess effects of agricultural policies on the environmental impacts of the French dairy sector
S. Ades, INRA / UMR SMART; M. Levert, A. Forslund, J. Hercule, INRA Agrocampus Ouest / UMR SMART; H. van der Werf, INRA, UMR SMART / Environment et Agronomie European daily production is facing great changes. Removal of milk quotas, increased farm size and increased demand for dairy products are the drivers for these changes that are expected to affect the volume of raw milk produced, the structure of the milk production sector and the environmental impacts of the milk produced. Following the Common Agricultural Policy Health Check in 2008, impacts of quotas removal on prices and milk supply in the European Union (EU) and/or its Members States (MS) have been widely studied. However, supply and environmental impacts of this policy change through its effects on milk production systems and markets, and its effects on individual farms and farmers have been less studied, and, to our knowledge, never through a Consequential Life Cycle Assessment (CLCA) approach. We combined LCA and economic modelling to assess environmental impacts of public policies in the French dairy sector through CLCA. MATSIM-LUCA, a partial equilibrium model, was used to project agricultural markets to 2030. The impact of a change in policy, representing different policy and demand contexts, were simulated: i) SRef, which is the baseline scenario, representing the agricultural markets in 2030; ii) SRefL, representing the agricultural markets in 2030 if dairy quotas had still been in force; iii) S1 Ec, corresponding to SRef with reduced growth of world demand for milk as compared to OECD forecasts; iv) S1 Ec, where SRef is implemented with increased growth of world demand. For each scenario, MATSIM-LUCA gives results, for the various regions considered (including France and EU) for: i) the share of dairy production systems; ii) the quantity of milk produced, consumed and traded, the prices for milk and agricultural goods; iii) the areas for crop and grasslands. These outputs are used to feed a LCA model. CLCA, through scenario comparison, is then performed. A range of impacts are examined, including land use change and intensification of crop production.

TU254
Water use as a regional LCA indicator in the production of bio-based succinic acid using corn and wheat as potential primary resources in France
S. A. Leal Meza, Universite de Technologie de Troyes / Hetic; J. Kim, University of California, Davis / Agribalyse; SAD / URASTER; F. Jourjon, Ecole Supérieure dAgricultures; M. Thiollet-Scholtus, INRA Levert, A. Forslund, J. Hercule, INRA Agrocampus Ouest / UMR SMART. Bioeconomy is currently targeted as a strategic policy to increase their production (NNFCC, 2015). The present work aims to contribute to increase their production (NNFCC, 2015). Bioeconomy growth could imply putting more efforts to the integration of environmental benefits of this policy change through its effects on milk production systems and markets, and its effects on individual farms and farmers have been less studied, and, to our knowledge, never through a Consequential Life Cycle Assessment (CLCA) approach. We combined LCA and economic modelling to assess environmental impacts of public policies in the French dairy sector through CLCA. MATSIM-LUCA, a partial equilibrium model, was used to project agricultural markets to 2030. The impact of a change in policy, representing different policy and demand contexts, were simulated: i) SRef, which is the baseline scenario, representing the agricultural markets in 2030; ii) SRefL, representing the agricultural markets in 2030 if dairy quotas had still been in force; iii) S1 Ec, corresponding to SRef with reduced growth of world demand for milk as compared to OECD forecasts; iv) S1 Ec, where SRef is implemented with increased growth of world demand. For each scenario, MATSIM-LUCA gives results, for the various regions considered (including France and EU) for: i) the share of dairy production systems; ii) the quantity of milk produced, consumed and traded, the prices for milk and agricultural goods; iii) the areas for crop and grasslands. These outputs are used to feed a LCA model. CLCA, through scenario comparison, is then performed. A range of impacts are examined, including land use change and intensification of crop production.

TU254
Water use as a regional LCA indicator in the production of bio-based succinic acid using corn and wheat as potential primary resources in France
S. A. Leal Meza, Universite de Technologie de Troyes / Hetic; J. Kim, University of California, Davis / Agribalyse; SAD / URASTER; F. Jourjon, Ecole Supérieure dAgricultures; M. Thiollet-Scholtus, INRA Levert, A. Forslund, J. Hercule, INRA Agrocampus Ouest / UMR SMART; H. van der Werf, INRA, UMR SMART / Environment et Agronomie
Water availability and usage is of increasing concern in modern society (Vink & Davies, 2015). In LCA practice has been pointed out that water use is an indicator that should be assessed in the local or regional context, (Pfister, et al., 2009; Vink & Davies, 2015; others) so the use of regional data is more relevant to obtain more reliable values for this indicator. Bioeconomy is currently targeted as a strategic axis in the European Commission. Of the four regions, in France partionally, it is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015). Bioeconomy growth could imply putting more pressure on water availability, depending on local conditions, thus it is important to review the increasing biomass uses regarding its implicit water use as an LCA indicator at regional level. As part of the bioeconomy, several biobased chemicals like biopolymers or chemical building blocks are increasing and they are expected to increase their production (NNFCC, 2015). The present work aims to contribute on sharing the experience to calculate water use as an LCA indicator, using regional inventories and agricultural production data. The Contra model and the regional diet reduced, is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015). Bioeconomy growth could imply putting more pressure on water availability, depending on local conditions, thus it is important to review the increasing biomass uses regarding its implicit water use as an LCA indicator at regional level. As part of the bioeconomy, several biobased chemicals like biopolymers or chemical building blocks are increasing and they are expected to increase their production (NNFCC, 2015). The present work aims to contribute on sharing the experience to calculate water use as an LCA indicator, using regional inventories and agricultural production data. The Contra model and the regional diet reduced, is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015). Bioeconomy growth could imply putting more pressure on water availability, depending on local conditions, thus it is important to review the increasing biomass uses regarding its implicit water use as an LCA indicator at regional level. As part of the bioeconomy, several biobased chemicals like biopolymers or chemical building blocks are increasing and they are expected to increase their production (NNFCC, 2015). The present work aims to contribute on sharing the experience to calculate water use as an LCA indicator, using regional inventories and agricultural production data. The Contra model and the regional diet reduced, is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015). Bioeconomy growth could imply putting more pressure on water availability, depending on local conditions, thus it is important to review the increasing biomass uses regarding its implicit water use as an LCA indicator at regional level. As part of the bioeconomy, several biobased chemicals like biopolymers or chemical building blocks are increasing and they are expected to increase their production (NNFCC, 2015). The present work aims to contribute on sharing the experience to calculate water use as an LCA indicator, using regional inventories and agricultural production data. The Contra model and the regional diet reduced, is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015).
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results show that if these rules seem pertinent to allocate benefits of some crop
Biomarker analysis in soils of the Amazon rainforest after vegetation fire
for Agribalyse. For each case study, LCA was applied on a basic scenario and on
ARVALIS - Institut du végétal; M. Laustriat, Terres Inovia; h. HEITZ, ARVALIS
soil has become a more common procedure, since the abundance of these
whole crop rotation and not only a single crop. The method to allocate emissions
produced at the surface to the deeper layers occurred. After vegetation burning, a
Life cycle assessment of hemp concrete blocs
chromatography atmospheric pressure. The distribution of n-alkanes was
enriching thus the proportion of C<sub style="font-family: arial, helvetica, sans-serif;">24</sub>
environmental impacts of husbandsries by optimizing their feed. This project to improve
the environmental impacts of husbandsries by optimizing their feed. This project to improve
the soil availability to satisfy crop needs and therefore increases the
other allocation methods (e.g., physical, economic). The biophysical allocation
energy model was used to estimate the energy requirements for body growth from
birth to slaughter age. The allocation factors were calculated based on the energy
requirements attributed to build body tissues characterized by their chemical compositions (proteins, lipids, carbs, etc). Finally, this method was compared with other allocation methods (e.g., physical, economic). The biophysical allocation reflects a physical and biological relationship between the coproducts as required by ISO standard. It provided a moderate allocation factor for human food due to their chemical characteristics compared to the other physical allocation methods. In the data required is specific to species and less influenced within a predefined system than economic allocation. This study provides a generic and robust biophysical allocation method to handle the coproducts in beef cattle system. The method can be considered as an original contribution to the international debates on the allocation methods in LCA applied to livestock products, especially among the stakeholders of the meat value chains.
TU260
How to take into account the crop rotation into Life Cycle Assessment of crop at national scale ? Methodological development and results from ECOALIM project
A. Tailleur, ARVALIS - Institut du végétal; S. Dauguet, Terres Inovia; A. Wilfart, INRA Institut National de la Recherche Agronomique / UMR SAS; S. Willmann, ARVALIS - Institut du végétal; M. Laustriat, Terres Inovia; h. HEITZ, ARVALIS INSTITUT DU VEGETAL; S. Espagnol, IFIP
Animals' feeding contribute very significantly to the overall environmental impact of animal products. The French project ECOALIM aims to improve the environmental impacts of husbandsries by optimizing their feed. This project to improve the environmental impacts of husbandsries by optimizing their feed. This project to improve
the formulation of compound feed with environmental constraints in order to improve environmental footprint of animal products. Animal feeding optimizing could induce substitution from one crop product to another into the formulation. In the meantime, it is difficult to allocate the impacts of a production system to each crop in a crop rotation because emissions depend on practices implemented on the whole crop rotation and not only a single crop. The method to allocate emissions between crops and its results on 12 case studies are discussed here. Specific methodological developments were made in particular to allocate nitrate (NO<sub>3</sub>) and phosphorus (P) losses between crops. NO<sub>3</sub> leaching due to the nitrogen (N) fertilization of one crop depends on the applied quantity but also on the intercrop management and on the following crop. As P is immobile in the soil, some farmers use residual nutrients by applying P fertilizers to one crop only in quantities optimal for off take and needs of following crops. Hence, after assessment of different allocation rules, NO<sub>3</sub> was allocated equally between crops within a same rotation and a specific rule was defined to allocate P in function of off take and needs. Organic N was allocated according to the rule used for Agribalyse. For each case study, LCA was applied on a basic scenario and on 3 crop management scenarios, aiming to reduce environmental impacts related to loss of nutrients and use of mineral fertilizer: 1) introduction of intermediate crops, 2) introduction of legumes in the rotation, or 3) organic fertilization (with 2 options for the chosen organic fertilizers in each case study, 3-1 and 3-2). LCA results show that if these rules seem pertinent to allocate benefits of some crop management between crops, they raise questions for some others (cf table 1). Finally, these results represent a step forward in order to take into account system production improvements in LCA of single crop but also show that some developments are still required as results obtained with a same method are very variable in function of hypothesis.
TU261
Biomarker analysis in soils of the Amazon rainforest after vegetation fire J.B. da Costa, UFRGS / Analytical Chemistry; D.P. Dick, Federal University of Rio Grande do Sul / Physical Chemistry; M.O. Lima, L.Z. Lara, M.R. Peralba, Universidade Federal do Rio Grande do Sul; P.d. Costa, Embrapa Acre Biomarkers, also known as molecular markers, refer to compounds that have a relationship with their precursors and indicate the occurrence of a particular process within an organism. Recently, the evaluation of biomarkers in plants or soil has become a more common procedure, since the abundance of these compounds is sensitive to changes in the soil organic matter (SOM) caused by the treatment and management of this fraction compared to the SOM content of the soil aliphatic structures and may give information about the source of the organic matter present in soil (microbial origin or higher plants) and is therefore called biomarkers. Studies on soils biomarkers can contribute to the understanding of the dynamics and stabilization of organic matter, which is of great importance from an environmental point of view. The objective of this study was to determine the origin of organic matter incorporated in Amazon forest soils subjected to vegetation fire by analyzing the biodegradable fraction of lipid extracts of soil samples. The study area is located at Embrapa-Acre in Rio Branco, Acre/Brazil and consists of primary forest which was partially burned in 2011. Samplings were conducted in September 2012 (one year after burning) and September 2014 (three years after burning) in primary forest areas (PF) and burnt forest (BF) at two depths (0-5 and 100-150 cm). Approximately 4 g of sample was used for lipid extraction. The samples were extracted via soxhlet with dichloromethane/methanol (3:1 v/v) for 24 hours. Completed the extraction step, the lipid extract was concentrated in rotary evaporator. The extracts containing lipids were then subjected to fractionation using preparative liquid chromatography atmospheric pressure. The distribution of n-alkanes was determined by GC/MS. The SOM of the primary forest comprises contributions of microbial moieties and of higher plants along the whole profile. This similar distribution pattern of n-alkanes indicates that a percolation of SOM fragments produced at the surface to the deeper layers occurred. After vegetation burning, a lipids fraction original from higher plants are fragmented in smaller chains, enriching thus the proportion of C<sub style="font-family: arial, helvetica, sans-serif;">24</sub>-alkanes was
249
enriching thus the proportion of C<sub style="font-family: arial, helvetica, sans-serif;">24</sub>-alkanes was
life cycle. Within the frame of this project, Isohemp (BE) hemp concrete bloc impact is evaluated in a cradle-to-gate LCA. Functional unit is a pallet of hemp concrete blocs ready for shipping. It represents about 1.3 m<sup>3</sup> of blocs. Hemp blocks are made by pressing a mix of hemp shives, hydraulic and hydrated limes, and water. Long term carbon storage due to lime carbonation induces a large benefit in Climate Change (CC) category. The balance of the CC indicator for hemp cultivation is also favorable due to carbon dioxide uptake by the photosynthesis occurring during the plant growth. Life Cycle Assessment of hemp concrete blocs ready to ship has also highlighted some improvements that can be easily made at the design and engineering level in order to improve the global environmental impact and increase the sustainability of this insulation material. Data are processed in SimaPro 8 software, with Ecoinvent 3 and ELCD 3 databases, and analyzed with the CML 1A method. This method is compliant with the indicators required by EN15804 standard in order to communicate on the environmental performance of Isohemp blocs.
TU263
A regionalised life-cycle analysis structure for sustainability assessment of bioenergy systems K. Aliyeva, University of Exeter / Renewable Energy; P. Connor, T. Taylor, X. Yan, University of Exeter sustainability assessment of bioenergy systems is often challenged by various uncertainties including spatial variables. Life-cycle assessment (LCA) is a reliable methodology for sustainability assessment and it has been applied extensively to bioenergy and agricultural systems. The sustainability assessment is capable of taking into account environmental repercussions of the whole life cycle of bioenergy production, but the results are often only valid for specific case studies or too general to be used for regional decision making. By enhancing the generic structure of LCA and expanding the current datasets, we are proposing a more efficient procedure which is capable of producing region-specific results. The suggested framework includes techniques for making the life cycle inventories more relevant by regionalised compilation procedures. In addition, the framework integrates improved selection of appropriate impact assessment models. This methodology can specifically be used by local stakeholders or decision makers, who are considering producing energy biomass, as a guide for improving the environmental performance and efficiency of their activities.
the use and management of soil. The content of this fraction comprises much of the process within an organism. Recently, the evaluation of biomarkers in plants or humans has been used to assess these changes.

Finally, these results represent a step forward in order to take into account system dynamics. The method can be considered as an original contribution to the field of environmental science.

TU264

Influence of crop management on water use of agricultural products. Case study: soybeans (Glycine max) in conventional tillage versus no-tillage in Argentina

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The aim of this study is to analyze the influence of tillage systems in assessing consumptive use of water in agricultural products. A new procedure which allows the incorporation of the water balance of the soil on the green and blue water footprints calculation is presented. For this, an adjustment of crop evaporation-inspiration and surface runoff is performed, taking into account the effects related to the presence of stubble, crop cover and surface roughness. The case study of soybean (Glycine max) in conventional tillage and no-tillage in Argentina constitutes the first application of this procedure. The results confirm that no-tillage reduces soil evaporation, prolongs the water storage period in the soil, delays runoff and increases crop yield. "Cleaner" crop systems allow the improvement of the yield and the water availability to satisfy crop needs and therefore increases the consumption of green water. The increased water availability increases the crop productivity, resulting in a diminution of green water footprint. If the soybean is grown under irrigation, the increase on the green water consumption corresponds to blue water savings. The considerations of tillage systems in assessing green and blue water footprints would be highly relevant in products derived from crops whose productivity is easily influenced by hydric stress through the growing cycle. Likewise, tillage effects on the green and blue water footprints would be of great magnitude for crops grown in regions with regular rainfall, or where the periods of greatest rainfall take place during the stage of crop development.

TU265

Modelling agricultural production: systematic analysis of secondary Life Cycle Inventories

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The analysis of agricultural production with life cycle based methodologies is very data demanding. In order to build comprehensive life cycle inventories, the use of secondary datasets is a common practice when primary data are not available. However, different inventory data and modelling approaches are used to populate secondary datasets, leading to different results. This can limit the perception of the reliability of the LCA tool especially when it is used for comparison purposes, e.g. among different food products and competing approaches. We analysed the features of secondary datasets for agricultural production with the aim to identify how different inventory data and modelling choices can influence the LCA results. The outcomes of the analysis can support LCA practitioners in the selection of secondary datasets and proper interpretation of the results and can contribute to the challenge of defining a common modelling framework for agricultural production. Wheat grains production in France was assessed using three datasets from different databases (Agricultfoot v1.0, Ecoinvent v3.1 and Agribalyse v1.2). Firstly, we compared the system boundaries and the general assumptions. Secondly, we focused on the foreground systems by comparing inventory data, data sources and modelling approaches. Thirdly, we performed a comparison de ans analysis of the impact assessment results to identify those modeling choices that contribute the most to differences in the results. We identified and assessed nine relevant elements: definition of system boundaries and modelling of agricultural practices; characteristics of inventory data; agricultural operations modelling; fertilisers’ application and fate; plant protection products application and fate; heavy metals inputs to the agricultural system and fate; irrigation assumptions; land use and transformation. These elements included crop-specific data, such as the amount of inputs provided to the field, and database-specific modelling approaches, common to all the datasets modelling agricultural production within the database. The three datasets differed greatly with respect to those elements. Hence, we drew recommendations from the analysis of database-specific modelling approaches, supporting LCA practitioners in the selection of the datasets coherently with the goal and scope of their study and in the interpretation of the results and fostering the development of common rules to model agricultural production.

Ecotoxicological assessment and water quality monitoring in support of marine and freshwater legislation in Europe (P)

TU266

PAH exposure, genotoxicity and histopathological alterations in Scottish flatfish

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The UK has long been committed to monitoring and assessing the quality of the marine environment, and setting the vision for its seas to be Clean, Safe, Healthy, Biologically Diverse, and Productive. Along with other countries, the UK reports marine monitoring data to international bodies such as the International Council for the Exploration of the Sea (ICES) and the OSPAR Convention for the Protection of the NE Atlantic which will be used in assessments under the EU Marine Strategy Framework Directive. As part of the Scottish monitoring programme flatfish blood samples were collected from the Firth of Forth, Moray Firth and offshore Scottish east coast and the North Minch. Samples were assessed for the presence of micronuclei, nuclear buds and binucleated erythrocytes and the results compared to observed liver histopathologies and exposure to polycyclic aromatic hydrocarbons, as indicated by biliary 1-hydroxypropane concentrations; the significance of the data was assessed using OSPAR Assessment Criteria. The differences between polychaete and flatfish 1-hydroxypropane concentrations and the histopathological response, when differentially examined, alongside differences between European plaice (Pleuronectes platessa) and common dab (Limanda limanda). The highest biliary PAH concentrations and greatest frequency of micrornuclei erythrocytes were observed in flounder from the industrialised lower Forth estuary; early toxicopathological lesions and foci of cellular alterations were also more commonly observed in fish from the Forth. PAH exposure and toxicological responses were lower in offshore dab, particularly those from the North Minch (NW Scotland).

TU267

ANALYSIS OF COMMUNITY-LEVEL MESocosM DATA BASED ON ECOLOGICALLY MEANINGFUL DIsSIMILARITY MEASURES AND DATA TRANSFORMATIONS

C. Tebby, INERIS / METO; S. JOACHIM, INERIS / CIVS; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; J. Porcher, INERIS / INERIS UMRI SEDIO ECOT; S. ANDRES, INERIS / Toxicological Ecotoxicological Assessment of Marine Substances; R. Beaudouin, INERIS / Models for Ecotoxicology and Toxicology METO

The Principal Response Curves (PRC) method is a constrained ordination method developed specifically for the analysis of community data collected in mesocosm experiments which provides a friendly summary of the results, and at the same time allows for the extraction of patterns in the data that are otherwise masked by the representation of the species in the datasets. The PRC method is based on redundancy analysis (RDA) usually performed on log-transformed abundance data. The log-transformation is used to lower the weight of the most abundant species in the analysis and implies that the variations in abundance are scaled to the total abundance of each species. Many different measures to calculate dissimilarity between samples on the basis of the abundance of each species have been developed, in particular in the field of ecology. The measure of dissimilarity between samples and the data transformations have a very large impact on the results of the multivariate analysis. The Euclidean distance between samples is implicitly used in PRC, but in the field of ecology, many more dissimilarity measures are currently used. Distance-based redundancy analysis provides a basis for integrating more ecologically meaningful distance measures into the PRC than Euclidean distance alone. In this paper, we investigated the ordinations produced with a small selection of ecological meaningful dissimilarity measures, namely the Euclidean distance on log-transformed data, the Hellinger distance, and the Bray-Curtis dissimilarity on raw and log-transformed data. We compared unconstrained ordination as well as PRC results obtained with these dissimilarity measures for two different macro-invertebrate community datasets, resulting from experiments performed with anti-inflammatory drug diclofenac and the insecticide chlorpyrifos. In both cases, the ordinations obtained with unconstrained ordinations and RDA were similar, indicating that the time effects had been partialled out, the treatment effects were the major source of variability in the data. In the diclofenac dataset, the Hellinger distance produced interesting significant results that completed those obtained with the Euclidean distance on log-transformed data. The chlorpyrifos dataset showed similar results for the different dissimilarity measures, with slight differences in the number of significant dates. The fraction of the total variance belonging to between replicate and time-dependent variation appeared to be an important criterion in the selection of a dissimilarity measure.

TU268

A complete pesticide monitoring in small Swiss rivers over a growing season applying a screening method and a continuous sampling strategy

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Agricultural pesticides are regularly found in surface waters at concentration levels that raise ecotoxicological concerns. Due to large fluctuations in concentration over time and the potentially high number of pesticides in agricultural watersheds, it is difficult to obtain a comprehensive overview of the influence of the actual pollution level. This collaborative project between research and Swiss federal and cantonal authorities aimed for a comprehensive analysis of pesticide pollution in five small agricultural streams to address this knowledge gap. The five rivers are located in catchments with intensive agriculture covering a wide range of crops, such as potatoes, vegetables and orchards. Twelve-hour composite samples were collected continuously from March until the end of
August with automatic sampling devices, resulting in 360 samples per site. Using precipitation and water level data, we differentiated between discharge events and low-flow periods. Samples taken in dry weather between rain events were pooled for the analysis. This procedure resulted in a complete concentration profile over the entire monitoring period covered by 60 samples per site. The analysis, using liquid chromatography coupled to high resolution mass spectrometry (Orbitrap technology), involved a target screening of 248 pesticides including fungicides, herbicides, insecticides, as well as important transformation products. Data on the total number and distribution of pesticides, their detection frequency, crop specific applications and concentration time profiles will be presented. Preliminary results indicate the potential of low discharge samples as a tool to detect and quantify: it was observed in sticklebacks, sediment and watercress for the first time in these systems.

This article presents the results of a literature review performed with a multidisciplinary approach to evaluate the impact of the growing use of these compounds. The present study shows the importance of the design of some public policies on the very scientific research and/or publication process. Policy makers have to be aware of this potential bias when designing new policies. A hierarchy of potential biases develops over time, from the ‘most likely’ bias to the ‘worst case’ bias. Each case has a potential bias linked with the geographical location of authors, the Funnel graphs reveal the existence of an asymmetrical publication bias (PB) of NA vs. EU studies. This PB is then statistically tested by proposing an adaptation of the Funnel Asymmetry Test (FAT) of Stanley (2005). This test reveals a systematic bias of NA studies results under the minimum thresholds for life cycle GHG emission savings specified by the RFS2, which is not the case for EU/Studies. This result is of primary importance as it highlights the influence of the design of some public policies on the very scientific research and/or publication process. Policy makers have to be aware of this potential bias when designing new policies.

No effects of Polyoxometalates Encapsulated in Silica Nanoparticles in the soil invertebrate Enchytraeus crypticus
R.C. Bicho, CESAM University of Aveiro / BioScience and CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; H.J. Nogueira, Universidade de Aveiro / Department of Quimica; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. Polyoxometalates (POMs) are metal-oxo clusters that have been investigated for many years in many fields including catalysis, art, medicine, and neuroscience. Despite the fact that effects were observed for concentrations higher than current regulatory levels, nevertheless long-term exposure can have negative effects on biotic communities due to their intrinsic biological activity. Diclofenac (DCF) is one of the most frequently detected human pharmaceuticals in water and it has lately been included on the “watch” list of the European Union. However little data is available on the detection of this substance and its transformation products in aquatic environments. In this context, analytical methodologies based either on QuEChERS or on ASE extraction followed by LC-MS/MS analysis were developed to quantify traces of DCF along with nine of its biotic and abiotic transformation products in bivalve, fish, sediment and watercress collected from lotic mesocosm experiments. The analytical protocol was first independently optimized and validated for each matrix of interest: sticklebacks, zebra mussels, sediment and watercress. For the optimization steps, uncontrolled matrices, corresponding to the matrices introduced into the mesocosms before the contamination with DCF were used. Considering the great diversity of transformation products and metabolites, a focus was made in this study on chemically stable and commercially available substances. Next target compounds were measured in samples collected from 6-months mesocosm experiments where DCF was continuously introduced in triplicate channels at three concentrations (5; 0.5 and 0.05 μg/L) and three more channels without DCF were used as control ones. DCF was quantified in zebra mussels and sediment for both highest exposure conditions and in watercress solely for the highest concentration. In sticklebacks DCF recovery was found to be lower than other compound limits probably due to rapid depuration as fishes were collected one week after the end of experiments. Among the selected metabolites, 4’OH-DCF was the most often quantified: it was observed in sticklebacks, sediment and watercress for the highest exposure concentration. The metabolites DCF-lactum and 5-OH-DCF were also measured as well as the 2-indole either in sediment, watercress or bivalves. In this presentation, the optimization of the extraction and clean-up steps will be described. The results of the quantification of DCF, along with nine of its degradation products and metabolites, in organisms and watercress collected from mesocosms will be presented.

Science Integrity and Publication Bias (P)
TU270
What if Science follows Policies? The case of advanced biofuels
L. Patoullidou, CIRAIQ - École Polytechnique de Montréal; B. Cheze, IFP Energies nouvelles / Economics and environmental evaluation department
This article presents the results of a literature review performed with a meta-regression analysis (MRA) that focuses on the estimates of advanced biofuel GHG emissions reductions with a Life Cycle Assessment (LCA) approach. 47 LCA studies are included in the database, providing 593 estimates. Each study estimate of the database is characterized by i) technical data/characteristics, ii) author’s methodological choices and iii) typology of the study under consideration. 82% of these GHG emissions results from North American (NA) authors are compliant with their more restrictive GHG emissions minimum threshold whereas it is the case only for 59% from European (EU) authors. This systematic difference between NA and EU observations may come from the use of a different set of technical variables, for instance, but it may also reveal the existence of a potential publication bias in the literature. Then, we conducted a MRA in order to identify the key factors influencing the results. Among others, we set the geographical location of authors as well as some technical data (type of technology, mass yield, etc) as explanatory variables. Results show that the geographical location of authors always has an influence on the LCA GHG emissions and that NA authors are always lower than EU. Investigating more in-depth a potential bias linked with the geographical location of authors, the Funnel graphs reveal the existence of an asymmetrical publication bias (PB) of NA vs. EU studies. This PB is then statistically tested by proposing an adaptation of the Funnel Asymmetry Test (FAT) of Stanley (2005). This test reveals a systematic bias of NA studies results under the minimum thresholds for life cycle GHG emission savings specified by the RFS2, which is not the case for EU/Studies. This result is of primary importance as it highlights the influence of the design of some public policies on the very scientific research and/or publication process. Policy makers have to be aware of this potential bias when designing new policies.

Tendency towards higher complexity in environmental risk assessment of Plant Protection Products: to accept or to
avoid? (P)

TU273
Sequences of pesticide applications: Is the prospective RA representative for the
agronomic reality?
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Ecotoxicology; L. de Baan, Agroscope / Institute for Plant Production Sciences
IPS; A. Aldrich, Research Station Agroscope ACW / Ecotoxicology; B. Fulda,
Agroscope; D. Danzi, Research Institute for Biodiversity Production Science
Cons ICP
The prospective ecotoxicological risk assessment (RA) conducted during the
registration of plant protection products (PPP) is a complex procedure, based on
tiered assessments of toxicity and exposure. The exposure profile is directly
affected by the application pattern of the PPP by the farmer. Thus, in order to
assess the ecotoxicological risks of PPP, information on the PPP application is
needed. Application parameters, i.e. application rate, number of applications and
interval between applications, have to be established by the regulatory authority
for each registered indication, i.e. use of a PPP on a specific crop and pest.
Application parameters are established based on the following criteria: ensuring
effective treatment of pest and reducing resistance risk, while keeping the risk of
humans and the environment in an acceptable range. In this study we investigated,
whether the prospective environmental RA is in accordance with the agricultural
practice. Since 2009, data for PPP use of approximately 300 farmers are recorded
within the framework of agri-environmental monitoring (AEM) in Switzerland.
Specific information per lot of land is being collected about the actual use of PPP,
including application rates, treated crops and application times. Based on this data
we set the application of PPPs, considering application rates, number of
application and intervals between applications. These data from the AEM were
compared to the authorised uses of PPP. First results indicate a high variability in
the analysed AEM application parameters. However, despite their heterogeneity,
the AEM data seem to largely be in accordance with authorised uses, considering
parameters set in the registration such as application rates, numbers of applications
and intervals. A high complexity of a RA is only justified if it does reflect the
reality better than a less complex RA. However, by stating the parameters along
with the registration a higher transparency and reliability may be achieved.
General questions as to whether further standardisation and standardisation of application
parameters is necessary or feasible and how sequential PPP applications could be
considered in the registration are discussed.

Chemical Transport via the Global Food System (P)

WE001 Development of a method to extract chemicals from food for toxicity testing
K. Muttert, RWTH Aachen University / Department of Ecossystem Analysis; P.A. Neale, F. Leusch, Griffith University / Smart Water Research Centre
Many environmental chemicals are known to be present in or on food. In
particular, low income countries can suffer from the application of mostly banned
and restricted pesticides in agriculture, combined with misapplication, which can
lead to high food contamination. Owing to potential human health concerns,
residues in food are generally identified with targeted chemical analysis. There is
increasing awareness of assessing the biological effects of contaminants present in
food, but few studies have applied in vitro bioassays for monitoring food based
chemicals. To overcome this problem, the present study focuses on the
development of a simple extraction method for bioanalytical testing of chemicals in food. The bioassays were selected throughout the cellular toxicity
pathway, including induction of xenobiotic metabolism and cytotoxicity. The Chemically Activated Fluorescence gene expression assay (AhR-CAFUX) detects compounds activating the xenobiotic mechanism via
activation of the arylhydrocarbon-receptor (AhR). While this assay is typically
used to screen for dioxin-like compounds, a set of other compounds, such as
polychlorinated biphenyls (PCBs) and some pesticides, are known to activate the
AhR. To screen for non-specific acting toxicants, the bacterial
luminescence toxicity screen (BLT) assay was applied. This assay reflects the
response of all chemicals present in a sample, causing different damages on
bacterial cell viability. Liquid-liquid extraction, which was selected due
to reduced solvent demand and rapid sample treatment, was applied for a range of
types of food. Additionally, washing water was also screened for chemicals to assess
food processing. Regarding extraction efficiency, the method seems
to be more efficient for high watery samples (strawberries, tomatoes) instead of
fatty (fish, meat) and high fiber (apples) samples. Hence, higher biological
effects were obtained for watery samples in the studied assays, including response of
the washing water. Future work should focus on method improvement of the
liquid-liquid extraction to address the urgent need of a reliable
hazard assessment of food for low income countries including toxicity issues

WE002 AMINOGLYCOSIDES ANTIBIOTICS RESIDUES ANALYSIS IN
BOVINE MILK AND BOVINE, SWINE AND Poultry MUSCLE BY

LC-MS/MS AND LC-QTOF-MS: A SIMPLE AND FAST NON SPE
METHOD
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The presence of several different substances in animal products has been a major
public concern. The use of antibiotics drugs as veterinary medicines might lead to
the development of bacterial resistance, which might undermine the efficacy of
drugs in human use. The Brazilian government implemented a control plan
that assesses the residues of veterinary medicines in animal products. The
aminoglycosides are antibiotics that have been extensively employed in animal
husbandry for the treatment of respiratory and urinary tract infections, as well as
some infections caused by chemically stable and commercially available substances. Next target compounds
were developed for determination of ten aminoglycosides (spectinomycin, tobramycin,
gentamicin, kanamycin, hygromycin, apramycin, streptomycin, dicyclomycin
dihydrostreptomycin, amikacin and neomycin) in bovine milk and bovine, swine
and poultry muscle. A simple, fast, cheap and ecofriendy extraction method with
non SPE steps was developed using trichloroacetic acid and clean up with low
temperature precipitation and C18 bulk. A 2° factorial design with center point
was performed to assess changes in the steps of the extraction process and the
best condition was adopted. The LC–MS/MS and LC-QTOF-MS methods were
developed for determination of ten aminoglycosides. The metabolites DCF-lactam and 5-OH-DCF are
probably due to rapid depuration as fishes were collected one week after the end
of experiments. Among the species, the sticklebacks DCF resulting concentration was inferior to our quantification limits
been included on the “watch” list. This article presents the results of a literature review performs with a
systematic approach. 47

WE003 Levels of pharmaceuticals and endocrine disruptors in commercially
available seafood before and after cooking
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Research has shown that eating fish and shellfish regularly is beneficial to our
bodies in many ways. However, it can also be a source of harmful environmental
contaminants with potential to impact negatively on human health. The presence of
“contaminants of emerging concern” like pharmaceuticals and endocrine
disrupting compounds in seafood for human consumption needs to be monitored.
Before this study the effect of cooking and processing on contaminants levels in
seafood becomes a relevant issue since it has been observed that an increase in
the concentration may occur. Therefore, the main objectives of this study were
to detect the possible presence of pharmaceuticals and endocrine disruptors in
seafood, to investigate the effect of cooking on contaminants levels, and to
establish whether the measured concentrations were within the maximum levels set
by the European Union. Besides, a risk assessment was performed in order to
evaluate the human health impact of the contaminants present in seafood. An
European sampling survey of seafood collected in different European regions have
been carried out. Several commercial seafood species including seabream,
mackerel, tuna, salmon and shellfish have been sampled in different seasons
and geographical locations (Spain, Italy, Portugal, Denmark, Norway, Netherlands and France). Based on previous results a list of
priority PhACs and EDCs was targeted. The presence of compounds such as
diclofenac, diazepam, sotalol, carbamazepine, cilastrol, fentanyl, azithromycin and sulfamethoxazole, TBEP, bisphenol A, and tricosanol
were researched. In the majority of the samples analysed PhACs compounds were not
detected or below method quantification limits. In few occasions positive
identification of some pharmaceuticals was done reaching up to 11.7 ng/g dry weight of sulfamethoxazole in mussels from Netherlands. Regarding EDCs’ methylparaben, triclosan, and bisphenol A were frequently detected in seafood.

Besides, an increase in these contaminants levels was observed after cooking by steaming. The highest level measured corresponded to bisphenol A in canned tuna reach up to 646 times higher compared to its levels in seafood (methylparaben, triclosan, and bisphenol A were selected for performing risk assessment, and for researching whether intake of these contaminants through seafood consumption involves a risk for human health. This piece of research is currently ongoing and results will be presented at the meeting.

WE004 Heavy metals in freeze dried milk from Brazil

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The production of milk is one of the most important rural activity involving and more than 30 billion of liters being produced each year. Essential food for different but important population groups like children and the elderly, milk is an invaluable source of important micronutrients like zinc, copper and iron. However mineral supplementation or even corrosion in metallic equipment may also represent a source of non-essential and toxic elements, like mercury, cadmium and lead that may sometimes be a route of contaminants to human beings.

Since soil particle ingestion is frequently observed during cattle grazing, our objective with this study is to determine if the levels of these elements in freeze dried milk can reveal a route for the transference of the selected metals to man.

Around two grams of freeze dried milk were analyzed in triplicates after catalase hydrolysis [45]. A question in this study was that all analyses were performed at the Radioisotopes Laboratory of the Biophysics Institute of the Federal University of Rio de Janeiro. In spite some cases of contamination by Fe in some imported brands of milk in powder; the recurrent problem in Brazil is milk adulteration by addition of caustic soda and formaldehydes in the production of UHT milk. As expected, there is a higher contribution of Zn in the overall results, indicating once more that milk is an important source of this micro-element. Although most farms are equipped with stainless steel devices for refrigeration and storage, the use of less noble steel more found of corrosion can be considered a source of toxic metal contamination, especially Ni and Cr. As for Pb, this metal may be present in antimonial or paint. In the present study, with only few exceptions for Cr and Pb, the determined values were not higher than the provided maximum allowance for the agricultural Brazilian legislation. In the absence of new voltametric and anodic stripping methods for direct measurements of the metallic contents of milk, the so-called old fashion methods are still a choice and can help in the characterization of the inorganic contamination of dairy products.

More research is needed to clarify the relation on proper maintenance of metal equipments, soil contamination and milk quality in order to acquire better standard production parameters for milk source quality confirmation, in the view of an organic farmer green product verification/certification.

WE005 UV filters maternal transfer through breastfeeding

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Organic UV filters are the preferred protection against adverse effects of UV radiation in all kind of cosmetics, as well as in common materials and industrial products. This fact leads to a continued human exposure. Many studies show the tendency of UV-F to bioaccumulate in living organisms due to their lipophilicity and stability versus biotic degradation [1]. The extended use and the ubiquity of these compounds in the environment make necessary studies on the bioaccumulation in human tissues and elimination from the body of these compounds through biological fluids. Breast milk is a biological fluid that has a fundamental role in new born nutrition and has direct impact on growth, neurodevelopment and health [2]. Breast milk is a compositionally variable fluid that is known to change during the lactation period, and it is highly influenced by maternal dietary intake and life style. In this framework, the aim of this study was to assess the potential maternal transfer of UV filters to newborn through breastfeeding.

BPs, BP1, 4HB, 4DHB, OD-PABA, OC, EHMBC, E1-PABA, BZT, TCP, BPB, UV345 and UV4 were investigated. Breast milk samples, provided by mothers from Barcelona were first extracted by USE and internal standard addition, analysed by Turbulent Flow-Chromatography-HPLC-MS/MS. Results indicated that 7 out of the 14 UV filters investigated were detected in the 79 milk samples analysed. 28 samples were positive in at least one compound. BP3 was the most frequently detected compound (68%) and the one observed at the highest concentration, 1599.8 ng/g milk. Two human metabolites of BP3, 4HB and 4DHB, were also identified in the samples. Finally, we concluded that breast milk samples contained a number of UV filters, mostly BP3 and two of its human metabolites, which suggest that not only UV filters are accumulated but also the more polar metabolites formed. Direct breastfeeding is one of the maternal “own contaminations” that transfer it to the neonate. References [1] Kim JW, Ramaswamy BR, Chang KH, Isole T, Tanabe S. 2011. J Chromatogr A 1218: 3511-3520. [2] Miller, E. M.; Aiello, M. O.; Fujita, M.; Hinde, K.; Milligian, L.; Quinn, E. A. Am. J. Hum. Biol. 2013, 25, 1–11. Acknowledgement - The authors thank the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”) and MINECO (Project: Transferencia de filtros solares UV madre-hijo a través de la lactancia materna (SOLA)).

WE006 Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Breast Milk from Serbia First-Time Mothers

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Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are two highly lipophilic classes of persistent organic pollutants (POPs) able to resist degradation and with the ability to bioaccumulate through the food chain. The international community has responded to the threat from POPs by negotiating a global treaty, the Stockholm Convention on POPs, with the objective of protecting human health and the environment from POPs. Human milk may provide a significant source of exposure to POPs in early life, and data on levels of POPs in breast milk from different regions of the world have been generated in many previous known. However, the determination of POPs in milk of Serbian mothers, especially concerning PBDEs. Objective of study was to investigate contamination of PBDEs and PCBs in breast milk from Serbia. A total of 11 breast milk samples from the first time mothers were collected in Department of Neonatology in Belgrade, Serbia from November 2014 to April 2015. Total lipids from breast milk samples were extracted by Soxhlet method (ISO 8262-1) and subsequently the PCB and PBDE congeners were analyzed by HRGC/HRMS. Average concentration of PBDEs in samples was 753.8 ng/g fat, with highest share of BDE #47 more than 40%. Average concentration of PCBs in samples was 79.8 ng/g fat, with highest share of PCB #153 more than 14%. The PBDE (#118, #47, #49, #100, #135 and #154) and PCB (#28, #52, #77, #81, #101, #105, #114, #118, #123, #126, #138, #152, #156, #157, #167, #168, #180, #189) contamination in breast milk as well as congener profiles was comparable to those in other neighbour countries in region. These results represent first published data of PBDE contamination of human milk in Serbia. Considering human milk’s nutritional and immunologic effects, society must take steps to decrease human exposure to these chemicals by restrictions in their usage, while continue encouraging women to practice breastfeeding.

WE007 Using RISK21 matrix to assess the potential human health risk of DDT exposure from flower tea

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The Health and Environmental Science Institutes-coordinated RISK21 is an integrated evaluation strategy which provides a straightforward, efficient, systematic, and transparent way for health risk assessment, RISK21 takes advantage of existing information to graphically represent the comparison of exposure and toxicity data on a highly visual matrix, termed RISK21 matrix. Recently, the long-banned pesticide DDT (dichlorodiphenyltrichloroethane) and its metabolites were found in the rose leaves as the flower tea drinks sold at Taiwan’s popular tea chain store. The purpose of this study was to adopt RISK21 approach to assess the degree of health risk of DDT exposure from drinking flower tea or directly consuming rose leaves. Based on the foundation of risk assessment, we integrated the estimates of age-specific total daily intakes (TDI) of DDT with toxicity data on liver lesions and developmental toxicity to assess the level of health concern by RISK21 matrix. The TDIs through direct intake of flowers for various age groups were estimated based on the concentrations of DDT, DDD (dichlorodiphenyldichloroethene), and DDE (dichlorodiphenyltrichloroethylene) in rose leaves as well as age-specific intake rate of flower tea, whereas additional factors of compound-specific water solubility and dilution factor on the flower tea were taken into account to calculate the TDIs from flower tea drinks. By considering the uncertainties of TDI estimates and toxicity estimates, Risk21 matrix shows that drinking DDT-contaminated flower tea seems unlikely to constitute a public health and safety concern for all age groups due to the extremely low exposure level. However, the human health risk assessment DDT exposure and drinking flower tea reveals a conservative point of view in regards to direct intake of flowers. Risk21 matrix is an easy-to-use tool that not only promotes the understanding of risk assessment but also fosters the risk communication.

Interactions between traditional, novel and green carbonaceous materials and contaminants (P)
Polyaromatic hydrocarbons (PAHs) are organic contaminants which are harmful to human health and organisms. One of the source of these contaminants is industry e.g. coal and bitumen production. The emitted PAHs can migrate into soil where they can accumulate in biota and negatively affect organisms. Due to this, there is a need for providing efficient tool for remediation of industrially contaminated soils by PAHs. One of the current remediation strategy of contaminated sites is using a biocarbons for PAHs immobilization. To enhance the biocarbons PAHs reduction in contaminated soils, we supported soil remediation by nano zerovalent iron (nZVI), known from its effectiveness in the organic contaminants degradation. Therefore, two types of soils were investigated in this study: 1) KOK soil - sampled from the area of coking plant (Dabrowa Gómezica, Poland), and 2) POPi soil – sampled from the area of bitumen processing plant (Wólka Lancuchowska, Poland). Moreover, biocarb produced from the wheat straw (BCS) and nano zerovalent iron, were used. Each soil were amended by BCS or and nZVI in few variants: 1) by nZVI with a dose of 2 and 10 gEquiv/kgDm as a water suspension, 2) by biocarb BCS with a dose of 5% w/w and equal amount of water needed for preparation of nZVI suspension, and 3) by nZVI with a dose of 2 and 10 gEquiv/kgDm as a water suspension together with biocarb at a dose of 5% w/w. Pure soils samples amended with water as in 2) were taken as a control. Prepared samples were rolled end over end at 10 rpm for 7 or 30 days in dark and at room temperature. After 7 and 30 days, chemical and ecotoxicological analysis were carried out. Freely dissolved concentration of PAHs (C_{Freely}) was assessed by polyoxymethylene (POM) and ecotoxicity was determined using Vibrio fisheri bacteria (Microtox). Application of pure biocarb and biocarb together with nZVI to contaminated soils decreased a PAHs C_{Freely} and its potential environmental risk to the organisms. We also observed negative effect of decreasing bacteria bioluminescence after pure nZVI amendment to soils (especially in case of KOK soil). Nevertheless, the combined addition of biocarb and nZVI resulted in elimination of toxic effects of PAHs. Moreover, this variant in most cases showed the highest reduction of ecotoxicity and also provoked stimulation effects of bioluminescence in both soils.

**WE1009**

Supporting of PAHs bioavailability and ecotoxicity reduction by nano zerovalent iron (nZVI) and biocarb in industrially contaminated soils

**M. Koltowski, P. Olezczuk, Maria Curie Skłodowska University / Department of Environmental Chemistry**

The dual-mode model also proved useful to fit sorption isotherms and compare the contributions of solvent-driven and sorbent-driven processes (described by lineal and Langmuir model, respectively). Multivariate analyses were also applied to identify the factors driving the great differences in sorption observed. Overall, the results provide a better understanding of the effects that biocarbon properties have on sorption behavior and thus possibly identify groups of biocarbons, whose sorption behavior could be described using common approaches.

**WE101**

Adsorption of Polychlorinated biphenyls to activated carbons with different surface and textural properties

**Q. Spitalny, A. D. Netto, Universidade Federal Fluminense / Quimica; J. Torres, Universidade Federal Fluminense / Departamento de Quimica**

The extended use and the ubiquity of metallic equipments, soil contamination and milk quality in order to acquire better products. More research is needed to clarify the relation on proper maintenance of the metallic contents of milk, the so called difference reduction pathways. Applicability of Fe(0)-embedded biocarbons in natural and engineered systems was also discussed.

**WE1011**

Removal of 2,4-dinitrotoluene and 2,4-dichlorophenol with zero-valent iron-enabled biocarbons

**S. O., University of Ulsan / Department of Civil and Environmental Engineering; Y. Seo, University of Ulsan / Civil and Environmental Engineering**

The extended use and the ubiquity of metallic equipments, soil contamination and milk quality in order to acquire better products. More research is needed to clarify the relation on proper maintenance of the metallic contents of milk, the so called difference reduction pathways. Applicability of Fe(0)-embedded biocarbons in natural and engineered systems was also discussed.

**WE1012**

Effects of Carica papaya seeds biocarb and its Goethite modified amendments on Lead mobility in soil

**B.J. Olu-Owolabi, B. Agbagaag, O.A. Oluyinka, University of Ibadan / Department of Chemistry**

Presence of heavy metals (HM)-most especially Pb, in the environment has been worrisome because of its health implications on man and other biota. The labile fraction of HM in contaminated soils is however the most important. It is the portion that is readily available for plant uptake, thus accumulates in plants and eventually gets to humans who consume the plants. Therefore, any efforts that could lead to immobilization of HM in contaminated soils should be of utmost scientific interest. This study reports the effects of Carica papaya seeds biocarb (CB) and Goethite modified Carica papaya seeds biocarb (GCB) on the mobility of Pb in a contaminated soil from the abandoned dump site of the defunct Exide-battery Manufacturing Company at Lalupon, Southwestern Nigeria. Ten treatments pots (in duplicates) were set up with the soil sample. First pot contained the sample at 0% amendment, and this served as the control. The second, third and fourth pots were amended with CB at 2%, 5% and 10% levels respectively; the fifth, sixth and seventh pots were amended with 5% Goethite modified CB (5%GCB) at 2%, 5% and 10% levels respectively, while the eighth, ninth and tenth pots were amended with 10% Goethite modified CB (10%GCB) also at 2%, 5% and 10% levels respectively. All treatments were brought to the field capacity with deionized water, and then incubated at room temperature for 30 days. The field capacity moisture was maintained throughout this period. At the end of the incubation period, 1g of the sample was extracted with 1M MgCl2 solution and the Pb content was determined by AAS. The Pb content of the control was found to be 1680 mg/Kg. This was reduced by 80-89% by CB, 90-98% by 5%GCB, and 70-90% by 10%GCB. In conclusion, all the amendments significantly reduced the bioavailable Pb in the soil, with the 5%GCB being the best, followed by CB. This has opened further opportunities for similar researches with other agricultural biomass. Keywords: Heavy metals, Carica papaya, Biocarb, Goethite

**WE1013**

Sorption of substituted aromatic hydrocarbons by fullerenes - Influence of functionalization on molecular interactions

**T. Hiffer, H. Sun, M. Kah, T. Hofmann, University of Vienna / Department of Environmental Geosciences**

The interpretation and prediction of molecular interactions between organic compounds and carbon-based nanomaterials is of major importance to understand phase transfer processes in environmental matrices and to assess the potential implications of these materials as special sorbent materials. While there is a lot of sorption data available for carbon nanotubes, little information is available on the
sorption behavior of C60 fullerenes. In a previous study we have investigated the effect of UV-induced surface oxidation on sorption behavior of C60 [1]. Changes in surface chemistry led to significant alteration in both sorption capacity and affinity. The results indicated that in addition to non-specific van der Waals and electron donor-interactions, the presence of –OH and –COOH groups on C60 surface potentially allowed for the contribution of hydrogen bonds. However, sorption data for a much broader set of probe compounds, covering various chemical classes, are necessary to further investigate the contribution of individual interactions to overall sorption. To systematically study this process, the combined effects of –OH/NH2 functional groups of aromatic organic compounds and surface O/C ratios on the interactions were evaluated. We combined two multi-phase sorption batch experiment approaches using a passive sampling technique applying polyoxymethylene and a headspace in-tube microextraction. This allows for an systematic comparison of the interactions between C60 and organic compounds with significantly different physico-chemical properties (e.g., aromaticity, functional groups, etc.). [1] Höffler T., Kah M., Hofmann T., Schmidt T.C. 2013. Environ Sci Technol 47: 6935-6942.

WE014 Immobilization of organic contaminants and heavy metals by carbonized sorbents - mechanisms and prediction

G. Sigmund, H. Sun, T. Höffler, M. Kah, T. Hofmann, University of Vienna / Department of Environmental Geosciences

Carbonized sorbents typically comprise only a small proportion of the soil or sediment matrix. However, they do significantly enhance sorption affinity and capacity of contaminants. Hence, carbonized sorbents are commonly used for the immobilization of organic contaminants. In a contaminant group, different interactions drive sorption, and different parameters may be used to predict the sorption potential of a given carbonized sorbent for a given contaminant. Therefore, the aim of this study was to identify (i) interactions that drive sorption and (ii) key parameters for the prediction of sorption for neutral organic contaminants, charged acids and heavy metals. Hence, we combined comprehensive characterization of selected carbonized sorbents with data from sorption batch experiments for selected polycyclic aromatic hydrocarbons, organic acids and heavy metals. Generally, sorption strength (Ks, kg/L) followed: polycyclic aromatic hydrocarbons > heavy metals ≥ organic acids. We found that the immobilization of neutral organic contaminants can occur via hydrophobicity driven interactions and specific interactions (i.e., π-π electron interactions and H-bonds), and depends on various factors including the grade of carbonization and availability of sorption sites. The immobilization of organic acids can additionally occur via electrostatic interactions and cation bridging and depends on factors including ionic strength and solution pH. The immobilization of heavy metals can occur via specific and non-specific electrostatic interactions, exchange, and precipitation, and depends on factors including ionic strength, pH and redox-conditions. The sorption of neutral and acidic organic contaminants can be well predicted by combining parameters commonly used to describe sorbent and sorbate properties, i.e. specific surface area, and octanol-water partitioning behavior (Kow and DOW for neutral and acidic compounds, respectively). For the prediction of heavy metal sorption, a case by case evaluation is needed, due to the complexity of factors influencing speciation and precipitation processes. Our findings help better predict the sorption potential of carbonized sorbents to a large range of contaminant groups. For neutral and acidic organic contaminants we present a simple assessment tool for the potential use of carbonized sorbents in environmental applications.

WE015 Vicia-micronucleus test as a newly standardized tool to assess soil genotoxicity: Application to the evaluation of the effects of biochar in contaminated soils

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The risk assessment of industrial contaminated soils is an important challenge in remediation process. Information on bioavailability of pollutants requires the development of biological tests, especially with plants. Ma (1999) described higher plants as the most sensitive organisms for the detection of mutagens and genotoxic effects of polluted soils. However, the application of soil toxicity assessment, plants are surprisingly not the most commonly used organisms for genotoxicity tests (White and Claxton, 2004). This is the reason why genotoxicity tests with higher plants have been promoted (IPCS – United Nations Environment Programme, 1999). Genotoxity - simply defined as the toxicity on the genome - is an indicator of dysfunctions appearing at sub-lethal concentrations. An easy endpoint to observe is the formation of micronuclei, that are small nuclei appearing whenever a chromosome fragment or a complete chromosome is not incorporated into the nuclei during mitosis. This endpoint is very important to include in a battery of ecotoxicity tests for a better risk assessment of contaminated soils and of the impact of remediation techniques applied to them. Among techniques of in-situ remediation, soil amendments with biochar, i.e., the solid product from biomass pyrolysis, have recently been investigated for decreasing the bioavailability of metals in industrial soils. Biochar has been shown to immobilize metals both by direct sorption at its surface and by an indirect effect through an increase of soil pH (Rees et al., 2014). The effect of biochar on the actual genotoxicity potential of metal-contaminated soils is however unknown. The aim of this study was to test the genotoxic potential of a range of contaminants. This aim is drawn to the use of Vicia faba heavy metals (Zn, Pb, Cd) and amended by a wood-derived biochar to create a gradient of metal availability. We recently obtained international standardization of the Vicia-micronucleus test (ISO 29200) in 2013 and we performed it in this study by direct exposure of root tips to soils. The analysis of root morphology and root metal content completed the experiment. Results showed that these industrial soils induced genotoxicity, revealed by an increase of micronuclei frequency. Their genotoxic potential strongly decreased with the addition of biochar. Relationships between genotoxicity, soil metal extractability and root metal content will be discussed.

WE016 Modulation of metal bioavailability for two earthworm species by the use of biochar

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Anthropic activities may lead to depletion in soil organic matter contents and to soil contamination, particularly by trace elements. Biochars i.e. stable carbon-rich by-products synthesized through pyrolysis/carbonization of plant- and animal-based biomass, are used as soil amendments to improve carbon sequestration and crop yields while valorizing crop residues and stabilizing soil contaminants. The aim of this study was to assess the effect of a poultry manure-derived biochar on the bioavailability of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) for two earthworm species i.e. Aporrectodea icterica and Aporrectodea longa exposed in microcosms to an in situ contaminated soil during 28 days. For that purpose, we assessed (i) the environmental availability by measuring total concentrations and concentrations in pore water, (ii) the environmental bioavailability by measuring concentrations of trace elements in earthworms, and (iii) the toxicological bioavailability by measuring survival, weight gain, energy reserves (protein, lipid and glycogen contents) and the GST (glutathione-S-transferase) enzymatic activity of earthworms, as an indicator of detoxification processes. Contrary to what we expected, the presence of the studied poultry manure-derived biochar did not greatly influence the bioavailability of trace elements for earthworms under our conditions. We discussed assumptions to explain these results.

WE017 Modulation of trace element bioavailability, yield and seed quality of rapeseed (Brassica napus L.) by biochar addition to a contaminated technosol

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Rapseed (Brassica napus L.) is a Cd/Zn-accumulator whereas soil conditioners such as biochars may improve its growth and yield. These potentially complementary soil remediation options were trialed, singly and in combination, in a pot experiment with a metal(loid)-contaminated technosol developed on dredged sediments. The efficiency of these remediation options were assessed using both phenotypic and physiological plant responses. A trace element (Cd, Cu, Pb, Zn) contaminated technosol was amended with a poultry manure-derived biochar. Rapseed was cultivated under both soil treatments during 24 weeks up to harvest in greenhouse. Based on the soil pore water, biochar incorporation into the technosol promoted the As, Cd, Cu, Mo, Ni, Pb and Zn solubility. It decreased foliar B, Cu and Mo concentrations, and Mo concentration in stems, pericarps and seeds. Conversely, it did not impact neither the mass of aerial rapeseed parts (except a decrease for seeds), nor their C content (except a decrease for stems), seed fatty acid content, seed sugar content.
an interdisciplinary COST Action (FA TD1107) initiative
A. Bastos, University of Aveiro / Department of Biology Centre for Environmental and Marine Studies CESAM; X. Domene, CREAT-Universitat Autonoma de Barcelona / CREATUnit of Ecologia Università Autònoma de Barcelona; C. Kannmacher, Hannover; C. GEISENHÖR and Université de Lorraine Centre de Recherche pour l’Environnement et l’Aménagement RURAL (CERTHR); D. Nunez-Carrasco, CESAM, University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; R. Morgado, University of Aveiro / Department of Biology and CESAM; M. Prodana, University of Aveiro & CESAM / Department of Biology; T. Bucheli, Agroscope ART / Environmental Analytics Natural Resources and Agriculture; J. Hilber, Agroscope ART; H. Schmidt, Ifakara Institute for Carbon Intelligence; F. Verheijen, University of Aveiro / Department of Environment and Planning and CESAM; J. Keizer, Université de Lorraine / CESAM Department of Environment; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; S. Lounerie, Universidade de Aveiro / Biology

The use of alternative activated carbon derived from biomass, as agricultural and/or environmental management (0, 10, 50, 100, 200 t/ha). Avoidance behaviour, survival and reproduction bioassays were performed, with soil nematodes Eisenia fetida, Eisenia fetida and Eisenia fetida respectively. In adipoise tissue, a similar decrease has been observed. This study leads to conclude that 1/AC could dramatically reduce the bioavailability of CLD 2/ extent of this limitation depends of the nature of AC. This result obtained with artificial soils is considered as a first major step prior to on-field experiments.

WE021
Activated carbons prepared from plum, apricot and cherry kernels: Influence of operational parameters and elemental composition on the maximum adsorption capacity of products
M. Mork-Sekulić, University of Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; S. Pap, M. Brboric, M. Vojinovic Miloradov, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; M. Prca, University of Novi Sad Faculty of Technical Sciences / Department of Graphic Engineering and Design; Z. Djukic, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; J. Radonic, Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health

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Effects of activated carbon on PCB bioaccumulation and biological responses of Chironomus riparius

J. Nyborg, S. Abel, University of Eastern Finland / Department of Biology; G.C. Waissi-Leinonen, University of Eastern Finland; K. Vaananen, University of Eastern Finland / Biology; K. Maempaa, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute; J.V. Kukkonen, University of Jyväskyla / Biological and Environmental Science; J. Akkanen, University of Eastern Finland / Biology

Activated carbon (AC) amendments have been studied as a new stabilizing method for contaminated sediments. The sorption efficiency of AC toward hydrophobic organic compounds (HOCs) has been shown in several studies. Recently, the focus has been turned also to the possible secondary effects of AC amendments. At the present study the sorption efficiency and possible secondary effects of AC were studied with *Chironomus riparius* in a full life cycle test. Coal based AC (63-200 μm) was mixed in natural sediments from PCB contaminated area. AC amendment reduced the PCB concentrations determined with both modelled by Freundlich and Langmuir isotherms. Obtained results show that CNTs can be considered as superior to ACs in terms of sorption kinetics. Moreover sorption of ILS on CNTs is highly effective process. The sorption mechanism has been proposed. Acknowledgement „Program rozwoju Uniwersytetu Gdańskiego w obszarach Europa 2020 (UG2020)” project is co-financed by the European Union under the European Social Fund.

Remediation of PCB contaminated sediments using activated carbon: Assessing efficiency and robustness using equilibrium sampling

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When released into aquatic environments, polychlorinated biphenyls (PCBs) accumulate in sediments due to their low water solubility and association with sediment organic carbon. PCB contaminated sediments have been successfully remediated using activated carbon (AC) amendments, which strongly adsorb lipophilic compounds and thereby reduce their exposure/risk to benthic and pelagic organisms. For such remediation methods, it is important to carefully assess both the method efficiency in reducing exposure and the method robustness towards ongoing influx of contamination. In the present study, a series of mesocosm experiments were performed in order to reveal how AC amendments can be most effectively applied to reduce exposure and enhance the resilience of *in situ* sediment remedies challenged by ongoing influx of contamination. In these mesocosm-scale experiments, we evaluated adsorption and bioaccumulation rates of PCBs in sediments amended with activated carbon, (ii) covered by a sand cap, (iii) covered by an AC amended sand cap, and (iv) covered by a mobile AC amended sand cap. Half of the mesocosms received ongoing influx of contamination during the 3-months experiments. Careful exposure assessments were conducted before and after treatment in order to reveal how AC amendments can be most effectively applied in sediment remediation. As part of the exposure assessments, equilibrium sampling of the sediments were used to determine: (1) the freely dissolved PCB concentrations in sediment pore water (C_{free,pmol/L}), (2) the PCB chemical activities in sediment (a, unitless), and (3) the PCB concentrations in lipid at thermodynamic equilibrium with sediment (C_{lipid,pmol/kg}). The analysis dissolved concentrations were then perceived as the effective concentration for bioconcentration and toxicity, while chemical activity (a medium-independent exposure parameter that quantifies the energetic level, and not the concentration, of an organic compound relative to its energetic level in pure liquid [0-1, unitless]) governs diffusion and equilibrium partitioning. Both freely dissolved concentrations and chemical activities express the effective exposure as opposed to the more traditionally used measure of total concentration. Still, the main novelty of this approach is that C_{lipid-	ext{sediment}} can be determined in a practical and precise manner to express the thermodynamic potential for bioaccumulation (i.e. the concentration in the lipid of an organism at thermodynamic equilibrium with sediment).

Effects of biobar on the dynamic of metals in soils polluted by mining wastes under changing hydric conditions

The aim of the present study was to assess the effects of biochar and the presence/absence of vegetation on the fate of metals in wetland soils polluted by metal mining wastes to ascertain the effectiveness of these techniques on the improvement of soil conditions, the mitigation of the environmental risks associated to metal pollution. A column (15 x 30 cm) experiment with two types of soils (one with pH 4.7 and another one with pH 7.4), two types of biochar (one from municipal solid wastes -MSW- and another one from sewage sludge -SS-) and with the presence/absence of plants (halophyte species Sarcocornia fruticosa) was performed with alternating flooding/drying conditions inside a greenhouse. The water level was kept at 5 cm above the soil surface for one month and at 15 cm below the soil surface for the following month. This cycle was repeated several times along the experiment. The pH and redox potential (Eh) were regularly checked as well as the dissolved organic carbon (DOC) and soluble metal concentrations (Cd, Mn, Pb and Zn) in pore water. In the basic soil, the biochar application did not have any effect on soil pH. In the acidic soil the pH increased the pH from 4.5 to 6.0-6.5 while the SS biochar to ~5.5. The presence of vegetation did not have any effect on soil pH. The SS biochar greatly decreased the Eh values in both study soils due to a higher input of DOC and then probably to a greater microbial activity. In the acidic soil the higher pH due to biochar application led to a decrease in the soluble metal concentrations regardless the Eh values and the presence/absence of plants. In the basic soil the drop of Eh values due to the flooding conditions favoured higher soluble Mn and Zn concentrations probably due to the solubilisation of metal oxides. The MSW biochar favoured higher soluble Cd concentrations after one month of flooding although the concentrations decreased when decreasing the water level. The dynamic of Pb was similar among all the treatments assayed, regardless the biochar and the presence/absence of plants, and was conditioned by the flooding regime. The Pb concentrations in pore water increased during the flooding periods and decreased during the drying ones probably due to the metal immobilisation/mobilisation in relation to the dynamic of sulfur. The effectiveness of the treatments assayed (biochar application) depended on the soil type, the hydric regime and the presence/absence of vegetation.

Passive sampling of organic micropolllutants and toxicity assessment: opportunities, challenges and innovations (P)

WE027 Bioaccumulation of hydrophobic organic contaminants in mussels in the Istanbul Strait: Field monitoring by means of passive water sampling and modelling

S.D. Yakın Dündar, Istanbul Technical University / Faculty of Naval Architecture and Ocean Engineering; A. Feeks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team

Determination of the concentrations of hydrophobic organic contaminants (HOCs) such as PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls) from water samples is difficult due to the low water solubility, high lipophilicity, strong adsorption tendency to suspended materials and tendency to accumulate in organisms. Biomonitoring and passive sampling methods are alternative ways of assessing the sources and fate of HOCs in the marine environment. Bivalve molluscs and Semi-Permeable Membrane Devices (SPMDs) are widely used in monitoring studies to collect information about water concentrations of HOCs. The simultaneous consideration of concentration data of bivalves and SPMDs enables conclusions about accumulation of HOCs in bivalves, even without information about water concentrations, because of their similar uptake processes. In this study, field monitoring data from the Istanbul Strait is presented. The Istanbul Strait is a busy route for both national and international navigation and hence has high potential for contamination. Specimens of the Mediterranean mussel species Mytilus galloprovincialis were deployed and stayed together with SPMDs in the water column for up to 21 days. A published model was adapted and used to calculate deproportionation rate constants and accumulation factors for the mussel and a series of PAH and PCBs. Model interpolations based on separate PAH and PCB data match the trend in the data well, model interpolation using a hyperbolic fit of BAF factors show an inverse trend as compared to the single-substance models. Elimination rate constants are decreasing with Log Kow>26 values and can be fitted best by a hyperbolic function for the combined PAH and PCB data. In conclusion, bioaccumulation and deproportionation kinetics of HOCs in M. galloprovincialis can be modelled properly based on Log Kow. While deproportion constants show a clear hyperbolic trend, for the bioaccumulation factor’s dependency on Log Kow, it is not completely clear whether linear or hyperbolic regression fits best. Using the hyperbolic regression equations in interpolation of SPMD/mussel results in a completely switched response of Cb/Co ratios for changing Log Kow. Inclusion of more HOCs in monitoring studies will help to further investigation of this relation. Differences in the Cb/Co ratios for different exposure durations can be modelled, and the duration of future monitoring experiments can be decided effectively.

WE028 Calibration of Polar Organic Chemical Integrative Sampler (POCIS) in wastewater treatment plant, and pesticide monitoring in wastewater network

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Because of generalized uses of pesticides by agriculture, private consumers, industries or public authorities, pesticides are released into the environment, with potential affecting ecosystem. Many xenobiotics considered for some compounds. Wastewater via wastewater treatment plants (WWTP) have shown to be an important source of pesticides to the environment. Wastewaters are specific matrices, difficult to monitor and heterogeneous, with high amount of organic matter, variable water depths and high variability of pesticide concentrations. All these drawbacks can compromise the regular sampling of wastewater, so passive sampling seems to be a good alternative to high frequency grab sampling for pesticide monitoring. This study evaluated the potential of Polar Organic Chemical Integrative Sampler (POCIS) as medium term samplers. POCIS were calibrated in-situ, in a WWTP influent, and on a small peri-urban river, generating sampling rates for a large amount of pesticides. Some POCIS were also deployed on wastewater network and in the river for short term monitoring of pesticides, conducing to calculation of time weighted average concentrations. These experiments brought to light that the time of exposure in wastewater was short (about 3 days) for a quantitative purpose. Moreover the devices appeared to be not so appropriate in such environments due to solids that have been accumulated on them and could have led to a misestimation of time weighted average concentrations in wastewater. New designs of the sampler were tested in lab and on field to improve the sampling capacity of this disposable. A better knowledge of the contamination of micropolllutants in wastewater can lead to the identification of sources in wastewater network, reducing contamination at source, in the perspective of protecting water resources. Acknowledgement - This study benefited from funds in relation with RESEAU and “Plan Micropollutants” projects (associating as partners: Bordeaux Métropole, SUEZ, IRSTE A, Water Agency, CNRS, University of Bordeaux, CHU and Aquitaine Regional Council). This work was funded by the Aquitaine Regional Council and SUEZ. This study has been carried out with financial support from the French National Research Agency (ANR) in the frame of the Investments for the future programme, within the Cluster of Excellence CÔTE (ANR-10-LABX-45)

WE029 Effects of Flow Conditions on POCIS Sampling Rate for Constant Pesticides Concentration Exposures

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The effects of hydrodynamic conditions on polar organic chemical integrative sampler (POCIS) uptake rates for a range of pesticides were investigated in a controlled laboratory setting. The POCIS devices were exposed to constant concentrations of pesticides under different flow conditions (static, 1 cm/s, 3 cm/s, 4 cm/s and 5 cm/s) over a period of 20 days. The results showed that for most compounds, there was an increase (up to more than one order of magnitude) of uptake rate with increasing flow velocity. However, this trend was reversed for the noncoincident thiamethoxam, where a lower sampling rate was observed at 5 cm/s compared to flow conditions of 1, 3 and 4 cm/s. Generally, a positive correlation was observed between the sampling rates and hydrobophicity (log octanol/water partition coefficient, log Kow) for pesticides with log Kow ranging from 1 to 3. Study results also include the first reported estimates of POCIS sampling rates for bicyclopyrone and cyproconazole.

WE030 Passive sampling: A viable technique for post registration monitoring? A study in seaweed and coral reef communities

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Passive sampling techniques have been developed and successfully deployed for the assessment of contamination in marine sediments and in air. However, the potential of passive sampling for bystander exposure to pesticides is yet to be explored. Pesticide monitoring in seaweed and coral reef communities has not been performed previously. This study is an attempt to assess the potential of passive sampling and sampling for bystander exposure. To date, passive sampling has not been recognised as a method suitable for data collection within regulatory studies, possibly due to uncertainties involved with the extensive calibration procedures and matrix selection usually required. However, with the extended provision for post registration monitoring of pesticides under the current pesticide Regulation (Regulation EC No. 1107/2009), passive sampling may provide a cost effective alternative to conventional sampling techniques. Although no specific guidance on the use of passive samplers for pesticide monitoring in the regulatory context currently exists under Regulation (EC) No. 1107/2009, use is mentioned in Directive 2013/39/EU “Priority Substances”, stating: “novel monitoring methods such as passive sampling and other tools show promise for future application, and their development should therefore be pursued”. Additionally, although not
directly referred to in Directive 2000/60/EC “The Water Framework Directive”, passive sampling is listed as a complementary sampling method within the WFD guidance document, stating that “passive sampling can be used alongside spot sampling to confirm or refute the results of spot sampling”. This review will consider current developments in passive sampling technology in relation to monitoring pesticides, and their relevance in relation to data submission for regulatory requirements.

WE031 Pesticide quantification in POCIS extracts: matrix effect due to polymers in polythethersulfone membranes and washing protocol R. Guibal, S. Lissalde, A. Charriau, G. Guibaud, University of Limoges / Research Group on Water Soil and Environment GRESE Passive samplers are exposed in the aquatic environment for some days and a time-weighted average concentration (TWAC) can be calculated which improves the representativeness of the contaminant concentration, compared to punctual sampling. For pesticide residues, the Polar Organic Chemical Integrative Sampler (POCIS) prepared from a sorbent material (Oasis HLB) enclosed between two polythethersulfone (PES) membranes can be used. To mitigate the effects of environmental parameters (fouling, variations in flow velocity), performance and reference compounds (PRC) are applied. The presence of polyethylene glycol compounds (PEG) released by PES membranes in POCIS extracts was shown by high resolution time-of-flight mass spectrometry. PEG compounds induce strong matrix effects during PCR and target molecule quantification. Dilution of POCIS extracts can reduce this matrix effect but decreases POCIS performance (quantification limit). As confidence in quantification is the key to a satisfactory indication of the concentration of the contaminant and investigating the effect of analytical interferences on pesticide and PCR quantification and also proposing a washing protocol for PES membranes [1]. To study interferences, a matrix collected from POCIS exposed in freshwater was used. POCIS extracts were diluted at different levels (1/10; 1/25) and spiked with a pesticide mixture (from 10 pg L⁻¹ to 1000 µg L⁻¹). A suppression of DIA-δ5 signal (despropylortizatine) was induced by interferences. As this substance is used as a PRC, the quantification bias hamper the TWACs calculated for the other pesticides. A washing procedure consisting of two successive baths with ultra pure water (UPW) / methanol (50/50) and a final rinse with UPW was proposed to limit PEG release. Total ion current chromatograms of extracts from POCIS built with washed PES membranes did not display a significant PEG fingerprint. The removal of PEG interference allows proper quantification of DIA-δ5 and pesticides during the POCIS retention time range (3.5 – 5.5 min) with a 10-time dilution. With a slight extract dilution (10 instead of 25 times for POCIS with unwashed membranes), pesticides at very low concentration can be properly detected and quantified which further improves the ability of POCIS to quantify molecules at very low content in water, particularly those eluting with PEG contaminants. [1] R. Guibal, S. Lissalde, A. Charriau, G. Guibaud. 2015. Talanta. 144:1316-1323.

WE032 Sorption of structurally different ionized pharmaceutical and illicit drugs to a mixed-mode coated microsampler s. droge, Utrecht University / IRAS; H. Peltenburg, N. Timmer, J. Hermens, Utrecht University / Institute for Risk Assessment Sciences The mixed-mode (C18/SAX) solid-phase microextraction (SPME) fiber has increased sensitivity for ionic compounds compared to more conventional sampler coatings such as PDMS and polyacrylate. However, data for structurally diverse compounds to this sampler coating are too limited to define structural limitations of this type of passive sampler. We determined C18/SAX fiber partitioning coefficients of 17 cationic structures without hydrogen bonding capacity besides the charged group, stretching over a wide hydrophobicity range, and 8 strongly basic pharmaceutical and illicit drugs with additional hydrogen bonding moieties. In addition, several neutral benzodiazepines and the acid diclofenac were tested. All tested compounds showed nonlinear isotherms above 1 mMOL/L coating, and linear isotherms below 1 mMOL/L. The affinity for C18/SAX-SPME for tested organic cations without H-bond capacities increased with longer chain lengths, ranging from logD_{spme-water} 1.8 (benzylamine) and 5.8 (trifluromazine). Smaller amines than benzylamine may thus have limited detection levels, and cationic surfactants with alkyl chain lengths >12 carbon atoms may be appropriate for H-bonding systems and the cationic head groups of the fiber-water relationship. The logD_{spme} for these simple cation structures closely correlates with molecular volume and the octanol-water partition coefficient of the neutral form (KOW). Oxygen moieties in organic cations decreased the affinity to C18/SAX-SPME, in comparison to the relationship with molecular volume established with the simple cationic structures, within a factor of 3 of the KOW relationship for the anionic analogues. For these polar cations to a more than 10 times lesser extent than predicted by KOW.N. Neutral benzodiazepines sorbed a log unit stronger than their KOWs. Results for anionic diclofenac species (KOW = 4.5, pKa 4.0, logD_{spme,water} 2.9) indicate that C18-SCX, despite the negatively charged SCX moieties, may even be useful for sampling a wide range of other distilled compounds, such as hydrophobic organic compounds (sulfates, sulfonates) that are notoriously difficult to sample with bulk polymer phase coated samplers. This data demonstrates the applicability of C18-based SPME in the measurement of freely dissolved concentrations of a wide range of ionizable compounds in many types of environmental and pharmaceutical studies.

WE033 Equilibrium sampling in a semi in situ pot experiment to measure freely dissolved concentrations of hydrophobic organic compounds. N. Bartolomé, Agroscope I SS / Analytical Chemistry Natural Resources Environmental Protection in Agriculture; I. Hilber, Agroscope ART; R. Schulin, Institute of Environmental Chemistry ETH Zurich / Department of Environmental System Science; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; G. Witt, HAW Hamburg / Department of Environmental Engineering; T. Bucheli, Agroscope ART / Environmental Analytics Natural Resources and Agriculture Pesticides have been made to dissolve sufficiently in situ for passive sampling techniques for sensing hydrophobic organic contaminants (HOC) in the field, mainly in air, water and sediments. However, there is very little information on the capability of these methods to assess the bioavailability of HOC in soil. Under field conditions, environmental factors such as temperature, ionic strength or water content may influence the phase distribution of HOCs. Conducting passive sampling of HOCs in situ has the advantage of taking these actual environmental conditions automatically into account, in contrast to standard laboratory tests, which are performed under saturated water conditions (i.e. in suspensions). Here, we present an in situ pot experiment to assess bioavailability of polycyclic aromatic hydrocarbons (PAHs) in a historically contaminated soil with two different passive sampling techniques: low density polyethylene (LDPE) [1] and polydimethylsiloxane (PDMS) glass fibers [2]. The objective of this study is to investigate the concordance of the passive samplers, with the more widely used ex situ laboratory method using soil suspensions. Soil was excavated from the upper 20cm of a pigeon shooting range in Embrach, Switzerland, which was contaminated with 270 mg kg⁻¹ total concentration of the sum of the 16 EPA PAHs. The soil was thoroughly mixed according to Bucheli et al. [3], and the samplers were deployed directly into the pots with 1.5 kg soil and put into the greenhouse. The samplers were exposed for 3, 6 and 9 months. Some pots were kept at 60% water saturation, which is close to field capacity, and others at full 100% saturation. Results of the two passive samplers from both experiments will be presented and correlated to each other, and the outcomes will be compared to PAH bioaccumulation by earthworms and plants also derived from the very same pot experiment. This research will help to evaluate the applicability of these passive samplers directly in situ under soil unsaturated field conditions. References: 1. Booji, K., F. Smolders, and E.M. van Weereld. Chemosphere, 2002. 46(8): p. 1157-1161. 2. Witt, G., et al. Environmental Science & Technology, 2013. 47(14): p. 7830-7839. 3. Bucheli, T.D., et al. Journal of Analytical and Applied Pyrolysis, 2014. 107: p. 25-30.

WE034 Soil-atmosphere exchange of persistent organic pollutants: Determination of concentration gradients with passive samplers. J. Meierdierks, Hydrogeochemistry; C. Zarfil, University of Tuebingen / Center for Applied Geosciences; P. Grathwohl, Eberhard Karls Universität Tübingen / Center for Applied Geosciences Since the last century soil have accumulated plenty of anthropogenic chemicals like the persistent organic pollutants (POPs) which might revolatilize due to decreasing atmospheric concentrations during recent decades. As a contaminant class within the group of POPs the polycyclic aromatic hydrocarbons (PAHs) feature characteristic properties as probable human carcinogens with relatively high concentrations in surface soils, allowing accurate measurements of concentration gradients, and are therefore a relevant class of chemicals of concern. The direction and magnitude of diffusive contaminant flux across the soil–air interface is controlled by the gradient of gas-phase concentration. Past investigations into the soil–air exchange of contaminants have relied on estimation methods for calculating contaminant fugacity in soil because appropriate methodologies for measuring these gradients are not available. This study aims to overcome these problems by using time-integrating passive samplers to determine concentration gradients in the soil and across the soil–atmosphere interface. Passive samplers circumvent the need of estimating a contaminant’s soil partitioning properties by acting directly as a sensor for contaminant activity (e.g. fugacity or availability) in environmental media. 80 µm thick low-density Polyethylene (LDPE) sheets spiked with performance reference compounds (PRC) were deployed in the field to sample atmospheric concentrations of PAHs. Deployments during summer, winter and spring were conducted to check on seasonal variations and to analyze the climatic impact. Comparing PAH concentrations within covered and uncovered sheets the impact of photodegradation on the accumulated PAHs was tested, which can be considered exclusively significant with respect to the PRC and depending on the season. Soil samples were taken at 5 intervals, up to 30 cm depths and equilibrated ex-situ with 30 µm thick LDPE sheets. Evaluation of concentration gradients in the soil, soil moisture content, as well as the sum of the two are factors such as soil texture, porosity, water content and amount and type of organic matter was
performed to verify further correlations with the exchange at the soil-atmosphere surface. The monitoring outcomes from the field and laboratory studies are used in the validation of a numerical model of soil-atmosphere exchange of persistent organic pollutants.

**WE035** Calibration of PDMS and XAD-coated PDMS for measuring organophosphate flame-retardants (OPFRs) and perfluorinated compounds indoors

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Passive air samplers (PAS) are increasingly employed for monitoring semi-volatile organic compounds (SVOC) concentrations in the environment, although less work has been done on their use indoors. We report on passive air samplers consisting of polydimethylsiloxane (PDMS) and a newly designed PDMS coated with styrene divinyl benzene co-polymer (PDMS-XAD), in comparison to the popular polyurethane foam (PUF) with a single bowl shelter. An indoor air calibration study was conducted to determine passive sampling rates by deploying these PAS and comparing with air concentrations measured using two low-volume active air samplers analyzed for gas and particle phases separately. Analysts of interest are brominated and organophosphate flame retardants, phthalate esters and polycyclic aromatic hydrocarbons (PAHs) and perfluorinated compounds. The study extended for 50 days. Surface-area normalized uptake rates of PDMS were comparable to PUF using the single bowl shelter, ranging between 0.6 to 1.5 m$^2$ d$^{-1}$ for brominated flame retardants and phthalates. Preliminary results showed that the number of target chemicals was increased with the PDMS-XAD design because of differences in chemical properties of PDMS and XAD.

**WE036** Estimating Uncertainties in Air-Water Diffusive Exchange Flux and Gross Volatilization Loss of PCBs: a Case Study based on Passive Sampling in the Lower Great Lakes

Y. Liu, S. WANG, Tongji University / State Key Laboratory of Pollution Control and Resources Reuse; R. Lohmann, University of Rhode Island / Graduate School of Oceanography

Air-water diffusive exchange is a significant process in PCB geochemical cycling across the Great Lakes. Compared with dry and wet deposition fluxes, the diffusive flux cannot be directly measured experimentally. Its model-based calculation contained considerable uncertainty because of uncertainties in atmospheric and aqueous concentrations and meteorological conditions. To capture inherent variability of the diffusive flux and load of PCBs, 57 pairs of air and water samples from 19 sites covering the Lake Erie and Ontario were collected using passive sampling during 2011-2012. Error propagation analysis and Monte Carlo simulation (MCS) were applied to estimate uncertainty in the diffusive flux. Passive sampling technique can provide a more precise estimate on direction of air-water diffusive exchange of PCBs. Best-fit values of wind speed and air-water surface exchange probability distribution function can quantify more precisely the site-specific diffusive flux. We confirmed that 30-40% of relative uncertainty in overall mass transfer velocity was a reasonable and acceptable assumption in error propagation to a large extent. Results from error propagation analysis were comparable with those from MCS simulation, but the former would not be recommended in net deposition situation. Parameters sensitivities were different in net deposition, volatilization and equilibrium situations. Volatilization flux of 7 PCBs with maximum frequency across Lake Erie and Ontario was 618 pg m$^{-2}$ d$^{-1}$ and 426 pg m$^{-2}$ d$^{-1}$, respectively, and annual gross volatilization loss of 7 PCBs was 45.65 kg year$^{-1}$ for Lake Erie and 32.42 kg year$^{-1}$ for Lake Ontario in 2011-2012.

**WE037** Persistent organic pollutants (POPs) at Ross Sea (Antarctica) and through Circumpolar Deep Water

A. Gedra, University of Pisa / Chemistry and Industrial Chemistry; R. Ficoci, S. Francesconi, University of Pisa / Dept of Chemistry and Industrial Chemistry

The Antarctic region is ideal place for environmental studies of the marine ecosystem both near the coastal line and off-shore, due to the almost total absence of local pollution sources. In fact, pollutants reach Antarctica almost exclusively by long range transport processes involving both the atmosphere and the hydrosphere. The Circumpolar Deep Water (CDW), the largest and most important feature of the Southern Ocean, is mainly responsible for the rather limited exchange processes between the Antarctic seas and the outer oceans, thus it may be a possible source of persistent organic pollutants (POPs). The most significant findings on the presence of POPs in the marine ecosystem at Ross Sea and in CDW circulation will be discussed. Seawater samples were collected in many sampling sites located in a large area of the Ross Sea and during the travel to and from Antarctica during the XXVIIth Italian expedition. Two classes of POPs are considered, namely polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Some data was also obtained on the mixing process between two important water masses present in the area of the Ross Sea under ice coverage: the Modified Circumpolar Deep Water (MCDW) from the South Shelves Water (HSSW). The MCDW, relatively warmer, comes from the external oceanic circulation in the Pacific sector of the Southern Ocean, whereas the HSSW, relatively colder, is generated inside the Ross Sea basin. Both move towards Cape Adare where they mix. The intrusion of MCDW in the colder HSSW is clearly evident: the very sharp change in the temperature at about 170 km distance (~1.5 °C typical of the HSSW) to ~0.2 °C (typical of the MCDW) is in very good agreement with a sudden change in the PCB concentration by a factor of two. The same behaviour was also observed for the total content of PAHs. This is the first experimental evidence of the pollutant input in the Ross Sea basin from the external oceanic circulation, which happens in a period of the season when the ecosystem is particularly sensitive since the biological activity is at its peak.

**WE038** Laboratory calibration of two passive samplers for aquatic organic contaminants: POCIS and a new mixed polymer sampler

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Uncertainty in the determination of the compound-specific sampling rates is a major issue leading to errors in the conversion of Polar Organic Chemical Integrative Sampler (POCIS) measurements into environmental dissolved concentration, and is one of the main challenges with kinetic passive sampling. The variability of compound-specific sampling rates and membrane properties of POCIS, as well as the sampling rate, are all compound dependent. Therefore, it is important to better understand the uptake kinetics of such passive samplers aimed at sampling polar organic compounds. Furthermore, relating these uptake kinetics to the compound properties will allow the sampling rates of other compounds to be estimated when measurements are not available. Polydimethylsiloxane (PDMS) silicone is commonly used for the passive sampling of neutral hydrophobic organic compounds in aquatic environments. PDMS passive samplers are directly exposed to the surrounding aquatic environment, which maximizes accumulation but also simplifies the uptake process. Other advantages include reversible sorption, which is relevant when considering the application in passive dosing mode for toxicity test. Unfortunately, PDMS is not an ideal choice for polar organic compounds because of its lower partitioning affinity which leads to problems with analytical detection. The above issues raise the question whether it is possible to combine the advantageous properties of PDMS sampler with the higher affinity of a sorbent such as Oasis HLB to make a sampler for both polar and non-polar compounds. In this study, Oasis HLB were embedded within PDMS disks to make a mixed polymer sampler (MPS). MPS disks had a high affinity for both polar and non-polar hydrophobic organics and were much easier to handle since they consisted of a single polymer block. The uptake kinetics of a range of compounds from the aqueous phase to the MPS and the traditional POCIS were compared using a batch set-up. The sampling rate to each sampler was calculated by fitting a numerical model. Subsequently, the desorption of the compounds from the equilibrated samplers was measured. These measured uptake and desorption kinetics were related to the physico-chemical properties of the chemicals as well as the sampler architectures. Therefore, the MPS had a similar performance to the traditional POCIS which the advantage that it was easier to handle and can potentially be used for passive dosing in toxicity testing.

**WE039** Investigating methods for in tissue equilibrium passive sampling of POPs in lean fish

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Estimation of bioaccumulation factors (BAF) of hydrophobic substances requires measurement in aquatic biota as well as in the water phase at a steady state. Passive sampling is used extensively to quantify freely dissolved concentrations of persistent organic pollutants (POPs) in sediment pore water and surface water as a relevant parameter for the assessment of organism exposure. Furthermore, passive sampling can also be applied in fish tissue. Passive samplers that are equilibrated with water, sediment and fish would contain equal POP concentrations if the fish was at the state of a thermodynamic equilibrium with water and/or sediment. If the POP concentrations in one of these phases differ from the other, the equilibrium is not achieved and the sampling phase is no longer in equilibrium, which is equally important for biodistribution and bioaccumulation studies. The presently applied method for in tissue passive sampling is restricted to application for lipid rich fish tissues since the uptake capacity in lean fish of the sampler may easily exhaust the POPs from the tissue in contact with the sampler. That ultimately leads to an underestimation of the POPs concentration level in the tested tissue. We investigated several ways to solve this problem using specimen of lean fish (e.g. pike, Esox lucius) and more lipid-rich fish such as carp (Cyprinus sp.).
Equilibrium partitioning of PCBs and PBDEs between tissue and silicone rubber samplers was investigated by daily moving samplers to another position in the tissue to avoid depletion. Sampler movement was performed manually and done 4 times (4 days exposure in total). A parallel experiment was performed by rolling pieces of fish tissue together with passive samplers in a glass jar for 4 days. All incubations were done at 20 °C. In all experiments the degree of equilibration was monitored by quantifying the release of performance reference compounds (PRCs) dosed to the sampler prior to exposure. Data were compared with measurement of total POP concentration of analytes in tissue samples. Derived method figures of merit include the minimum exposure time to reach partition equilibrium for all investigated substances and the temperature range as well as a minimum mass of tissue required to reach quantification limits. This method has a large application potential for bioaccumulation studies, also for some emerging environmental contaminants.

**WE040 Quantifying the effects of temperature and salinity on partitioning of hydrophobic organic chemicals to silicone rubber passive samplers**

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Nowadays, passive sampling is a widely applied technique to determine freely dissolved aqueous concentrations of hydrophobic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Crucial to the measurements are sampler-water partition coefficients, which are generally determined in the laboratory under ‘standard conditions’ (in freshwater at 20 °C). Theoretically, however, the coefficients are dependent on environmental conditions, such as temperature and salinity. Yet, there are insufficient experimental data in the scientific literature to prove this for different polymers. Several polymers are already being applied during field monitoring however, and neglecting any effects may lead to imprecise results. In the present study, we therefore evaluated the effects of temperature and salinity on the sampler-water partition coefficients of PAHs and PCBs for silicone rubber, a material used in Dutch passive sampling monitoring campaigns. The results demonstrated a chemical-specific and hydrophobicity-dependent temperature effect, being independent of salinity; and a chemical- and equilibrium calculations for EQS evaluation can introduce great bias.

**WE043 Evaluating pharmaceuticals in rivers as tracers for WWTP performance in whole catchments**

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Pharmaceuticals display variable fate in waste water treatment, but also following emission to rivers. Some compounds like carbamazepine can be used as a conservative tracer and can be directly related to population equivalents emitted in a river catchment. Other compounds reflect the performance of treatment plants and their presence in rivers can be used to evaluate the overall degradation efficiency in a river catchment. This requires the in-river fate of the compounds to be quantifiable: Indeed some compounds are subject to photodegradation or microbial transformation during transport in a river. Here we investigate the fate of a dozen pharmaceuticals using 1) diel autosampler campaigns to evaluate temporal trends, and 2) a sampling method when the "sampling frequency" is high. However, high analytical method by using LC/MS/MS. The other is their monitoring method to measure neonicotinoid insecticides in surface water is effective sampling method when the "sampling frequency" is high. However, high monitoring method to measure neonicotinoid insecticides in surface water is effective sampling method when the "sampling frequency" is high. However, high
systems were conducted in order to reveal sampling rates for 64 pesticides in our laboratory. Absorbed mass of 64 pesticides were analyzed by GC/MS. The calibration tests were performed under four different conditions of water velocity. Some chemicals like isopropyl aromatic hydrocarbons were added as performance reference compounds and flow monitors. We also applied this passive sampling technique to some rivers and revealed seasonal trends of 64 pesticides TWA and compared TWA and grab sampling data.

WE045
Developing ceramic passive samplers to monitor cytoxic drugs in river water
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Cytoxic drugs have recently been detected in river waters as a result of their and increasing consumption, becoming new water contaminants. Currently, grab sampling is the method to collect samples from rivers although this might not be completely representative of the river quality due to time dependent variability. In a previous study, grab sampling monitoring was performed in a drinking water treatment plant (DWTW) and along the Besós River (near Barcelona, Catalonia, NE Spain). In the DWTW intake, mycophenolic acid was detected at concentrations between 17 to 52 µg L⁻¹. In the Besós River, two sampling campaigns were performed and different concentration profiles were obtained along the river for 14 cytoxic drugs, showing that their emission to the waters is reiterative but with high concentration variability. Therefore, passive samplers can be used to better evaluate the presence of cytoxic drugs in the aquatic environment, as they can be efficient, relative inexpensive and provide time integrated concentrations. Ceramic passive samplers had been developed, using porous ceramic tubes (45 mm x 13 mm, ID 9 mm) and 200 mg Zepha ZZ (30 µm, 85 Å, Phenomenex) adsorbent. The adsorbent was conditioned with MeOH and water, and the tubes were filled. If needed, more water was added to completely fill the internal volume in order to facilitate the drugs diffusion. Then, they were kept in multi-Q water until deployment. For laboratory test, 10 devices were immersed in a 4L solution containing a mixture of 16 cytoxic drugs at 20 µg L⁻¹. They were deployed from 2 to 11 days to evaluate their adsorption, and two samplers were removed every 2 days. The adsorbent was removed from the tube and was extracted using MeOH in an ultrasonic bath. Linear adsorption was observed for most of the drugs, being up to 200 ng after 11 days, which allowed calculating the diffusion coefficients and the sampling rates. Then, 6 devices were deployed in the DWTW intake during 1 to 3 weeks to see the fouling effect. Finally, the passive samplers were deployed in the DWTW intake and in the Besós River.

WE046
Passive sampling technique combined with ELISA assay detection for monitoring neonicotinoid insecticides in Japanese surface water
Y. Kameda, Chiba Institute of Technology / Civil Architectural and Environmental Engineering
Neonicotinoid insecticides are widely used in Japan. Especially, seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiacloprid, Clothianidin, Dinofeturan, Thiamethoxam and Nitipyratom are popular. Their usage began from the beginning of 1990 and is increasing till 2008 in Japan. Recent annual total usage of seven insecticides is not increasing, approximately 400 tons per year. However, in recent years and in future, their occurrence, their behaviors and their ecological risk in Japan. Although their monitoring in surface water including storm water, wastewater and sewage treated water, which can be important to Japanese aquatic environment, is necessary to their ecological risk assessment, two big problems must be solved. One problem is their difficult analytical method by using LC/MS/MS. The other is their monitoring method which is constituted by only grab sampling. Grab sampling is a very basic and effective sampling method when the “sampling frequency” is high. However, high frequency is difficult in most investigations and screenings. In this study, a novel monitoring method to measure neonicotinoid insecticides in surface water is developed. The most common way to collect samples from rivers although very simple, their concentration and their occurrence in surface water in Japan.

WE047
Does the settling of the resin beads have an impact on DGT measurements?
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DGT (Diffusion Gradients in Thin Films) is one of the most popular passive samplers for the determination of inorganic and organic fluxes (availability). Recent publications have shown that in presence of partially labile complexes, most of the metal accumulation in the DGT device comes from dissociation of the complex in the resin disc. The distribution of the resin beads (in the resin disc) might, then, play an important role in metal accumulation. For simplicity, it is usually assumed that there is homogeneous distribution of the ligand in the resin. However, it is well known that, during the preparation of the resin, the binding beads settle to one side of the disc. The influence of this inhomogeneity on metal accumulation (in presence of ligands) is here assessed using numerical simulation of DGT devices with resin beads in only one half of the resin disc. Results indicate that, due to the settling, there is a deficit in mass accumulation which increases as K (stability constant of the metal complex) increases. The maximum deficit takes place for complexes with lability degrees close to 0.25, while very labile or inert complexes remain unaffected. A maximum deficit of 13% can be found when K<sub>app</sub> < 10⁴, but this deficit can increase up to 30% when K<sub>app</sub> < 10⁵ or 30.6% when K<sub>app</sub> < 10⁶. Additionally, DGT devices with a stack of two resin discs can be used to estimate kinetic dissociation constants of complexes. The influence of the inhomogeneity on the recovered constant is also analysed. It has been found that the recovered kinetic dissociation constant, k<sub>app</sub> (k<sub>app</sub>inherent), and the true value, k<sub>app</sub>, are practically proportionals k<sub>app</sub> = k<sub>app</sub> (k<sub>app</sub>inherent) being the fraction of volume of the resin disc occupied by resin beads. This relation is quite independent of the value of K and k<sub>app</sub>. Funding from the Spanish Ministry MINECO (projects CTM2012-39183, CTM2013-48967) and from FEDER is acknowledged. References: (1) Galceran, J. et al. Environ.Chem. 2015, 12, 112. (2) Shafaei-Arjavag, M. R. et al. Environ.Sci.Technol. 2013, 47, 463. (3) Jiménez-PrietoRéhula, M. et al. Anal.Chim.Acta 2015, 885, 148. (4) Puy, J. et al. Anal.Chim. Acta 2014, 86, 7740.

WE048
Investigation of trace element speciation alteration by fouling development on DGT passive samplers
D. Devillers, R. Buzier, A. Chargiat, M. Grybos, G. Guibaud, University of Limoges / Research Group on Water Soil and Environment GRESE
The Diffusive Gradient in Thin Film technique (DGT) has been mainly developed for passive sampling of trace elements and its use for water quality monitoring is currently considered. Among the potential biases induced by sampler deployment in water systems, fouling of the protective membrane has been identified as potentially interfering elements diffusion in the sampler. Although numerous in situ deployments have been performed in water systems such as rivers, influence of fouling development on element quantification has been nearly not studied. This work /Mines an investigation on fouling development on sampler and its influence on element quantification. For this purpose, three contrasted systems are studied: a conventional river, a wastewater impacted river and a stagnant pond. In each system, three types of membrane (cellulose acetate, polycarbonate and polyethersulfone) are deployed several weeks for fouling deposition and development. Membranes are then brought back to laboratory, incorporated into DGT systems and deployed in controlled solution (pH 7 and 10 ± 0.2, Eq strength fixed with NaNO₃) containing metals (Cd, Cu, Ni, Cr(III), Cr(VI) or Pb), metalloid (As) or nutrient (PO₄³⁻) in order to determine their effective diffusion coefficient in the sampler. In parallel, fouling of the membrane is characterized through elemental analysis and observation with scanning electron microscopy coupled with X-ray microanalysis. Diffusion coefficient derived for fouled samplers will be presented and sampling biases induced by fouling will be quantified. Furthermore, link between diffusion coefficient alteration and composition of the fouling will be discussed.

WE049
Bioavailability assessment of copper in sediments using chemical methods and bioassays and the effects of potential adsorbents in remediation
K. Vaananen, S. Hietaketo, T. Oksanen, University of Eastern Finland / Biology; S. Abell, University of Eastern Finland / Department of Biology; G. Waissi, J. Akkanen, University of Eastern Finland / Biology
Metal contamination in aquatic environments is a growing concern. Bioavailability is the main factor determining toxicity of metals and therefore the use of bioavailability analysis is encouraged. Using adsorbents to reduce bioavailability and transport has been introduced as a novel remediation method for polluted soils contaminated with metals. In the present study, the use of passive samplers (DGT) and pore water extracts (Rhizon) in assessing the bioavailability of Cu in a natural fresh water sediment. We also compared the results to a bioassay and evaluated the remediation potential and possible adverse effects of the adsorbent materials: chitosan and a biochar. The natural uncontaminated sediment was spiked with CuCl₂ (0–460 mg/kg dw) and subsamples were spiked with 0.5 and 0.5 % (dw) chitosan or biochar. Total Cu concentration and bioavailable concentrations (DGT and Rhizon) were analyzed and the effects of Cu and/or amendments to biota were evaluated with a biotest (Lambiricultia variegatae). The functionality of the adsorbents was analyzed using DGT and Cu body reside in L. variegatus. There was a strong correlation between DGT and sediment Cu concentrations between DGT and Rhizon samples. The growth of L. variegatus was reduced in chitosan-spiked sediments,
but no adverse effects of biochar were seen. The bioaccumulation or Cu into L. variegatus increased together with total sediment concentration. On the basis of chemical methods, 5% chitosan reduced the availability of copper by 75–87%. For biochar, no remediation potential was seen. DGT and pore water sampling gave comparable results for the bioavailability of Cu and they seem to be usable methods. Environ.Chem. even though chitosan nor biochar reduced the uptake of copper in the test organisms. Most of the studies of adsorbent materials for metals are focused on the chemical analysis of remediation potential, but this study shows that biological responses needs to be taken into consideration as well. The main uptake route of Cu to L. variegatus is seen to be through ingestion of sediment particles, which could partly explain the results. Since the uptake route of metals varies between different metals and species, further studies are still needed.

**WE050**

**Does bioavailability of Zn from hydroponic media correlate with the free metal concentration or with the flux of a labile fraction?**

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Zinc is an essential element for plant nutrition. In solutions, zinc exists in different chemical forms such as free hydrated ion, inorganic and organic complexes. The bioavailability of elements in the soil solution depends on their chemical speciation. Lipophilicity, global charge and lability of the complexes are the main factors that determine metal uptake by organisms and therefore information about speciation is needed. The necessity of being able to measure not the total metal in solution, but the free fraction or the labile ones. Zn concentration. DGT, ASV provide availability fluxes associated to different labile fractions. An innovative approach based on PIMs has also been applied after optimization of parameters. Zinc accumulation in the roots and shoots of Solanum tuberosum (potato plant) grown in hydroponic media with added Zn, and with or without the fraction of retained PRCs according to Rusina et al. [1].

For biochar, no remediation potential was seen. DGT and pore water sampling gave comparable results for the bioavailability of Cu and they seem to be usable methods. However, neither chitosan nor biochar reduced the uptake of copper in the test organisms. Most of the studies of adsorbent materials for metals are focused on the chemical analysis of remediation potential, but this study shows that biological responses needs to be taken into consideration as well. The main uptake route of Cu to L. variegatus is seen to be through ingestion of sediment particles, which could partly explain the results. Since the uptake route of metals varies between different metals and species, further studies are still needed.

**WE051**

**Identification of contamination hotspots along a large river by silicone based passive sampling**


Silicone-based passive sampling allows the quantification of time-weighted average concentrations (C_{TWA}) of hydrophobic organic chemicals (HOCs) in water, even in the low pg L^{-1} range. In previous studies, the suitability of passive sampling for the monitoring of HOCs was tested in different rivers in Germany. Thereby, the sampling site in the river Saar showed higher contamination with polycyclic aromatic hydrocarbons (PAHs) compared to the sampling sites in the rivers Elbe, Saale, Rhine and Danube. The Saar river originates in France and passes the Saarland conurbation before it flows into the river Moselle in Germany. The Saarland conurbation around the city of Saarbrücken is known as a former highly industrialised region with mining, iron and steel industries. In this study, we applied passive samplers for the identification of contamination hotspots along the downstream river Saar. Silicone rubber reduced the uptake of Zn, in France and permitted to reach lower LOQ for polar compounds and recent developments have been done for few organic compounds, i.e., bisphenols, antibiotics, 4-chlorophenol, glyphosate and aminomethyl phosphonic acid. This work aimed at testing the applicability of an innovative sampling technique for metal pollution assessment in the Saarland region during long-term deployments. Despite the presence of target and suspect compounds in the collection medium, PACSiR passive sampler were 1.3 to 12.5 higher than for SR depending on the analytes.

**WE052**

**An innovative receiving phase for passive sampling of polar and apolar micropollutants in surface waters**

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Silicone rubber (SR) has been successfully implemented as a receiving phase in various passive samplers (MESCO, Passive SBSE and silicone rod/sheet). Indeed, SR is mechanically resistant, insoluble in common solvents used for compound desorption, and enable a thermal desorption at high temperature. Nevertheless, SR is an hydrophobic material which permit mainly enrichment of apolar organic compounds (log K_{ow} > 3) from water (organochlorine pesticides, PAHs, PCBs, etc.). To remedy this lack of measurement representativity, the use of an other passive sampler in co-deployment for polar compounds, as Polar Organic Chemical Integrative Sampler (POCIS), is the strategy preferred. This work aimed at testing the efficiency of SR as a receiving phase for apolar polar organic compounds in passive sampling with a single receiving phase. In this way, a Polar/Apolar Composite Silicone Rubber (PACSIR) was developed for sorption of a wide range of organic compounds. First, this innovative composite material was assessed in laboratory for pesticides sorption with a large range of physico-chemical properties. Then, PACSIR was shaped and calibrated as a passive sampler in laboratory, to be finally applied for the monitoring of several pesticides in surface waters. Extraction recoveries determined experimentally for 22 pesticides were similar or higher for all pesticides than for SR. The enhancement of extraction performances is the highest for polar pesticides, with log K_{ow} below 3, which are poorly sorbed on SR. Kinetics of uptake of pesticides in calibration system were mainly described by a pseudo-linear equation model for 14 days exposure. Sampling rate and partition coefficient were determined for calculation of time weighted average concentration (TWAC). Sampling rates for PACSIR passive sampler were 1.3 to 12.5 higher than for SR depending on the pesticide. Consequently, PACSIR enable to reach lower LOQ than SR for polar pesticides. Finally, results of field exposition of passive samplers in rivers were compared with grab sampling to access the occurrence of pesticides and to estimate the TWACs during field exposition. This study demonstrated that the PACSIR is a sensitive receiving phase for pesticides which can greatly improve sorption properties compared to SR and permitted to reach lower LOQ for polar pesticides. Further experimental investigations on accumulation of other organic compounds will be carried out (hormones, pharmaceuticals, PAHs and PCBs).

**WE053**

**Passive sampling of anionic herbicides (bentazon, chlorsulfuron, isoxaflutole and metribuzin) by DGT technique**

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PACSiR is a sensitive receiving phase for pesticides which can greatly improved sorption properties compared to SR and permitted to reach lower LOQ for polar pesticides. Further experimental investigations on accumulation of other organic compounds will be carried out (hormones, pharmaceuticals, PAHs and PCBs).
by solution pH. For bentazon and mecoprop, the performance of DGT seems to be unaffected by pH in the range 4 to 8. For isoxixan, an increase of pH from 5.0 to 8.0 led to the decrease of CDGT / Corga by approximately 17%. Ionic strength increase was found to slightly affect bentazon and chlorosulfuron sampling only. The tested binding phase was found to be suitable with long term deployments and high concentrations of natural waters. DGT appears therefore to be a promising tool for measuring time-weighted average concentrations of anionic pesticides in waters.

**Persistent and mobile contaminants in the aquatic environment:** how to identify, analyse and regulate a potential threat for drinking water resources (P)

WE055

**Using REACH registration data for the identification of persistent, mobile and toxic (PMT) substances to protect raw water resources**

M. Neumann, Federal Environment Agency UBA / Section IV Chemicals; L. Vierke, D. Saitler, Federal Environment Agency UBA / Section IV Chemicals Chemicals substances and their uses that fall within the scope of the REACH Regulation (EC No. 1907/2006) have to be registered at the European Chemicals Agency (ECHA) in Helsinki. Registrants are requested to ensure a high level of protection of human health and the environment. By doing so, industry guarantees the safe use of chemicals throughout the whole life cycle. Raw water resources used for the production of drinking water need a high level of protection. In Europe drinking water is obtained mainly from groundwater, reservoirs or river bank filtration. If these environmental compartments are exposed to hazardous chemicals, the confiscation of drinking water is possible. In the last decade the fate and behaviour of substances was investigated both scientifically and from a regulatory perspective. The finding is that the intrinsic hazard potential of a substance with respect to raw water contamination is maximised if the substance is at the same time persistent in the environment and mobile in the water cycle. Once emitted, these substances remain in the aquatic environment and the contamination is irreversible. If in addition they also fail the properties of being toxic any emission into water resources should be avoided during the production and downstream use of these substances. Kalberlah et al. (2014) present a proposal for an assessment concept of persistent, mobile and toxic chemicals (PMT substances). We show that the assessment concept for PMT substances uses the same set of data provided as standard information requirements for the registrations under REACH. It may build the basis for regulatory measures by authorities. From an authority point of view such a contamination may be of equal concern as those substances with persistent, bioaccumulative and toxic (PBT) properties. So far, a potential for raw water contamination by PMT substances is not part of the standardized assessment under REACH. Kalberlah et al. (2014) also published a proposal for a practical guide. Therefore our assessment concept may also be applied by registrants under REACH to fulfill their responsibilities under REACH and ensure a safe use of chemicals.

**Interpretation of Residue Data from a Groundwater Monitoring Study to Define Environmental Safe Use**

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Current regulations governing the registration of plant protection products in the European Union (1107/2009) mandate that an assessment for the potential to reach groundwater is made. A recent Sanco opinion (Sanco/13144/2010 rev. 3) has provided guidance on demonstrating "safe use" through monitoring: specifically, 90% of the analyses, obtained from at least 50 locations would need to be less than EU trigger values in order for the Commission to consider a proposal for EU level approval. In 2012, a groundwater monitoring study was initiated with the objective of deriving a statistically valid 90th and 95th percentile concentration for the target substances S-metolachlor (CGA57473) (ESA) and CGA51202 (OXA). From 796 quarterly samples collected from 124 locations across 11 European countries over a time period of 12-24 months , for OXA, 40% (321 samples) were < LOQ and >99% (789 samples) were < 10 µg/L. Despite the seven isolated OXA detections >10 µg/L, no site was found to have a long term average > 10 µg/L which provides statistically valid confirmation of safe use. For ESA, 16% (126 samples) were < LOQ and 97% were < 10 µg/L (774 samples). Of the samples >10 µg/L (22 samples; 2.7%), only 5 sites were found to have a long term average > 10 µg/L with no sites demonstrating analytical residues consistently > 10 µg/L. The 90th and 95th percentile of ESA concentration was derived using sampling survey statistics and these were determined to be significantly below the EU trigger. Even using a worst-case assessment of the maximum site average in each relevant FOCUS zone, Chloraunad, Picazenc and Thiva residues were 10 µg/L. This indicated that exceedances are not widespread. The relative vulnerability of the sites was determined by linking the actual field properties back to the original European cumulative distribution of ESA mass flux, placing 35% of the sites above the 90th centile of vulnerability. In conclusion, this exceptionally thorough monitoring study across 124 randomly selected locations supports the "safe use" of S-metolachlor on the basis that both the 90th and 95th confidence intervals for OXA and ESA were < 10 µg/L.

**Carbamazepine and degradation by products in watershed and effluents**

A. Topola, C. SOULIER, BRGM / Laboratory Division

Carbamazepine, as anti-epileptic drug is well-known as anthropic pollutant in wastewater and stormwater, especially in riverine and groundwaters. This study proposes to assess the potential of known and unknown degradation products. The extrinsic and intrinsic degradation pathway and the method performance were investigated. High resolution mass spectrometry (HRMS) has gained increasingly in importance for monitoring these organic compounds. Its high resolving power, mass accuracy and the sensitive full spectrum acquisition are the key points. On the other hand, the high complexity for the identification of monitoring is sometimes low and fluctuating concentration levels and complex mixture of pollutants. Therefore there is a strong interest to combine passive sampling to HRMS. Passive samplers allow accumulating compounds during exposure that improve trace detection and integrating pollution fluctuations. It is a promising tool for measuring time-weighted average concentrations of anionic pesticides in waters.

WE056

**Identification of pesticides and their transformation products in groundwater**

C. SOULIER, A. Topola, BRGM / Laboratory Division

Nowadays it is increasingly important for public policy to know what compounds are present in groundwater and which of them must be monitored. This implies the need of specific analytical methodology to identify these micropollutants, emerging substances and transformation products present at low concentrations. The high resolution mass spectrometry (HRMS) has gained increasingly in importance for monitoring these organic compounds. Its high resolving power, mass accuracy and the sensitive full spectrum acquisition are the key points. On the other hand, the high complexity for the identification of monitoring is sometimes low and fluctuating concentration levels and complex mixture of pollutants. Therefore there is a strong interest to combine passive sampling to HRMS. Passive samplers allow accumulating compounds during exposure that improve trace detection and integrating pollution fluctuations. The Polar Organic Chemical Integrative Sampler (POCIS) was employed to sampling polar and semi-polar compounds (pesticides, pharmaceuticals, phenolic compounds, triazoles...). Two drinking water supplies were investigated and sampled during several months. Passive sampling was deployed monthly and analyzed by LC-QToF. To process data, different approaches were investigated. The first one is based on research from compounds listed on our homemade database (around 450 with experimental data on our system as retention times, masses for molecules and fragment ions) and suspect database (from bibliography and online databases). This suspect list was supplemented by crop protection agents and their degradation products of interest for these sites. The second approach concerns the non-target screening that could give information on the presence of other degradation products or unknown compounds present in all samples. These approaches allow highlighting the use of passive samplers as storage tool because more compounds are identified with POCIS. The use of these two techniques (passive sampler and HRMS) identified some target and suspect compounds present in groundwater on several month.

WE059

**Neonicotinoid Pesticides in Drinking Water from Agricultural Regions of the Great Lakes Basin, Ontario**

C.D. Metcalfe, Trent University / Water Quality Centre; S. Kleywegt, Ontario Ministry of the Environment / Standards Development Branch; T. Sultana, Trent University / Water Quality Centre

Neonicotinoid insecticides (NNIs) are widely used in regions of the Great Lakes basin in Ontario, Canada where there is intensive agriculture. Because of the persistence and high mobility of NNIs, there is concern that these insecticides may contaminate surface waters and groundwater in agricultural regions, including sources of drinking water. The objective of the project was to evaluate the distribution of NNIs and their degradation products in raw, treated (groundwater) and finished (treated) drinking waters in selected agricultural regions of southern Ontario, Canada. Raw and finished drinking waters were monitored with Polar Organic Chemical Integrative Samplers (POCIS) and grab samples collected from six drinking water treatment plants that draw their water in agricultural areas. The data from POCIS deployments indicated that neonicotinoid pesticides in raw water at concentrations < 25 ng/L, with clothianidin, imidacloprid, thiacloprid and thiamethoxam being
the compounds most widely detected. The frequency of detection of NNI s was much lower in finished drinking water, but some of these compounds were detected with POCIS samplers at estimated concentrations in the low ng/L range. The data from grab samples of raw and finished drinking water collected at the time of POCIS deployment and retrieval, respectively, showed good agreement between measured concentrations and the values estimated from POCIS, although concentrations of NNIs in grab samples collected from finished drinking water were all below detection limits. Even though NNIs have been shown to have low toxicity to vertebrates, including humans, contamination of drinking water by this class of insecticides is still a cause for concern.

**WE060**

**Fate of Persistent Organic Pollutants: Surface Water and Sediment**

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For monitoring of surface water and sediment, we took and analyzed the probable content of persistent organic pollutants (POPs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) of the Republic of Armenia. The study involved Sevan Lake, rivers Masrik, Martuni, Gavaraget, Hrazdan, as well as water reservoirs. “Yerevanyan” and “Akhtpara”, Gas-Chromatograph/ Mass-Spectrometer GCMS-QP2010 SE EI 230V CELV incl. GC-2010 Plus/Shimadzu Corporation, Japan was used for analyses. The following pesticides were revealed in studied samples: hexachlorocyclohexane; DDT; Heptachlor; Hexachlorbenzene; Aldrin; Dieldrin; Endrin; Mirex. As revealed, in all tested samples of surface water the residual amounts of pesticides presented in marine environment as a consequence of their continuous input from agricultural and industrial soils. In this study, effects of clay minerals on diethyl phthalate degradation were investigated. Recent results showed the absorption of DEP to different clay minerals could significantly reduce DEP degradation. The clay minerals with low Fe content would quench free radicals and reduce DEP degradation in solution, and adsorbed DEP would not be degraded. However, the clay minerals with high Fe content would produce more free radical and accelerate DEP degradation in solution, and adsorbed DEP could be degraded. This study implied that clay types, compound structure, exchangeable cation played important roles in phthalate ester’s degradation.

**WE063**

**Evaluation of polyfluoroalkyl (PFASs) contamination profiles in water and sediments for contrasting lakes in southern and northern Sweden**

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Perfluoralkyl and polyfluoralkyl substances (PFASs) are becoming an increasingly global concern. PFASs are highly fluorinated organic chemicals that represent a serious risk for environmental and human health. Due to their toxicological impact, PFASs are not included in several emission regulations. Even if the global level of PFASs use is decreasing much of present concentrations in the environment are results of usage and exposure in the past. Therefore, observation of current environmental concentrations and transport patterns may lead to better prediction of their potential environmental distribution, health impact, and future fate. Investigation of PFASs distribution profiles was carried out in surface waters and sediments of the two lakes Sänskjön and Langsjön in southeast and northeast of Sweden. In total 11 sediment cores and 6 water samples were collected to investigate the PFAS concentrations by using high-performance liquid chromatograph-tandem mass spectrometer (HPLC-MS/MS). The concentration of PFASs in sediment ranged from 2.7 to 216 ng g⁻¹ dry weight for the Sänskjön lake and from 0.8 to 78 ng g⁻¹ dry weight for the Langsjön lake. The PFAS concentrations and distribution were correlated to investigate transport conditions for different PFASs and to determine the possible exposure time sequence for each sampling site. Selected water and sediment properties such as pH, conductivity, and total organic carbon were correlated to observed PFAS concentrations. The results for water samples reflect the high contamination of surface water in both lakes. The maximum concentrations were 79 and 1885 ng L⁻¹ for Sänskjön and Langsjön, respectively. Analysis of sediment cores reflects the temporal evolution of discharge patterns to the two lakes. The correlation of selected samples suggests transport and degradation mechanisms.

**WE064**

**Evaluation of a suspected-target and non-target high-resolution mass spectrometry method for the preliminary detection of contaminants of emerging concern in water samples impacted by wastewater**

A. Higa, Florida International University / Chemistry Biochemistry; P.R. Gardinalli, Florida International University / Chemistry Biochemistry and SERC

In our continuously growing society, there is a constant production, use, and disposal of products such as antibiotics, flame retardants, personal care products, pharmaceuticals, and pesticides/herbicides. Such products eventually enter the wastewater, and sometimes negatively affect ecosystems, which represents one of the primary current areas of environmental concern. Further, all of these compounds, which fall under the broad term of contaminants of emerging concern (CECs), can undergo multiple transformation processes, such as photolysis, oxidation, or microbial degradation, and thus be modified into new, sometimes equally as concerning, compounds. Recent focus has shifted from a targeted analysis, which limits the monitoring to a small set of predefined compounds, to a suspected-target or non-target analysis. This workflow allows for a much more comprehensive screening of analytes, requires no “a priori” knowledge on what compounds are of interest, and allows for the monitoring of transformation products. The ability to do such analyses can be mainly attributed to developments in high-resolution mass spectrometry (HRMS), allowing the assignment of molecular formulae, which in combination with other confirming tools (e.g., retention time, isotopic pattern, MS/MS fragmentation data) can be used to identify specific compounds. The current study uses an Orbitrap Q Exactive mass spectrometer, which provides excellent sensitivity and selectivity, and allows in both high-resolution-target and non-target screening of CECs and their TPs. The analysis includes

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**WE062**

**Effect of Clay Minerals on Diethyl Phthalate Degradation**

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Phthalate esters are a group of plasticizers used to increase plastic flexibility, transparency, durability, and longevity. They have been detected in China’s agricultural and industrial soils. In this study, effects of clay minerals on diethyl phthalate (DEP)’s Fenton reaction were investigated. The results showed the absorption of DEP to different clay minerals could significantly reduce DEP degradation. The clay minerals with low Fe content would quench free radicals and reduce DEP degradation in solution, and adsorbed DEP would not be degraded. However, the clay minerals with high Fe content would produce more free radical and accelerate DEP degradation in solution, and adsorbed DEP could be degraded. This study implied that clay types, compound structure, exchangeable cation played important roles in phthalate ester’s degradation.

**WE061**

**Biliary PAHs metabolites in red mullet (Mullus barbatu s) from Spanish Mediterranean coast**

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Poly cyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants present in marine environment as a consequence of their continuous input from either land or marine sources. Fishes have a higher capacity to uptake metabolize and excrete PAHs than invertebrates such as molluscs, therefore the accumulation of PAHs metabolite in their bile fluid can be used to assess the environmental exposure to the parent compounds. In this study the bile concentration of PAHs metabolites in red mullet (Mullus barbatus) from 10 areas from the Spanish Mediterranean coast were characterized (Barcelona, Ebro Delta, Tarragona, Mallorca, Valencia, Santa Pola, Cartagena, Almería, Castell de Ferro and Mlaga). Red mullets from 12 to 18 cm were sampled in autumn 2012, 2013 and 2014. Bile samples were treated individually and maintained at -20°C until analysis. In addition the PAHs profile was analyzed in samples of muscle of the same specimens. The metabolites were separated with gas chromatography and the PAH bile levels. Phenanthrol and pyrenol were the major PAH metabolites detected in the red mullet bile, and their concentrations were analyzed by liquid chromatography with fluorescence detection using a standard solution for external calibration. Phenanthrol and pyrenol were found in all samples, with pyrenol reaching the highest concentrations. The metabolite concentrations were higher in specimens sampled close to Barcelona than in the rest areas. In contrast, the minimum values were found in Castell de Ferro. These results showed that accumulation of PAHs metabolite was related with the human pressure. Thus phenanthrol and pyrenol concentrations in red mullet bile represented better their PAHs exposition in the marine environment than PAHs concentrations in other tissues estimated from POCIS, although concentrations of NNIs in grab samples collected from finished drinking water were all below detection limits. Even though NNIs have been shown to have low toxicity to vertebrates, including humans, contamination of drinking water by this class of insecticides is still a cause for concern.
WE065 Stationary phase selection in HILIC: a study about stationary phase retention properties and the influence of mobile phase parameters

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Performing stationary and mobile organic micropollutants may pose a threat to drinking and raw waters, and thus need to be monitored and analyzed. A high polarity increases the chance of chemicals to be mobile in the water cycle significantly since it leads to good water solubility as well as low volatility and adsorption and thus enables those chemicals to pass most natural and artificial barriers unhindered. The same multiplicity of origins and chemical structures is highly relevant for drinking and raw waters also exacerbar their analysis. GC-MS and RP-HPLC-MS, the two predominantly deployed methods in organic trace analysis suffer from insufficient volatility or retention while many alternatives like the use of ion pair reagents, capillary electrophoresis, NP-HPLC and ion chromatography have major drawbacks in combination with mass spectrometry or are limited to ion-exchange and hydrophilic interaction liquid chromatography (HILIC). It has proven to be a versatile tool for the analysis of highly polar organic compounds and is well compatible with mass spectrometry, the technique is not yet widely established. This may be a consequence of the complexity of the multimodal retention mechanism, the availability of a variety of dedicated HILIC stationary phases, the importance of stationary phase selection and the lack of an almost universally applicable stationary phase chemistry such as C18 in RP-HPLC. As a consequence, stationary phase selection is one of the most crucial steps in HILIC method development and in-depth knowledge is required for a suitable selection. Several studies investigated the retention properties of HILIC stationary phases, but most limited to either analyses of a specific substance class or stationary phases with similar functionality. More broad attempts encompassing a wide range of stationary phases and analyte classes are still rare. In this study we investigated the retention behaviour of 19 model analytes on twelve HILIC stationary phases with different mobile phase compositions. The results allowed a grouping of stationary phases based on their retention properties and selectivity which facilitates stationary phase selection for method development and optimization. A comparison of the retention properties at different mobile phase compositions provides valuable information about the intrinsic optimization potential of important mobile phase parameters, possibly facilitating future HILIC method development.

WE066 Environmental and Metabolic Transformations of the Pisciocide, Antimycin A

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Antimycin A is an extremely potent inhibitor of mitochondrial respiration and has been extensively studied due to its potential to enter aquatic and freshwater aquaculture (e.g., catfish) for the selective removal of scaled fish from ponds prior to restocking. In spite of the long-term use of antimycin A, little information exists on its environmental fate and metabolism. Antimycin A is a bacteria fermentation product containing a nine member dilactone ring and exists as a mixture of eight structural isomers that differ in their dilactone alkyd chain (hexyl or butyl) and acyloxy side chain (butyl or propyl). A high performance liquid chromatographic analytical method was developed for the measurement of antimycin A isomers: 4a, 3a, 2a, and 1a in aqueous and microsomal samples. Laboratory hydrolysis studies were conducted using rat hepatic microsomes at pH 7.4. Abiotic hydrolysis of the antimycin lactone occurred more quickly than hydrolysis of the acyloxy moiety. In vitro antimycin A hydrolysis was increased with increasing pH. Antimycin 3a, 2a hydrolyzed significantly slower than 1a, above pH 6 while 1a hydrolyzed more slowly than 3a below pH 6. At pH 8 and below, the mixed batch systems exhibited faster rates of antimycin A hydrolysis than the static system; above pH 8 the rates were nearly identical for the two systems. In vitro phase 1 metabolism led to the preferential hydrolysis of the antimycin A acyloxy group and formation of the N-formylaniline moiety. The four hexyl-containing isomers (1a, 1b, 2a, 2b) yielded the same metabolite; the four butyl-containing antimycin isomers (3a, 3b, 4a, 4b) yielded a second unique metabolite. Based on reports that binding of the antimycin A formylamino group is critical for inhibition of mitochondrial respiration (i.e., antimycin efficacy), we hypothesized that the formylation of antimycin A could be a significant pathway for deactivating antimycin A—especially in the case of mammalian exposure (i.e., consumption of antimycin tainted fish). Additional work is ongoing to test this hypothesis.

WE067 Degradation Mechanism of Algal Derived Odorants by Chlorination and UV/Chlorination Processes

T. Kim, Seoul National University, Graduate School of Public Health / Department of Environmental Health Sciences; M. Kim, Environmental Health / Department of Environmental Health Sciences; K. Zoh, Seoul National University / Department of Environmental Health

Marine activities can degrade the quality of drinking water sources by the release of algae derived organic matters including algal toxins and undesirable taste and odor (T&O) causing compounds such as 2-methylisoborneol (2MIB) and geosmin. These T&O compounds have a possibility to be the precursors of trihalomethanes (THMs) as well as other hazardous by-products during chlorination and other treatment processes due to their strong oxidation potentials. Furthermore, the conventional water treatment processes (coagulation, sedimentation, and filtration) are not effective for removing these T&O compounds. For effective treatment of 2MIB and geosmin, the investigation including the identification of their by-products formed during processes needed. In this study, chlorination, UV photolysis, and UV/Cl2 reactions were applied to treat 2MIB and geosmin in water. While chlorination was ineffective to the removal of GSM and 2MIB, UV photolysis and UV/chlorination reactions effectively removed both compounds. This may due to the destruction of tertiary alcohol structure which has a resistant to chlorination. UV photolysis and UV/chlorination reactions followed the pseudo first-order reaction. Neutral pH was effective for the removal of geosmin and 2MIB, due to that OH radical scavenging by OCI ion at higher pH. The intermediates identified by GC/MS scan mode (m.w. range 50 to 300) during UV/Cl2; reaction were 1,4-dimethyl-adamantane, 1,3-dimethyl-adamantane, and cis-1-ethylidenecyclohexyl-1a-methyl-1H-Indene for geosmin, 2-methylisoborneane, and 2-methyl-2-borne for 2MIB). Also, 4-methyl-2-heptane, 2,4-dimethyl-1-heptene, and 2-methyl-3-pentanol were detected from both 2MIB and geosmin. These intermediates were produced by the rearrangement, bond scission, and ring opening. These intermediates decreased with further reactions, and the formation of chloroforom with further reaction. Using the obtained information, the degradation pathways of UV/chlorination of 2MIB and geosmin were constructed.

WE068 Chemical and microbial markers to identify human-associated contamination in Kokemäenjoki watershed

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Microbial and chemical contaminants can be used as source tracking markers in the environment. Accurate identification of pollutant sources in watersheds is crucial for ensuring a good water quality. Ideal human markers are specific i.e. their source is solely anthropogenic. They should also be detectable even at long distances from the source. Sources and distribution of chemical and microbial human markers in Kokemäenjoki watershed, Finland, were studied as a part of Aquatic contaminants - pathways, health risks and management (CONPAT) project. Municipal and industrial wastewaters, surface waters, and artificial groundwater were sampled 8–9 times during two years. The feasibility and ability of different markers (i.e. acetylsalimine, human adenosviruses and human-specific fecal bacteria markers) to indicate human fecal pollution was evaluated. Chemical source markers are typically water soluble compounds that are continuously released to environment at easily detectable concentrations. Artifical sweeter acetylsalimine is an ideal human marker, since it is commonly used, does not decompose in wastewater treatment, and is persistent in the aquatic environment. It was detected in all studied samples. Fecal indicator bacteria are commonly used for the evaluation of water quality in relation to the potential human health risks. However, the origin of fecal pollution can be either humans or animals and bacterial indicators might decay in the environment with different rate than pathogens such as viruses. Human infecting adenosviruses and human-specific Bacteroidetes species are commonly found in sewage and from human-impacted watersheds. We used DNA-based qPCR to characterize human-derived pollution. However, the DNA of the dead microbes may persist in the environment. Metabolically active cells usually contain higher numbers of ribosomes than dormant cells. Therefore we developed RNA-based techniques to characterize active microbial populations. Adenosviruses were common in the CONPAT study area; 62% of the surface water samples contained low numbers of adenovirus gene copies. Human-specific source identifier HF183 was present in all but 10 water samples. However, the H183 gene copy numbers varied greatly depending on the environmental factors such as the sampling effects and distance from the municipal sewage discharge point. Furthermore, the rRNA:rDNA ratio indicating the activity level of the HF183 bacteria group was highest in the most polluted locations.

WE069 Development of a compact continuous submerged water samplers for
monitoring micropollutant time-weighted average concentrations using a 3D printer

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Grab sampling is a very basic and effective sampling method to measure micropollutants in water. However, determination by this method depends on the "sampling frequency" which is very high. Some problems associated with the presence of some of the analytes on the filters and on the sorbents were encountered and reduced by using thorough clean-up protocols. Various methods were applied in this study, namely, precision in terms of relative standard deviation (RSD) and limits of detection and quantification. Finally, the method was applied to determine micropollutants in several mussels samples. Acknowledgement - This work is financed by MINECO, in the frame of the collaborative project EMECONFO (CTM2014-56628-C3-2-R). We also acknowledge the support of Xunta de Galicia ("Consolidación" funds) and FEDER funding (HCMAR). We also thank the staff of the CEMAR (Technological Institute for Monitoring of the Marine Environment in Galicia) for providing several mussels samples.

WE072

SimpleBox 4.0: improving the model while keeping it simple...

A. Hollander, RIVM / DMO; D. van de Meent, Radboud University / Department of Environmental Science

Chemical behavior in the environment is often modeled with multimedia fate models. SimpleBox is one of the most used multimedia fate model, initially developed in 1986. Since then, two updated versions were published. Based on recent scientific developments and experience with SimpleBox 3.0, a new version of SimpleBox was developed and is made public: SimpleBox 4.0. In this new model, eight major changes were implemented: removal of the local scale and vegetation compartments, addition of lake compartments and deep ocean compartments (including the thermohaline circulation), implementation of intermittent rain instead of the continuous sources and deposition of pollutants by wet and dry deposition.

WE070

Polycyclic aromatic hydrocarbons in water of Bangladesh

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in nature and widely found in plant, air, soil and water. Anthropogenic activities e.g. motor vehicles, industrial processes, domestic heating, waste incineration etc. are major sources of PAHs to the environment. Due to their carcinogenicity, the water pollution caused by PAHs is a great concern worldwide. Dhaka, the capital of Bangladesh with an inhabitant of 20 million people is vulnerable for the anthropogenic activities. In order to evaluate the level of PAHs, 30 water samples were collected from three big rivers namely Megna, Buriganga and Turag that surround the city and Lake Gulsan within the city. The target polycyclic aromatic hydrocarbons (PAHs) were anthracene, fluoranthene and benzo[a]pyrene. Solid phase extraction using C-18 cartridge was used for the pre-concentration, extraction and clean up, and finally acetonitrile was used for elution of PAHs. High performance liquid chromatography coupled with fluorescence detector (HPLC-FLD; excitation wavelength: 340 nm, emission wavelength: 425 nm) using water-acetonitrile (1:1) was used for separation and laborious calibration of sampling rates for micropollutants. Recently small continuous low-level aquatic monitoring "C.L.A.M." has been developed which can perform low flow rate extraction sampling at sampling sites. C.L.A.M. is a very effective tool to estimate micropollutant concentration in low turbidity surface water. Therefore it is very meaningful to develop novel samplers to monitor micropollutants in particulate and dissolved form even in muddy surface water during several weeks. In this study, a novel sampler which can concentrate dissolved micropollutants at constant flow rates in surface water even with high concentration of suspended solids. Disks which absorb micropollutants in surface water are Empore disks. A filter upon the disk is a 47mm hydrophilic PTFE membrane filter. A flow cell which houses an Empore disk and PTFE membrane is developed using a 3D printer. This presentation will show various developed flow cell models and their filtration characteristics which is very important to build a novel sampler.

WE071

Determination of several organophosphorus triesters in mussels by pressurized liquid extraction and liquid chromatography-mass spectrometry

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Phosphoric acid triesters are mainly employed as plastic additives, as flame retardants and as plasticizers, but are also used in many other applications, such as in hydraulic fluids, paints and several industrial processes. Many of them are considered as high production volume chemicals and moreover, phosphoric acid triesters usage is growing due to the ban on the use of most polybrominated diphenyl ethers. Other triesters that are mostly used as plastic additives, phosphoric acid triesters are not chemically bonded but simply mixed with the bulk material they are intended to protect, and so they are relatively easy to release into the surrounding environment. Thus, they have been detected in a range of environmental compartments, like air, water and sediment, and more recently marine biota, which could contribute a health safety issue. Information on these compounds analysis is however limited to very few compounds and several particular triesters only. The aim of this work was the development of a comprehensive method for the extraction and determination of several classes of organophosphorus triesters (including alkylated, aroylated and chloro- and bromo-alkylated) in mussels samples. Pressurized solvent extraction (PLE) involving a clean-up process was employed as sample preparation technique and liquid chromatography coupled to mass (LC-MS) for separation and determination. The extraction conditions (solvent, sorbent temperature, time and number of cycles) were optimized maximizing both extraction and clean-up efficiencies. Blank problems associated with the presence of some of the analytes on the filters and on the sorbents were encountered and reduced by using thorough clean-up protocols. Validation of the method was realized by the analysis of certified reference materials. Correlation coefficients (r2) were 0.994, 0.997 and 0.998 for anthracene, fluoranthene and benzo[a]pyrene, respectively. The calibration curves were linear and the correlation coefficients (r2) were 0.994, 0.997 and 0.998 for anthracene, fluoranthene and benzo[a]pyrene. Good recoveries were obtained for the target analytes (anthracene, fluoranthene and benzo[a]pyrene) and ranging from 91, 85 and 96%, respectively. Analysis of PAHs by HPLC-FLD method has successfully been employed in the study of these PAHs, which can be useful information to understand the pollution sources in the environment. In the study of these PAHs, which can be useful information to understand the pollution sources in the environment. In the study of these PAHs, which can be useful information to understand the pollution sources in the environment. In the study of these PAHs, which can be useful information to understand the pollution sources in the environment. In the study of these PAHs, which can be useful information to understand the pollution sources in the environment.
Southern European Seas) aims to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, linking them to the Marine Strategy Framework Directive (MSFD) descriptors and indicators. Since marine sediments can affect the resident biota, aquatic-independent wildlife, and human health, the analysis of contaminants in sediments is a key factor for understanding the environmental quality of coastal waters. The present research demonstrates that numerous groups of contaminants in sediments are still poorly studied and represent a complex issue in terms of understanding the interaction mechanisms between different chemical compounds and possible environmental consequences. In this context, a monitoring survey (ADREX 14) has been conducted in order to investigate the spatial distribution of contaminants in sediments from the Adriatic Sea, Italy. Three groups of regulated compounds were analysed: PCBs, DDTs (p,p’-DDE, p,p’-DDT and p,p’-DDE) and PAHs. Total PCBs, DDTs and PAHs varied between 0.05 and 4.2 ng g⁻¹, 0.05 and 4.3 ng g⁻¹ and 38.8 and 570 ng g⁻¹, respectively. Spatial trends revealed a common pattern, with decreasing concentrations from the northern to the southern Adriatic Sea, suggesting an influence from the Po River, which is the major Italian watercourse. Diverse groups of CECs were analysed, such as personal care products (PCPs), which include fragrances, UV-filters and antimicrobials, organophosphorus compounds and endocrine disruptors compounds. Concentrations of PCPs ranged from 1- to 100-fold from the Northern Adriatic Sea to the Southern Adriatic Sea. Spatial trends suggested the Po River outflow to be a major contributor of pollutants to sediments in the Adriatic Sea. However, the patterns of some PCPs indicate point sources of these compounds, particularly in areas with higher human presence.

**WE075**

**Polycyclic Aromatic Hydrocarbons in the Danube River Sediments: Potential source contributions and carcinogenic risk assessment**

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The present investigation was undertaken to investigate potential pollution emission sources and carcinogenic risk of 16 priority PAHs in bottom sediments from the Danube River, Serbia. Three models (Diagnostic Ratios, Cluster Analysis and Principal Component Analysis – Multiple Linear Regression (PCA-MLR)) were applied to analyze the results obtained from ten collected bottom sediment samples. The total concentration of 16 PAHs ranged from 99.48 to 5283.66 ng/g dw with a mean (median) concentration of 271.86 ng/g dw (235.37 ng/g dw). Individual PAH analysis showed that four to six rings PAHs were the most frequently detected isomers and accounted 85% of the total PAHs concentrations. Source apportionment results derived from three different models, considering the whole dataset, were similar, indicating that the highest contribution to PAHs was from: (i) coal combustion, (ii) vehicular emission and (iii) grass and wood combustion. PCA suggested that these three principal components could be the primary PAHs contributors, accounting for 73.7%, 16.0% and 5.5% of PAH concentrations, respectively. Diagnostic ratios showed that PAHs in the sediments of the Danube River were predominantly of pyrolysis origin (only two sites: SUMW/2HMMW=1.57 and 4.66, respectively). According to hierarchical cluster analysis, collected sediments cluster in three major groups, Low (4 localities), Moderate (4 localities) and High (2 localities) contamination that is a consequence of the proximity of the main industries complexes consisting of a petrochemical factory, oil refinery and a chemical fertiliser factory. The levels in the site from cities with different pollution levels in comparison with Sediments Quality Guidelines (ERL/TEL and ERM/PEL), which suggested that sediment of the Danube River did not show any toxicocological risk for benthic organisms. The values of carcinogenic PAHs were in the range of 26.54 to 277.54 ng/µg dw. The toxic equivalent concentrations of carcinogenic PAHs were 6.14 to 12.9 µg/g dw. The PAHs sediments suggested a low carcinogenic risk for this area. **Acknowledgement:** Ministry of Economy, Science and Technological Development, Republic of Serbia (III/46009) supported this research.

**WE076**

**Identification of pollution hotspots in Norwegian marine sediments using local fish data**

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In the present research, we characterized spatiotemporal trends of sediment concentrations of chemicals known to be widespread in Norwegian marine water. To do so, we made use of historical data available through the Norwegian state pollution monitoring programme and the coordinated environmental monitoring programme (CEMP). We used generalized additive models (GAMs) to infer the spatiotemporal trends of the concentrations of PCBs, PAHs and trace metals (Cd, Cu, and Zn) detected over the period 1986-2013 at 53 sampling locations. GAMs with time, organic carbon content, latitude and longitude as covariates explained ca. 80% of the variability in the log transformed sediment concentrations. For each of the compounds we identified historical pollution hotspots (e.g. Sorøfjord (Western Norway) for mercury and zinc and Greelandfjord (South-Eastern Norway) for PAHs). To identify the local and regional drivers of the contaminant concentrations at these locations we pair the contaminant data with other relevant environmental data (e.g. water chemistry, input data, climatic data and remediation measures). The present research demonstrates that long-term data can provide useful insights in the fate and distribution of chemicals in the environment. The extensive monitoring campaign results in a long series of data, however for some chemicals the design does not allow coherent temporal trend analyses.

**WE077**

**Spatial patterns of target metals (Cu, Pb, Zn) in recent sediments from the Adriatic Sea**

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The Adriatic Sea has been facing numerous environmental and climatic challenges due to increasing anthropic pressures, contributing to the input of nutrients and contaminants to this marine system. The final sink for most contaminants is the marine sediment, which can represent the final sink of a complex cocktail of priority substances. Hence, approaches combining multiple contaminants (e.g. metals and polychlorinated biphenyls - PCBs) are extremely important and can contribute to the overall environmental quality status of marine ecosystems. This work was developed under the PERSEUS EU Project (Policy-oriented marine Environmental research in the Southern European Seas), guided by the Marine Strategy Framework Directive (MSFD), which aims to achieve a Good Environmental Status until 2020 in European water bodies. Our aim was to evaluate spatial trends of trace metals (Cu, Pb and Zn) associated to the levels of PCBs in surface sediments of the Adriatic Sea. In order to assess contaminant levels and distribution, a monitoring survey was conducted from northern to the southern Adriatic in October 2014 (ADREX 14). Surface sediments were analyzed for sediment characteristics (e.g. grain size and organic carbon) and target trace metals. Generally, sediment samples collected around the Po River Delta (northern Adriatic) presented higher concentrations of metals and PCBs in comparison to the concentrations detected in central and southern Adriatic. This preferential accumulation in the northern Adriatic has already been documented in previous previous works on the Adriatic Sea and is mainly related to the influence of the Po River discharge, which is the largest Italian drainage basin. Zn and PCBs were positively correlated (r=0.85 to 0.96) between the northern and the southern Adriatic (r=0.70; p<0.01). These correlations suggest a similar distribution of these compounds in the Adriatic Sea, probably driven by the same transport mechanisms and indicating terrigenous input. Trace metals concentrations were above the background levels for the Adriatic Sea (Zn=85, Pb=18,Cu=35 mg kg⁻¹). These elements, mainly Pb and Zn, have a large anthropogenic component in the marine basin, coming from atmospheric inputs and from industrial tailings and wastes. Finally, due to Adriatic Sea semi-enclosed character, metals and PCBs accumulation in the northern part could represent the influence of human activities, which could, in turn, alter the environmental quality status of this European sea.

**WE078**

Do persistent and mobile organic contaminants fulfill the definition of substances of very high concern owing to their equivalent level of concern defined by REACH?

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In Article 57 of the REACH Regulation (1), criteria are given in order to identify substances of very high concern (SVHCs). Within this definition SVHCs can be categorized into two groups: substances identified as “CMRs as defined in article 57 a-c) as well as persistent, biocumulative and toxic (PBT)/vPvB, as defined in article 57 d-e) substances. Furthermore, Article 57 provides a definition of compounds that should be identified as SVHC based upon their equivalent level of concern to CMR or PBT substances and having probable serious effects to human health and the environment. The debate on the definition of “level of concern” is open to interpretation. This work investigates whether persistent and
mobile organic contaminants (PMOCs) fulfill the requirements and thus can be considered as compounds possessing an equivalent level of concern. In order to investigate whether the requirements are fulfilled, we will focus on which properties of PMOCs could lead to a probable serious effects to human health or the environment at an equivalent level of concern to CMR or PBT substances. This in part is based on the ability of some PMOCs to reach water treatment plants, to resist treatment processes and thus to reach drinking water supplies enabling exposure to humans and thus possible adverse effects on human health. A literature review will identify PMOCs that have reached drinking water supplies based on normal use and not through accidental spillage. One prominent example is that of perfluorinated compounds used historically in aqueous film forming foams to put out fires and which has resulted in contamination of drinking water sources in Sweden and has impacted water supplies in Norway. These case studies will provide a line of evidence to define scientifically why PMOCs are hazardous and will then be used in the context of Article 57 to assess whether PMOCs are of equivalent level of concern and have probable serious effects on human health or the environment. (1) ARTICLE 57: Substances to be included in Annex XIV, TITLE-VII: AUTHORISATION CHAPTER-1: Authorisation requirement

WE079
Fast and accurate screening of small polar organics in the water cycle with UHPLC-QTOF
V. Albergamo, University of Amsterdam/IBED Institute; R. Helmus, University of Amsterdam IBED Institute / IBED; P. de Voogt, University of Amsterdam / IBED

The contamination of water sources with anthropogenic polar organic micropollutants (MPs) raises ecotoxicological and toxicological concern. Polar substances can be highly water soluble, highly mobile and can accumulate in aquatic ecosystems when they are not completely removed by natural barriers (e.g. riverbank filtration) and conventional water treatment. Their chemical identities and concentrations observed in the water cycle are constantly changing as a result of development of new products, hence reliable and up-to-date analytical methods for polar contaminants are needed. The characterization of organic pollutants is essential to assess the potential ecotoxic and toxic effects of individual chemicals and mixtures. In the present work, we developed an analytical method for the screening of small polar MPs with UHPLC-ESI-QTOF-MS. The method was applied to a selection of chemicals having low molecular weights (pIC_{50} < 4.5). The selection of the analytes was based on literature data and consisted of 25 polar MPs that have been observed in groundwater, drinking water, and that are not fully removed by reverse osmosis. Liquid chromatography was performed with a core-shell biphenyl stationary phase featuring specific polar retention mechanisms and 100% aqueous stability. Various mobile phase solvents and modifiers were tested, and optimal peak shape and sensitivity were achieved with a fast 7 min linear gradient of 0.05% acetic acid (A) and methanol (B). Water samples were extracted, purified and concentrated using optimized solid phase extraction methodology with hydrophilic lipophilic balanced (HLB) sorbent. The robustness of combining SPE with the UHPLC-ESI-QTOF-MS method was tested with groundwater, drinking water and demi water samples spiked with 50 ng/L analytes prior to extraction. Satisfactory recoveries resulted in LOQs within the sub-ng/L to ng/L range for both matrices. Identification with high certainty was supported by HR mass spectra. The method was incorporated in a novel software tool for automated target and suspect screening, which allowed fast identification and quantification based on the analytical method and the screening database. Combining SPE with an automated screening proved to be an efficient approach to analyze trace levels of a broad range of polar MPs, and can contribute to the characterization of toxic and ecotoxic profiles in the water cycle.

WE080
Diffusion of a dechlorinated metabolite of terbuthylazine in Northern Italian groundwaters

During a screening campaign on groundwaters used as a source of drinking waters in the area North of Milano, an anxiolytic drug, called Membral, has been found in several wells. The origin of this molecule has not been yet identified, but it was identified in the tests carried out by high resolution mass spectrometry (HRMS) for confirmation purpose an isobaric compound (MW 198.222360 g/mol and molecular formula CH_{3}H_{4}N_{2}O_{8})has been determined in many groundwater samples. The interpretation of the mass spectrum allowed to identify the compound as 4-(tert-butylamino)-6-hydroxy-1-methyl-1,3,5-triazin-2(1H)-one, which is a described dechlorinated metabolite of the erbicide terbuthylazine, also known as LM6. Other structurally related metabolites were tentatively identified by retrospective analysis via HRMS. These metabolites have been described in the registration document from Syngenta and in the ECHA dossier, but they are neither described in the scientific literature neither registered in CAS. Because the societal relevance of these findings for the erbicide terbuthylazine, we developed an HPLC-HRMS method based on an Orbitrap system which allowed to determine these compounds in groundwaters and treated drinking waters. In order to characterise the compounds and their diffusion in Northern Italian groundwater we carried out a wide monitoring campaign which highlighted the large diffusion of these compounds. The diffusion has been related to specific soil uses, with special regards to maize cultivation. The results of this extensive study with literature toxicological data allowed to draft a preliminary risk assessment for these metabolites which should contribute to the derivation of threshold limits for groundwater.

WE081
Screening of chemical pesticides in Swedish surface water and groundwater
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In early 2015 the Swedish EPA was commissioned by the Government to carry out a screening for environmental pollutants including chemical pesticides. A broad screening of chemical pesticides in surface water and groundwater in areas with high agricultural intensity in southern Sweden was therefore requested. This screening program was carried out by the Centre for Chemical Pesticides at the Swedish University of Agricultural Sciences during 2015. The screening was aimed at achieving a better surface coverage compared to the regular national monitoring program and consisted of extensive sampling of both surface water and groundwater. Surface water samples were collected from 46 different sites during May-October, half of the sites were sampled on 5 occasions and half of them on 3-5 occasions. The groundwater sampling was focused on private wells as previous monitoring has indicated that these are more sensitive to pesticide contamination than municipal groundwater wells. The sampling was done in 54 private wells and 18 municipal water works (incoming ground water) on one occasion. Surface water and groundwater samples were analyzed for 131 and 108 different pesticides, respectively. The results include information on which pesticides are the most frequently detected in Swedish surface water and groundwater, as well as on exceedances of the drinking water limit or ecotoxicological guideline values. The results are also analyzed in the light of collected metadata to explore potential correlations with elevated concentrations of pesticides. For surface water correlations with the portion of agricultural land and the size of the drainage area are analyzed. For groundwater relationships to the depth of the well, the distance to agricultural fields and the age of the well are examined. Correlations are also made with well water concentrations of nitrate and E. coli as possible signs of well vulnerability. The preliminary results from the screening program are largely consistent with results seen within the regular, spatially limited, national monitoring program. The final results are currently being analyzed and will be reported to the Swedish Government by mid-March 2016. A comprehensive evaluation of pesticide occurrence in Swedish waters will be available for poster presentation.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health (P)

WE082
Chronic toxicity of cefadroxil and cefadroxil in Daphnia magna and freshwater fish Orzyas latipes
B. Kim, Department of Environmental Health; K. Ji, Yongin University

Pharmaceuticals in the environment are of growing concern for their potential consequences on human and ecosystem health. Cefadroxil and cefadroxil, two cephalosporin antibiotics for animals, have been frequently detected in freshwater environment, however their toxicological information is very limited. In the present study, the adverse effects of chronic exposure to cefadroxil and cefadroxil were evaluated using Daphnia magna and Orzyas latipes. Chronic toxicity tests with D. magna was conducted as outlined in the OECD test guideline 211. D. magna were exposed to cefadroxil and cefadroxil (0, 6.25, 12.5, 25, 50 and 100 mg/L) for 21 d. Fish embryos were exposed to cefadroxil and cefadroxil (0, 0.001, 0.01, 0.1 and 10 mg/L) for 30 dph. Thymocyte (T4) levels in juvenile fish were measured using enzyme-linked immunosorbent assay (ELISA), and transcriptions of 8 genes in the hypothalamic-pituitary-thyroid (HPT) axis (trh, tsh, thea, thfl, tshr, tr, tgs, tgi, dilo, dliz) were also measured using real-time polymerase chain reaction (qPCR). The no obvious effect concentration (NOEC) based on the endpoints of survival, reproduction, and growth in D. magna exposed to cefadroxil and cefadroxil were 100 mg/L, except growth NOEC for cefadroxil (50 mg/L). Survival and body length in juvenile fish were significantly decreased at ≥0.1 mg/L cefadroxil. While no significant change was observed in T4 hormone, the transcriptions of trh and tgs genes were significantly altered by exposure to cefadroxil. Our results suggest that cefadroxil has the potential to inhibit the normal growth in macroinvertebrate and early-life stage fish, and these were accompanied by an inhibition of mRNA expressions related to thyroid hormone endocrine system. Concentrations of the pharmaceutical studied that have been reported to occur in rivers of Korea are much less than the
thresholds for effects on the endpoints studied here. Further investigations on endocrine disruption potential in aquatic organisms exposed to environmentally relevant concentrations are recommended. In Acknowledgement – This study was supported by National Research Foundation of Korea (Project no. NRF-2013R1A1A01061684).

**WE083** Evaluation of potential effect of pharmaceuticals in concentrated animal feeding operation (CAFO) of Korea

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There is a growing concern about the effects of pharmaceuticals and other pollutants from concentrated animal feeding operation (CAFO), since farm wastes often reach the environment without appropriate treatment. However, limited information is available on monitoring of antibiotic residues and potential risks in water environment around the animal farms. In the present study, potential toxicity on steroidogenesis in water extraction samples were evaluated using H295R cell lines. Measurement of veterinary pharmaceuticals and ecological risk assessment were also performed. Water samples were collected from five locations in a stream that runs alongside a CAFO area in Yongin, Korea. Several upstream and downstream locations were sampled for ambient water during June (low-flow) and August (high-flow), 2013. Total 12 pharmaceuticals (acetaminophen, cephradine, ibuprofen, mefenamic acid, six sulfonamides, and two tetracyclines) were evaluated using HPLC/MS/MS. The effects on the hormone production and expression of mRNAs involved in steroidogenesis were determined with H295R cell bioassays. Hazard quotients were derived from the highest measured environmental concentrations and predicted no effects for the concentrations. The pharmaceutical concentrations measured near CAFO area were greater than those in upstream or far downstream areas, suggesting that elevated pharmaceutical concentrations in waters are associated with activities of CAFO. Except one sampling area, the level of detection of the target pharmaceuticals were decreased in the high-flow season. However, greater production of 17β-estradiol (E2) was observed in the samples collected during the high-flow season, suggesting that possibly non-point source contaminants other than pharmaceuticals from CAFO may also cause endocrine disruption. Hazard quotients for several pharmaceuticals including acetaminophen exceeded unity, suggesting potential ecological effects in this area. Acknowledgement – This study was supported by National Institute of Environmental Research of Korea.

**WE084** Effects of temperature, genetic variation and species competition on the sensitivity of algae populations to the antibiotic enrofloxacin

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Primary producers, particularly cyanobacteria, are amongst the most sensitive organisms to antibiotics and pharmaceuticals. In this study we investigated the effects of the fluoroquinolone antibiotic enrofloxacin on the cyanobacterium Microcystis aeruginosa and the green algae species Scenedesmus obliquus. The toxicity of the antibiotic was investigated in the laboratory under different temperature conditions (20 and 30°C) and using three different strains of each species. Furthermore, it was investigated how antibiotic pollution affects the competition between *M. aeruginosa* and *S. obliquus*. The competition experiment was performed using three competition treatments, defined as density ratios (i.e. initial bio-volume of 25/75%, 10/90% and 1/99% of *S. obliquus*/M. aeruginosa, respectively), one *S. obliquus* control (100% *S. obliquus* and one *M. aeruginosa* control (100% *M. aeruginosa*), and five different ENR concentrations (i.e., control, 0.01, 0.05 and 0.10 mg/L). In all experiments, growth inhibition based on cell number, bio-volume, chlorophyll-a concentration, as well as PSII efficiency were used as evaluation endpoints. In the majority of the cases PSII efficiency was found to be the most sensitive endpoint, followed by growth inhibition based on cell number. *S. obliquus* was found to be slightly more sensitive at 20°C than at 30°C (EC50-72h of 38 and 41 mg/L, respectively), whereas an opposite trend was observed for *M. aeruginosa* (0.047 and 0.037 mg/L, respectively). Differences in EC50s between strains were within a factor of two. The competition experiment showed that *M. aeruginosa* growth can be significantly reduced at 0.01 mg/L in the presence of *S. obliquus* at a density ratio of 25/75% *S. obliquus*/M. aeruginosa. The results of this study confirm the high sensitivity of cyanobacteria to antibiotics and show that temperature and genetic variation can influence their response to them. Furthermore, the results of the competition experiment suggest that the structure of primary producer communities can be affected at antibiotic concentrations close to those that have been monitored in the environment.

**WE085** Assessing sensitivity to antibiotics in freshwater cyanobacteria and microalgae

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Cyanobacteria (CB) and microalgae (MA) play fundamental roles in primary production and nutrient-cycling in aquatic systems. Disruption to these communities through chemical exposure can therefore have far-reaching ecosystem consequences. Antibiotics (AB) cause toxicity to prokaryotic cells. Although there are many antibiotic drug targets, prokaryotic enzymes are usually conserved in some eukaryotes, for example in MA that have chloroplasts, the vast majority of the targets are not conserved. The likely consequence is that eukaryotes such as metazoans will not show adverse effects to ABs at exposures of environmental relevance. Environmental risk assessment (ERA) for the assessment of AB requires testing with growing biological systems. The study encourages testing for a single species of CB for prokaryotes, as an alternative to the test with a MA. Current ERA of ABs therefore may not be protective for prokaryotes as a whole. Given their importance to ecosystem services we argue that wider understanding on the effects of ABs on prokaryotes and algae is required. It has been suggested that the ERA of ABs should evaluate risk of antibiotic resistance and the predictive no effect concentration may be based on minimum inhibition concentrations (MIC) in clinically relevant bacteria. Increased knowledge regarding differences in species sensitivity will help ensure MIC based ERA will be protective for environmentally relevant bacteria. On the premise that knowledge of variation in species sensitivity to antibiotics in CB and MA is important, to better inform current ERA practice for the protection of prokaryotes and algae, we investigated the effects of 3 ABs with different MOAs (ciprofloxacin, sulfamethoxazole and cefotaxime) on CB and on MA. We set out to determine whether one species of CB is representative of a diverse class of taxa and across different MOAs. Inhibition of population growth (a standard regulation endpoint), expression of relevant target genes (via qPCR) changes in the cell phenotype (through TEM), and bioavailability were for each species and AB studied. Significant differences in the growth rate for the different AB exposures were observed between the CB and MA. Moreover, the three CB species varied in their sensitivity to the AB effects by up to one order of magnitude. Data will be presented illustrating how sensitivity in responses relates to bioavailability and pharmaco-dynamic properties of each antibiotic.

**WE087** Selection for Antibiotic Resistance in Complex Microbial Communities

L. Stantoon, University of Exeter / Medical School

Antibiotics have been regularly used since the 1900’s to treat bacterial infections and have revolutionised modern medicine. However, after development of a particular antibiotic class, resistance genes to counteract the effect of the antibiotic have always emerged. Therefore, there is potential to return to a pre-antibiotic era where treating bacterial infections becomes impossible. Antibiotics are found throughout the environment. They are naturally produced by certain bacterial species; however human activity also leads to the release of measurable quantities into environmental systems. Release into the environment can occur through use of antibiotics in the clinic, as the more stable compounds are excreted into water systems. However, other routes of antibiotic release through agriculture and as by and as growth promoters in agriculture, release from pharmaceuticals and use in personal care products. Antibiotics in the environment have the potential to exert a selective pressure on environmental bacteria causing resistance. Recently (2015), antibiotics have been added to the priority substances watch list of the Water Framework Directive for the first time. Macrolides, the group added, have been found in concentrations of up to 90µg/L within water environments. This study will investigate the evolution of macrolide resistance in aquatic systems by studying the genetic mechanisms of macrolide resistance in complex microbial communities and studying experimental evolution of macrolide resistance using culture independent and next generation sequencing methods.

**WE088** Minimum inhibitory concentrations and antibiotic resistant genes in the freshwater cyanobacteria Plankothrix agardhii

E. Dias, National Institute of Health Doutor Ricardo Jorge / Department of Environmental Health; M. Oliveira, National Institute of Health Doutor Ricardo Jorge / Department of Health Sciences; D. Jones-Dias, Infectious Diseases / Department of Infectious Diseases; V. Maneiro, National Institute of Health Doutor Ricardo Jorge / Department of Infectious Diseases; C. Churro, National Institute of Health Doutor Ricardo Jorge / Department of Environmental Health; V. Vasconcelos, Faculty of Sciences and CIBIR - Porto University; E. Ferreira, National Institute of Health Doutor Ricardo Jorge / Department of Infectious Diseases; M. Canica, National Institute of Health / Department of Infectious Diseases

Objectives: Cyanobacteria are ubiquitous prokaryotes in aquatic ecosystems and although they can be exposed to antibiotics and antibiotic resistant bacteria, their role on water resistome was never investigated. *Plankothrix agardhii* is one of the

SETAC Europe 26th Annual Meeting Abstract Book 271
most common cyanobacteria species in Portuguese freshwater reservoirs, often exhibition long residence time in those reservoirs. This work aimed to evaluate the antibiotic susceptibility patterns and resistance mechanisms in P. agardhii in order to assess their putative contribution to the global pool of resistance determinants in freshwater. Methods: We investigated 8 strains of P. agardhii, previously isolated from freshwater reservoirs. Susceptibility was evaluated by a microdilution method previously adapted for cyanobacteria, against beta-lactams, aminoglycosides, quinolones, trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to cell density (OD, 450nm) and microscopic examination of cultures integrity. All strains were screened for resistance to antibiotics using clinical breakpoints for Enterococcus spp. The presence of integrons (class 1 or 2) was detected by PCR/sequencing. Results: The results showed that P. agardhii is not susceptible to trimethoprim and quinolones within the tested concentrations (0.0015-1.6 mg/L). However, the cell growth is strongly inhibited by amoxicillin (median MIC value of 0.1mg/L). The other antibiotics presented the following minimum inhibitory concentrations (MICs): cefazidime, 1.6 mg/L; ceftriaxone, 0.8 mg/L; tetracycline, 0.4 mg/L; kanamycine, 0.2 mg/L and gentamicine, 0.1mg/L. None of the P. agardhii strains exhibited genes associated with trimethoprim and quinolones resistance, which suggests that these cyanobacteria are intrinsically non-susceptible to this antibiotics. Conversely, genes associated with streptomycin (smr-arb) and sulfonamide (sul) resistance, as well as a 1-type integron, were detected in three of the strains. These strains were isolated from the freshwater reservoirs where Planktothrix blooms are frequent and. This suggests that the presence of a common antibiotic resistant determinant in P. agardhii might be a result from a similar selective pressure within those reservoirs. Conclusions: The presence of antibiotic resistance genes and integrons, as well as the reduced susceptibility to antibiotics, suggest that cyanobacteria may play a role on freshwater resistant and eventually contribute to the dissemination of antibiotic resistance in freshwater environments.

WE089 Antimicrobial susceptibility of E. coli isolates from meat industry wastewater M. Stosic, Faculty of Technical Sciences, Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; D. Cucak, University of Novi Sad Faculty of Sciences; S. Kovacevic, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; M. Perovic, Jaroslav Cerić Institute for the Development of Water Resources; J. Radonic, Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; M. Turk-Sekulic, University of Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; M. Vojnovic Miloradov, Faculty of Technical Sciences Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; D. Radnovic, University of Novi Sad Faculty of Sciences Wastewater originating from the meat processing industry is characterized by the high presence of organic waste load and potentially pathogenic microorganisms. This study consisted of four sampling campaigns, during four seasons in 2013 and 2014. Wastewater was collected from three meat processing plants in the Province of Vojvodina, Republic of Serbia. The aim of this research was to determine the possible resistance of isolated E. coli strains to selected antibiotics. The evaluation of the antimicrobial susceptibility was performed on 37 strains of E. coli to 9 different antibiotics. Antibiotics, as emerging pharmaceuticals, used for susceptibility testing were: ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Monitoring of antimicrobial resistance in commensal bacteria E. coli is of great significance because this species is commonly present in animal faeces, and can often acquire conjugative plasmids from other enteric bacteria, thus serving as a valuable reservoir of resistance genes which can be transferred to other bacteria present in the same environment. From a total of 37 strains of E. coli, a moderate degree of resistance was shown to tetracycline (37.83%); low degree of resistance to ampicillin (21.62%), streptomycin (24.32%), trimethoprim-sulfamethoxazole (18.92%) and nalidixic acid (16.21%); and a very low to: chloramphenicol (13.51%), ciprofloxacin (2.7%), gentamicin and cefotaxime (0.0%). Ten of the tested E. coli strains (18.92%) were multirdrug-resistant. Antibiotics used in this experiment are present on the NORMAN list of emerging substances of concern, since they are most frequently detected in aquatic environment. Due to their detectable presence in the environment, it is of great importance to carefully monitor the susceptibility of antimicrobial resistance in microorganisms which have been in contact with antibiotics residues. High public concern considering the consequences of mass appearance of multi-resistant bacterial strains that have spread from the animals to humans, managed to significantly reduce the use of antibiotics, especially as growth promoters. However, the need to adopt this approach falls below the need to put more stringent control of law implementation is needed. Acknowledgement: This work has been supported within the NATO project (Ref. 984087), and it has also been financially supported by Ministry of Education and Science, Republic of Serbia (Project No. 46009).

WE090 Presence of multiple antibiotic resistant Enterococcus spp. harboring virulence genes in WWTP effluent and receiving water systems in the North West Province, South Africa L.G. Molale, North West University (Potchefstroom Campus) / Microbiology; C.C. Bezuidenhout, North - West University / Unit for Environmental Sciences and Management Facetals contaminants from poor operating waste water treatment plants pose a health risk to humans and animals. The latter is heightened when multiple antibiotic resistant bacteria harbouring virulence genes are present. The aim of the current study was to determine the antimicrobial resistance patterns and presence of virulence genes in Enterococcus spp. isolated from the final effluent of three municipal wastewater treatment plants and receiving water systems in the North West Province, South Africa. Sixty three Enterococcus isolates were isolated and antimicrobial susceptibility test performed on all isolates. Antibiotic inhibition zone diameter data was subject to cluster analysis. The cluster composed of Enterococcus spp. from all WWTPs final effluent was predominated by E. faecalis spp. followed by E. avium spp. Seventy three percent of Enterococcus spp. from downstream sites of receiving water systems was predominated by E. gallinarum, E. casseliflavus and E. mundtii. All 63 Enterococcus spp. were screened for the presence of five virulence determinants (asa1, cylA, esp, gelf and hly). All five virulence genes were detected and six multi-virulence profiles observed. Analysis of the antimicrobial susceptibility of the 63 Enterococcus isolates revealed that resistances to Ampicillin (67%), Vancomycin (62%), Tetracycline (58%), Penicillin (52%) and Erythromycin (51%) were most frequent. Sixty eight percent of the screened Enterococcus spp. were resistant to three or more antibiotics. Seventy six of the screened Enterococcus isolates resistant to multiple antibiotics had plasmids. Differences in M protein, and profiles were observed for plasmids with lower resistance profiles post plasmid curing. This study has demonstrated that Enterococcus spp. harbouring virulence factors and plasmids that mediate multiple antibiotic resistance are present in WWTPs final effluent and receiving water systems that support a variety of social needs in South Africa. Thus, it is recommended that Enterococcus spp. be used an additional faecal indicator in conjunction to E. coli. Keywords: Enterococcus spp.; WWTP final effluent; multiple antibiotic resistance; virulence genes

WE091 Antibiotic-resistant bacteria from air samples collected in nursing homes V. Salgueiro, V. Manageri, National Institute of Health Doutor Ricardo Jorge / Department of Infectious Diseases; D. Jones-Dias, Infectious Diseases / Department of Infectious Diseases; M.M. Cano, National Institute of Health Doutor Ricardo Jorge / Department of Environmental Health; E. Ferreira, National Institute of Health Doutor Ricardo Jorge / Department of Infectious Diseases; M. Canig, National Institute of Health / Department of Infectious Diseases Introduction Antibiotic resistance is currently a worldwide public health problem that can include diverse mechanisms and bacterial species. The understanding and monitoring of such phenomena is essential to avoid further dissemination. The main aim of this study was the characterization of antibiotic resistance mechanisms in bacterial strains collected in air samples from nursing homes and compare them with clinical samples isolated from the same geographic area. Materials and methods Air samples were collected from bedrooms, living rooms and outdoor of 4 nursing homes located in Lisbon, Portugal. Screening of antimicrobial susceptibility of 18 Gram-negative (5 Acinetobacter spp., 1 Klebsiella oxytoca, 4 Panteus spp., 7 Pseudomonas spp., 1 Sphingomonas paucimobilis) and 12 Gram-positive (3 Micrococcus luteus, 3 Staphylococcus spp.) isolates was performed by disk diffusion method. Different antibiotic resistant genes were searched by PCR. PFGE was used to evaluate clonality between K. oxytoca isolated from the air environment and other strains from clinical origin. Results and discussion The majority of strains were susceptible to all antibiotics tested. Among beta-lactam antibiotics, reduced susceptibility to cefoxitin was detected in Staphylococcus capitis, through expression of the mecA gene, and to ampicillin, piperacillin and piperacillin-tazobactam in 1 K. oxytoca expressing a blaOXY-type beta-lactamase. Nonsusceptibility to meropenem and piperacillin-tazobactam was observed in 2 Pseudomonas putida however no antibiotic resistant gene was detected. Regarding quinolones, non-susceptibility was found in 1 S. capitis, 2 Staphylococcus haemolyticus, and 1 K. oxytoca. The molecular characterization of the mecA-positive S. capitis from the nursing homes and from a hospital within the same region suggests a potential dissemination of strains between these two environments. The genetic relatedness of K. oxytoca from nursing homes (n=1) and clinical isolates (n=9) recovered within the same region, allowed to conclude that they were not genetically related. Conclusions Globally, nursing homes environments may act as complementary reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Thus, a better understanding of the antibiotic resistance mechanisms and dissemination pathways in other reservoirs than human is essential to control its emergence and spread.

WE092 Antimicrobial susceptibility and intrinsic diversity in organic and conventionally grown fruits and vegetables D. Jones-Dias, Infectious Diseases / Department of Infectious Diseases; J.B. Setac Europe 26th Annual Meeting Abstract Book
Moura, E. Ferreira, National Institute of Health Doutor Ricardo Jorge / Department of Infectious Diseases; M. Caniça, National Institute of Health / Department of Infectious Diseases

Introduction: During growth and harvesting fresh produce can become contaminated not only with environmental, but also pathogenic bacteria from animal and human sources. This study aimed to characterize the antibiotic resistant bacteria gathered from organically and conventionally grown fruits and vegetables, and to characterize the diversity of class 1, 2 and 3 integrons detected. Methods: Between 2013 and 2014, one conventionally and one organically produced batch of six fruits and vegetables were purchased at retail stores. Pears were grown with conventional practices. 50 g of each fruit random without washing or peeling, diluted and homogenized. The selection of resistant Gram negative bacteria was performed in VRBG plates containing ten different antibiotics separately. The isolates were identified through the amplification of the 16S rDNA. Antimicrobial susceptibility was assessed by disc diffusion method using Mueller-Hinton agar guide plates. Antibiotics of class 1, 2 and 3 integrons, through PCR amplification, and their variable region was explored by a strategy of Next Generation Sequencing. Results: A total of 333 isolates showing nonsusceptibility to, at least, one antibiotic were collected among 144 samples. Among others, Enterobacteriaceae (n=184), Moraxellaceae (n=88), Pseudomonadaceae (n=36), were detected. Nonsusceptibility was mainly identified among \( \beta \)-lactam antibiotics for Enterobacteriaceae recovered from conventionally produced samples (45.1%); overall, cefoxitin was the only ineffective antibiotic (19.6 % for organic and 26.6% for conventionally grown produce). The molecular screening for the integrase-encoding gene showed the presence of eight isolates with class 1 (2 Enterobacter, 2 Escherichia, 2 Morganella, 2 Pseudomonas spp.), three isolates with class 2 (1 Escherichia and 2 Raoultella spp.), and one isolate with class 3 integrons (Klebsiella spp.). The integrons revealed a variety of genes cassettes conferring nonsusceptibility to different classes of antibiotics. Conclusion: Resistance was more prevalent in the products grown in close contact with the soil. Besides environmental bacteria, some pathogenic agents were also detected. Leakage was significantly associated with the existence of class 1 and 2 integrons regardless of the origin. Both organic and conventional produced fruits and vegetables may constitute potential sources of resistant bacteria and of integrons.

WE093 Bioavailability - A concept to unravel the antibiotic effects on soil microbial functioning? Authors & affiliations: O. Crouzet, INRA Institut National de la Recherche Agronomique / UMR ECOSYS, A. GOULAS, INRA, UMR1402 ECOSYS, 78580 Thiverval Grignon, France; A. Richaume-Jolion, University Claude Bernard Lyon 1; C. HAUDIN, AgroParisTech, UMR1402 ECOSYS, 78580 Thiverval Grignon, France; P. BENOTO, INRA, UMR1402 ECOSYS, 78580 Thiverval Grignon, France The intensive uses of antibiotics (AB) in human and veterinary medicines contributed to widespread environmental contaminations, through the wastewater discharges and the recycling of organic wastes (OW) as soil amendments. While the occurrence and persistence of AB in the environment have become major environmental and human health issues, their ecotoxicological impacts are not well understood. AB inhibit active microorganisms and therefore can represent an important risk for the environment, especially for soil microbial functions (organic matter degradation, nutrient recycling). However, these side-effects depend on the bioavailability of AB. Organic matter quality is a key environmental factor influencing soil dissipation processes of AB which can control the bioavailability. Our objectives were to investigate the effects of the sulfamethoxazole (SMX) on microbial nitrogen transformations in relation with AB bioavailability in soil. A dose-effect approach was performed with different doses of SMX supplied to different OW (compost of sewage sludge / green waste (SGW), farmyard manure (FYM)) before their application to soil. The final doses of SMX in soil microcosms ranged from 0.022 to 2.22 mg kg\(^{-1}\) as, with control soils amended with OW without SMX. Nitrogen forms, potential nitrification and denitrification activities were determined in soils, after 7, 28 and 84 days following amendments. Also, Total bacterial and chemotaxonomic fraction were extracted respectively with organic and aqueous solutions and then quantified by UPLC-MS/MS. At these low doses, only nitrification activity was adversely impaired by SMX, following a dose-response pattern, in soils amended by SGW. Actually, as the SMX is a bacteriostatic antibiotic, the effects were stronger for growth inhibition of bacteria than for inhibition of activity which are largely promoted by the high levels of NH\(_3\) in SGW amended soils compared to FYM amended soils. The chemical assessment of SMX availability did not show strong differences between the SGW or FYM amended soils. However, throughout the experiment, the decrease of SMX availability was in accordance with a partial recovery of soil nitrification in SGW amended soils. Nitrification is a sensitive indicator of AB effects on soil microbial functioning. Its recovery after AB exposure may be explained by a strong decrease in the chemical availability and consequently in the bioavailable fraction of AB for microorganisms.

WE094 Bacterial community structure and biogeochemical activity in soil irrigated with treated wastewater in Tunisia C. Michiel, BRGM; O. Mahjoub, INRGEF; L. Lalane, BRGM; a. Mauffret, BRGM / Environmental biogeochemistry and water quality

In semi-arid regions, agricultural activity relies on water availability. For instance, during the dry season, from June to September in Nabeul region (Tunisia), the irrigation of citrus trees with treated wastewater is an old practice. Conjunctive use of treated wastewater (salinity, pH, g/L of N, P and K), provides enhanced water use efficiency. In this work, we focus on the effect of emerging substances, possibly present in the irrigation water, on soil bacteria diversity and function. Three irrigated plots were selected based on the type of irrigation water: treated wastewater, ground water, and treated wastewater and ground water together. Soils were sampled at 0-5 and 20 cm depth in June, July, August, and October 2015. Protein production (3-H leucine incorporation) and mineralization of different carbon sources (Biolog) were measured to assess activity of the microbial community in soil samples. Abundance of the universal marker (16S rDNA) and of nitrate-reducing bacteria (narG and napA) was assessed by qPCR. Presence of the ammonium oxidizing bacteria (AOB) was assessed by the detection of amoA gene. Biodiversity was assessed with a fingerprinting technique (CE-SSCP). Forty emerging substances, including human and veterinary antibiotics, were assessed in groundwater and treated wastewater by HPLC/MS/MS. Preliminary results showed that caffeine, carbamazepine and its metabolite 10.11-epoxycarbamazepine, ketoprofen, ofloxacin, pranoproanol hydrochloride, and sulfamethoxazole were quantified in both ground and treated waters at concentration higher by 2 orders of magnitude in the treated water (n=4). The results allowed determining whether soil microbial communities are affected by irrigation water and their implication in biogeochemical cycle.

WE095 Removal Characteristics and Mechanism of Antibiotics in the Constructured Wetlands Y. Choi, Seoul National University / Department of Environmental Health Sciences; M. Kim, Environmental Health / Department of Environmental Health Sciences; J. K. Zoh, Seoul National University / Department of Environmental Health

The occurrence and removal of antibiotics (sulfamethoxazole (SMZ), sulfathiazole (SFI), sulfamethazine (SMA), trimethoprim (TMP), tetracycline (TC), oxytetracycline (OTC), chlorotetracycline (CTC), and enrofloxacin (EFX)) using a constructed wetland (CWs) for treating livestock wastewater were investigated. The levels of antibiotics in the effluents of the CWs were in the order of CTC, SMA, SMZ, SMA, TMP, OTC, EFX and TC, ranging from 47.98 to 6883.646 μg/L, respectively. There was an inverse correlation (p < 0.0493) between the removal of sulfonamide group (SMZ, SFI, and SMA) and tetracycline group (TC, OTC, and CTC) antibiotics in the effluents of the CWs, indicating that sulfonamide-type antibiotics were more effectively removed in the CWs. Sulfonamide-type antibiotics have higher pKa values, resulting in more effective adsorption into negatively charged soils through electrostatic interaction. Therefore, the physicochemical properties (e.g., molecular weight, pKa value, ionic bonds, and functional groups) can be the important factors in CWs. Sunlight photo-degradation experiment showed that EFX was effectively removed (70%) compared to other antibiotics. The microcosm adsorption experiments using wetland soils under biotic and abiotic conditions showed that antibiotics in biotic system were more effectively removed than abiotic systems, indicating that soil-mediated microbial degradation is a major removal mechanism. The multi-residue adsorption experiments using wetland plants (Phragmites arundinaceus) showed that the biotic system also removed sulfonamide-type antibiotics more effectively compared to the abiotic system. Our results suggest that the removals of antibiotics in the CWs are mainly mediated by biodegradation and adsorption onto soil and plants. This study implies that the CW system can be used for the removal of antibiotics for syndromic livestock wastewater treatment.

WE096 Direct Photolysis of Ofloxacin and Sulfamethizidazole S. Chen, College of Chemistry and Environmental Engineering; S. Yang, Y. Lin, Harbin Normal University / College of Chemistry and Environmental Engineering

Due to large scale uses in animal husbandry and aquaculture, ofloxacin (OFL) and sulfamethizidazole (SMZ) are representative antibiotics found in the surface waters in China, and photolysis is considered as an important pathway for their degradation either in nature. In this study, photo-induced photolysis experiments using wetland plants (Phragmites arundinaceus) and simulated sunlight (SL) and mercury vapor lamp (MVL) were investigated with an Agilent Technologies 1260 Infinity HPLC as the analytical tool. Depending on the light intensity of the SL and MVL, typical half-lives of these two synthetic antibiotic compounds were in the range of minutes and the reactions seemed to follow first order kinetics. Mixed aqueous solution of OFL and SMZ was exposed to SL and MVL, respectively, air and light, and it was found that SMZ underwent faster photolysis than OFL, but the rates of degradation were slower in comparison with their single component individual rates. The presence of hydrogen peroxide or molecular oxygen in the aqueous solutions seemed to accelerate the photolysis for both the OFL and SMZ. MVL emitted more UV radiation than the SL and tended to enhance the photolysis efficiency. This study detected the existence of several photolysis products, and ion chromatography analysis of
the photo-products of OFL with a Metrohm 790 IC indicated the presence of fluoride, suggesting that fluoride atomic was removed from the molecule of OFL. Dihydroxy-olfoxacin (i.e. 8-Fluoro-3-hydroxy-9-(4-hydroxy-piperazin-1-yl)-6-oxo-2,3-dihydro-DH-1-oxa-3a-aza-phenalene-5-carboxylic acid), and hydroxyl-olfoxacin (i.e. 8-Fluoro-3-hydroxy-9-(4-hydroxy-piperazin-1-yl)-6-oxo-2,3-dihydro-DH-1-oxa-3a-aza-phenalene-5-carboxylic acid), defluoro-olfoxacin, demethyl-olfoxacin were suggested as the possible photolysis products and the analogues of the original antibiotic. Computations of the proton and carbon-13 NMR (nuclear magnetic resonance) chemical shifts (termed as NMR indices) of the possible photolysis products of OFL revealed the persistent molecular structure typical of quinolone, and the significance of such feature is discussed in terms of bacteria quinolone-resistance and nucleotide sequences of DNA gyrase in the clinical isolates publicized in scientific journals. It is postulated that sub-lethal exposures to the analogues helped bacteria develop resistance to original compounds. The NMR index of a given compound was proposed as a convenient parameter for estimation of antibiotic resistance.

Antibiotic residues in the polish coastal zone (southern Baltic Sea) - suggested as the possible photolysis products and the analogues of the original products of OFL revealed the persistent molecular structure typical of quinolone, and the significance of such feature is discussed in terms of bacteria quinolone-resistance and nucleotide sequences of DNA gyrase in the clinical isolates publicized in scientific journals. It is postulated that sub-lethal exposures to the analogues helped bacteria develop resistance to original compounds. The NMR index of a given compound was proposed as a convenient parameter for estimation of antibiotic resistance.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (P)

Transport and toxicity for bacterial communities of reactive iron nanoparticles used for nanoremediation

Nanoparticles (e.g. nZVI (Zero-Valent Iron), iron oxides, ferrate) applicable as in-situ reduction or oxidation agents for groundwater treatment show an extremely high reactivity leading to effective transformation of many contaminants into less toxic or benign products. However, these nanoparticles (NP) may also represent an additional contamination. Moreover, the different processes controlling the fate of NP and their toxicity towards microorganisms remain poorly understood. This study, a part of the EU-project NanoRem (Taking Nanotechnological Remediation Processes from Lab Scale to End User Applications for the Restoration of a Clean Environment; EU FP7/2013-2017), aims to (i) study the mobility of NP in the presence or absence of biofilm by columns transport assays mimicking real aquifer conditions and (ii) to evaluate NP toxicity on bacterial communities (planktonic and biofilm grown onto sand). Results show that the mobility of nZVI largely depended on the water flow and/or NP concentration. The recovery at the column outlet was 1% for a 100 mg L⁻¹ and 40 % for 10 mg L⁻¹ water flow. The presence of biofilm in the column decreased its total porosity from 35% to 25%. Though the recoveries of nZVI in the presence or absence of biofilm were similar, the analysis of the sand showed that the variation of labelled Fe content is concomitant with that of TOC, strongly suggesting NP-biofilm interactions. That was confirmed by CryoSEM images. Otherwise, the measures of the denaturing activity showed a toxicity of NP on planktonic bacteria starting at 50 mg Fe.L⁻¹ while cellular viability evaluated by Live and Dead method highlighted toxicity starting at 10 mg Fe.L⁻¹. It appears therefore that reactive NP, very useful for in-situ groundwater treatment, can represent a source of emerging contamination. Other studies about mobility and toxicity of such particles will have to be conducted before a prospective large-scale application.

Antibiotic residues in the polish coastal zone (southern Baltic Sea) - suggested as the possible photolysis products and the analogues of the original products of OFL revealed the persistent molecular structure typical of quinolone, and the significance of such feature is discussed in terms of bacteria quinolone-resistance and nucleotide sequences of DNA gyrase in the clinical isolates publicized in scientific journals. It is postulated that sub-lethal exposures to the analogues helped bacteria develop resistance to original compounds. The NMR index of a given compound was proposed as a convenient parameter for estimation of antibiotic resistance.

PharmCycle: Sustainable Pharmaceuticals

Engineered nanoparticles in cosmetics according to EU 2011/696/EU: sample preparation approach. Pre-characterization in order to identify possibly effects of AgNPs are mainly attributed to the silver ions (Ag⁺) released from dissolution for two AgNPs, differently coated with citrate (Cit) and branched (liver and kidney) presented a significant bioaccumulation. Interestingly, AuNPs from BPEI-AgNPs in 30 mg/L of PLFA, 1.08 ± 0.19 µg/L, was also slightly lower runs with sustainable antibiotics, including newly designed antibiotic peptides, produced within PharmCycle using molecular and biotechnological methods. The third cycle runs with sustainable antibiotics designed with physical-chemical and in silico methods. Every cycle includes the upgraded ecotoxicological risk assessment and investigations on wastewater treatment procedures. The approach of the project and first results are shown.
Fate of TiO2 nanoparticles in the aquatic environment in the presence of anthropogenic compounds

S. Ilina, N. Baran, N. Devau, BRGM; D. Slomborg, Labex Senerade/CEERGE/Aix-Marseille Université; N. Sani-Kast, ETH Zurich / Institute for Chemical and Bioengineering; J. Labillie, CNRS; M. Scherigner, ETH Zurich / Institute for Chemical and Bioengineering; P. Ollervod, BRGM

The increasing production and use of nanoparticles (NP) in consumer products inevitably lead to EPN emissions into the environment. The physicochemical properties of NP depend on various parameters (e.g., pH, cations, IS). In natural waters, the stability of NP can vary as a function of a sum of these parameters and occur by one of the numerous scenarios. In particular, the presence of anthropogenic organic molecules (AOM) can change the NP fate. Also, the presence of NP may affect the organic pollutants (fate and toxicity). The main objective of the work was to study the aggregation of TiO2 NP (pure hydrophilic 100% rutile and pure hydrophobic 100% anatase, 5-30 nm) in the presence of the nanocomposites containing TiO2 NPs in the water. The results demonstrated that the studied AOMs, glyoxal (4-pKa) and albumin (pH 5.5) affect the NP aggregation/stabilization in a wider range of physicochemical conditions. Overall, these results will aid in the evaluation of potential environmental risks posed by engineered NPs in the aquatic environments exposed to pesticide load.

WE102 Quantification of ionic silvers released from different surface-coated silver nanoparticles in the presence of fulvic acids

Y. Jue, KIST Europe; Y. Kim, S. Baik, KIST Europe / Environmental Safety Group

Studying the roles of natural organic matter (NOM) in fate and transport of nanoparticles (NPs) after their releasing into the aquatic environment has been performed by many other researchers. Silver nanoparticles (AgNPs) were chosen for this study due to their potential toxic effects from both ionized and nanoparticulate forms. Especially, previous researches suggested that the toxic effects of AgNPs are mainly attributed to the silver ions (Ag+) released from AgNPs. The objective of this study, therefore, is to assess the role of NOM in AgNPs dissolution for two AgNPs, differently coated with citrate (Ct) and branched polyethyleneimine (PBEI). Pony Lake Fulvic Acid (PLFA) was selected as a model NOM due to its higher contents of S and N with high affinity to metallic Ag and Ag+, compared to the other commercially available NOM standards. PLFA solutions were prepared in 10 mM NaNO3 with the concentrations of 5 and 30 mg/L. Time-dependent dissolution of AgNPs was monitored in the absence and presence of PLFA for 2 days and 6 days, respectively. Quantification of Ag+ released from AgNPs was performed using a single ion detection inductively coupled plasma mass spectrometer (ICP-MS). Prior to the analysis by ICP-MS, Ag+ ions were separated from AgNPs particles using a ultrafiltration filter with 10 kD of molecular weight cutoff (MWCO) and a dialysis membrane with 0.5-1 kD of MWCO in the absence and presence of PLFA, respectively. As comparing to the case without fulvic acids (FA), significant decreasing of Ag+ released from Ct-AgNPs was occurred in the presence of FA. This result may be from stronger interaction of Ag+ with S and N of FA or shielding the active sites of NPs by FA. In contrast, Ag+ released from PBEI-AgNPs was slightly increased in the presence of FA. Concentration of FA also played an important role in dissolution of Ag+. For Ct-AgNPs, the amount of Ag+ released in 5 mg/L of PLFA was 12.1 ± 2.16 µg/L, while the amount of Ag+ released in 30 mg/L of PLFA was significant lower as showing of 2.62 ± 0.44 µg/L. The amount of Ag+ released from PBEI-AgNPs in 30 mg/L of PLFA, 1.08 ± 0.19 µg/L, was also slightly lower than that in 5 mg/L of PLFA, 1.90 ± 0.02 µg/L. These lower releases of Ag+ in higher concentrations of PLFA, compared to the higher release of Ag+ released from AgNPs with FA. Our dissolution research suggests that FA in the environment may reduce free Ag+ and toxic effects of AgNPs to aquatic organisms.

WE103 Interference of signal transduction in zebrafish (Danio rerio) to zinc oxide nanoparticles

W. Kim, Korea Institute of Toxicology (KIT) / Environmental Toxicology Research; J. Choi, Korea Institute of Korea KIT

Zinc oxide nanoparticles (ZnO NPs) are being utilized in an increasing number of fields and special applications. While the general toxicity of oxidative stress has been extensively studied, it is still unknown what toxicological pathway may occur following developmental stage. In this study, the developmental toxicity of ZnO NPs were investigated in the embryo-larval zebrafish, the transcriptional expression profiles by ZnO NPs was also investigated to ascertain novel genomic responses related to specific toxicity pathway. Zebrafish embryos were exposed to 0.01, 0.1, and 10 mg/L ZnO NPs for 96 h post-fertilization (hpf). The ZnO NPs was found to exert a dose-dependent toxicity to zebrafish embryos and larvae, reducing the hatching rate and inducing malformation such as pericardial edema, yolk sac edema and physical deformities. As the results of gene expression profiling using microarrays, total 1,586 genes were differentially up- or down-regulated (fold changes > 2) in the zebrafish embryos exposed to ZnO NPs. The upregulated genes were involved in metals stress response and the downregulated genes were involved in silver ion (Ag+). We also focus on the involvement of zinc ion (Zn2+) in the expression of the proteins related to metal stress responses. The results suggest that the involvement of Zn2+ in the expression of the proteins related to metal stress responses.

WE104 Detection of metal-based nanoparticles in the river Dommel in the Netherlands

A. Markus, DELTARES / DSC; P. Krystek, Institute for Environmental Studies IVN VU University Amsterdam; J. Parsons, University of Amsterdam / IBEID; P. Tromp, TNO; P. de Voogt, University of Amsterdam / IBEED; E. Reel, DELTARES; R. Laane, DELTARES

We investigated the occurrence of inorganic nanoparticles in a natural river system, the river Dommel in the Netherlands. The river itself is un-studied as far as hydrology and water quality is concerned, easily inaccessible and one major wastewater treatment plant discharges into this river. We sampled the river water at various locations (commercial applications, three influents, effluent and sewage sludge) from the treatment plant. The sampling campaign was carried in June/November 2015 and these samples were analysed for seven metals (Ag, Au, Cu, Cr, Na, Zn and Zr) using inductively coupled plasma mass-spectrometry (ICP-MS), ultraviolet and scanning electron microscopy (SEM). From the results we conclude that there are indeed nanoparticles/apresent in the system we studied, as we found titanium and gold nanoparticles in the influent and effluent. The river water also contains inorganic nanoparticles, although they occur in clusters or attached to natural colloids, so that the effective size is above 0.4 µm. At most 10 to 20% of the mass concentration is made up of free particles within a size smaller than 20 nm or dissolved elements. We found evidence that there is no appreciable anthropogenic emission of Ag to the river, based on the geochemical relationship between cerium and lanthanum. Besides, the effluent of the treatment plant has lower concentrations of the examined metals than the surface water upstream. The treatment plant discharges much less of these metals than estimated using previous publications. However, the potential of titanium dioxide nanoparticles is their use in/with coatings of exterior surfaces, as the concentration of titanium/nincreased considerably in the urbanised area of the river Dommel.

WE105 Engineered nanoparticles in cosmetics according to EU 2011/696/EU: sample preparation and analysis

M. Velimirovic, Department for Environmental Geosciences; S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ / Department of Environmental Geoscience; F. Abdolahpur Monikh, University of Vienna / Department for Environmental Geosciences; F. Von der Kammer, Vienna University / Department of Environmental Geosciences; T. Hoffmann, University of Vienna / Department of Environmental Geosciences

The applicability of a previously developed generic scheme for detection, characterisation, and quantification of engineered nanoparticles (ENPs) in a complex matrix has been extended to real product samples containing unknown particles. The extended generic multi-step sample preparation procedure includes: 0) pre-characterization of the sample, I) homogenization of the sample, II) ENP separation from the matrix, III) ENP enrichment, and IV) ENP stabilization. In this work, we present as an example a sample of complex matrix, which potentially contains TiO2; as UV-filter was selected to apply the proposed extension of generic sample preparation approach. Pre-characterization in order to identify possibly present ENPs was designed and applied to the sunscreen sample. We demonstrated that the extended generic sample preparation scheme is valid and pre-characterization of the sample is a useful tool to isolate target ENPs for further analysis. TiO2 ENPs present in the sunscreen could be isolated by a combination of ultra-centrifugation and hexane washing with sufficiently high recoveries for performing further analysis on the particle size. To apply EU 2011/696/EU recommendation where materials are classified as nanomaterials based on number-based particle size distributions, the size distribution of the isolated TiO2 ENPs were determined by using flow cytometry equipped with 488 nm laser and a backscattering detector.
mass spectrometry (ICP-MS). AEP-MAVLSS-ICPMS analysis indicated only a slight shift of the size distribution towards larger diameters. Therefore the mass-based size distribution, which was derived from AEP-ICPMS analysis, was converted into a number based size distribution. The applied conversion algorithms are very sensitive towards mass-based signals of small particle sizes. Consequently, small particles with the lowest levels potentially introduce large errors in the number-based ENS size distribution. Acknowledgement – The NanoDefine project has received funding from the European Union’s Seventh Programme for Research, Technological Development and Demonstration under grant agreement No 604347-2.

WE106 Pitfalls and challenges of microplastic analysis with Raman micro-spectroscopy

P.R. Storck, DVGW-Technologiezentrum Wasser / Analysis and Water Quality; M. Sappl, O. Hackl, H. Brauch, DVGW-Technologiezentrum Wasser. Microplastic residues both in marine and freshwater systems have recently been a topic discussed in the public and in the scientific community. For freshwater systems, available information is still scarce. An increasing number of studies reports toxicological and ecotoxicological effects of microplastics. Particles with a diameter <300 µm are supposed to be very important and interesting for toxicological and ecotoxicological effects. A major problem is the lack of standardised methods for sampling and analysis of microplastics. Many studies so far published relied even on the naked eye to identify particles as polymers instead of chemical identification. Toxicological effects may be related to particle size, shape, material and particle concentration. A very promising approach to detect very small particles and to overcome the latter is the use of Raman micro-spectroscopy which provides a high spatial resolution of 1 µm. First, the focus was put on aqueous matrices and the extraction and detection procedure. An example of recently not investigated sources for microplastic particles could be slow or controlled-release fertilizers coated with polymer resins and super absorbents, both being used in terrestrial and agricultural areas. The latter could be transferred to surface waters or groundwater. Due to the novelty of the subject, this study provides some information on potential pitfalls and challenges of microplastic analysis.

Ecological traps for wildlife driven by pollutants (P)

WE107 Heavy metals, oxidative stress and immune function in common moorhens from natural and artificial wetlands

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different from SPS (p < 0.05). Assessment of PCB and DDT in Franciscana dolphins from FMA II in the last 15 years (2000 – 2014) suggests a slight decline in their concentrations. POPs data support the subdivision of FMAII, especially the southern area (i.e. SPS) that could be considered as a different Management Unit for future conservation plans on the species.

Fish model species in environmental toxicology (P)

WE110 Cross-species extrapolation of uptake and disposition of neutral organic chemicals in fish using a multi-species physiologically based toxicokinetic model
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The potential to bioaccumulate is generally considered an unwanted substance property. Consequently, chemical legislations, such as the European REACH regulation, require the chemical industry to provide bioconcentration data for chemicals that are produced or imported at volumes exceeding 100 tons per annum, or if there is concern that a substance is persistent, bioaccumulative and toxic. To fill the existing data gap for chemicals below this stipulated volume without the need for additional animal experiments, physiologically based toxicokinetic (PBTK) models can be used to kinetically predict whole-body bioconcentration as well as internal concentrations of neutral organic chemicals in different fish tissues. PBTK models have been developed for many different fish species with promising results. In this study, we developed PBTK models for zebrafish (Danio rerio) and roach (Rutilus rutilus) and combined them with existing models for rainbow trout (Oncorhynchus mykiss), lake trout (Salvelinus namaycush) and fathead minnow (Pimephales promelas). The resulting multi-species model allows for cross-species extrapolation of the bioaccumulation potential of neutral organic compounds. Predictions were compared with experimental data and were accurate for most substances. Our model holds strong potential for the probabilistic risk assessment of a chemical’s bioaccumulation potential with special emphasis on cross-species extrapolation of sensitivity.

WE111 Fish Metabolism Studies
G. Gomis

Stagnating growth in global food production is leading to an increased focus on alternative food resources. The global aquaculture industry continues to expand since fisheries have to deal with decreases in fish stocks and climatic effects. Fresh water fish farming is one of the most productive sectors for this industry. This leads to the problem: How to feed the fish? High quality food might be needed as a resource for fish growth. Therefore traditional high protein contaminated food will be fed to animals like fish. In most cases these residues will be metabolised. Therefore it is essential to identify metabolites which have a potential risk for human health. So far no specific guideline for fish metabolism is available. A testing approach will be presented with the focus on lab testing.

WE112 Investigations on biodiversity and ecosystem health of the river Nidda within the framework of the project 'NiddaMan'
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With almost 100 km length and a catchment of 2000 km², the river Nidda represents a great number of European streams. Being in its natural state near the spring in the Vogelsberg Mountains in Upper Hesse (Germany), the river Nidda rapidly changes its chemical and biological water status due to anthropogenic influences along its course. Discharges of local wastewater treatment plants, diffuse emissions of agricultural substances and substance inputs from tributaries as well as structural alterations are affecting the river on its way downstream, until entering the river Main near Frankfurt (Germany). The biodiversity and ecosystem health part of the BMF-project 'NiddaMan' investigates the ecologocal importance of diverse stress factors affecting fish in the Nidda catchment area. In an active monitoring approach, rainbow trout (Oncorhynchus mykiss) are directly exposed in cages to the water of the Nidda. Furthermore, feral fish are investigated at different sites in the catchment area with the aim to validate these responses obtained by active monitoring. To identify the influence of different input sources, like wastewater treatment plants, a local mineral spa and a papermill, samples from representative sites are also chemically analysed, and in addition, biotests addressing genotoxic- (micronuclear assay, cytotoxic- (histopathology), embryotoxic- (Daf) and dioxin-like (EROD assay) effects are conducted. The overall aims of this project part are the development of strategies to counteract water quality impairment induced by environmental stressors and to support river ecosystem recovery. Acknowledgement: The project NiddaMan is funded by the Federal Ministry for Education and Research (BMBF) within the ReWaM initiative under project code 02WRM1367.

WE113 Pollution induced effects in feral fish from the river Holtemme assessed by toxicogenomic techniques
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Anthropogenic and natural pollutants often result in adverse effects on different levels of biological organization. The aim of this project was the linkage of biochemical and physiological responses with parameters of demographic relevance (survival, growth, reproduction) by the use of toxicogenomic techniques. Deep sequencing technologies of the whole transcriptome enables the investigation of the specific mechanisms by which stressors such as xenobiotics can interact with molecular toxicity pathways. Therefore, a wide range of potential impacts of complex chemical mixtures can be examined. In this study, samples of wild brown trout (Salmo trutta) from the river Holtemme (Germany) were collected in a sampling campaign by the RWTH Aachen University and Helmholtz Centre for Environment and Health from 2013 to 2014. This river is of particular scientific interest due to its specific point sources of pollution. Therefore, four sampling sites were chosen to determine effects of agricultural use or discharges of waste water treatment plant (WWTP) effluents. Hepatic tissues of fish samples were used for RNA-sequencing-by-synthesis technologies in cooperation with the Toxicology Centre of the University of Saskatchewan (Canada). The first functional pathway analysis of the expression data shows more than 600 significantly altered genes at study sites downstream of WWTP effluents and agriculturally used area, respectively. Earlier studies detected pollution induced/inhibited effects on micronuclear formation, biotransformation enzyme activity, biomarker of exposure and oxidative stress in the same fish samples from the river Holtemme. This reveals that multiple known toxicity pathways have been altered, including changes of phase I metabolism enzymes expression or steroid biosynthesis. Further analysis should investigate pollution effects and specific mechanisms of actions with the ultimate goal to predict potential adverse outcomes as part of the environmental risk assessment of chemicals and to discover new biomarkers for ecotoxicological application.

WE114 Biomarkers measured in situ using native fish in three agricultural areas associated with environmentally valuable wetlands in Costa Rica
F. Garcia-Perez, Universidad Nacional / IRET; E. Fuentes, Universidad Nacional / IRET; R. de la Cruz, Universidad Nacional / IRET; C. Fuentes, Universidad Nacional / IRET; L. Castillo, Universidad Nacional, Costa Rica / IRET

Indigenous fish have been used to apply a biomarker approach in environmental evaluations in Costa Rica,. Caging experiments using the species Parachromis dori have been carried out along environments exposed to different agricultural activities. In situ 48-hour exposures of caged P. dori were performed at three different areas of the country, characterized by agricultural crops associated with wetlands: one at the North Pacific, close to the Palo Verde lagoon (Palo Verde National Park) four sites where studied in this area where mainly rice and sugarcane are grown. A second area is located at the North zone, upstream of the Refugio de Vida Silvestre Caño Negro (RAMSAR) four sites were sampled there, where the main crop is pineapple. The third zone is on the Caribbean side of the country; six sites were sampled on a region characterized by banana, rice and pineapple plantations, upstream from an estuarine lagoon (Laguna Madre de Dios). A set of biomarkers: cholinesterase (CHE), glutathione S-tranferase (GST) and catalase (CAT) activities; as well as lipid peroxidation (LPO) were measured in fish exposed in situ. Pesticide residue analyses were performed for every fish during the experiment, a relationship between pesticide pollution and biomarker responses was evaluated. Pesticides were present in all three studied areas. In Caño Negro, residues were only detected in one of the four sampled sites. The other two areas had different pesticide concentrations in all sampling sites. Biotransformation and oxidative stress-related biomarkers (GST, CAT and LPO) showed clear differences at one of the Palo Verde sites, where several pesticides were found in water. Only inhibited CAT responses were only detected in one of them. In the Madre de Dios area, all four biomarkers...
showed significant changes at different sites, agreeing with the presence of pesticides measured in water samples. Data suggest that biomarkers measured in caged *P. doovi* can give information on early effects caused by exposure to pesticides as significant changes were observed at most impacted sites. Regarding the areas and crops assessed, some differences in environmental conditions and agricultural practices might be enhancing runoff from agricultural areas in the Caribbean zone compared to the other two areas.

**WE115** Effects of Long-term exposure of different intensities magnetic field on biochemical and enzymatic parameters in Acipenser ruthenus

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Considerable attention is focused on the effects of electromagnetic field (EMF) due to its wide-ranging use in everyday life. Anthropogenic electromagnetic fields were induced to the aquatic environment by different sources such as underwater power and telecommunication cables, electrical heating cables for oil and gas pipelines as well as generating units and submerged substations. It is known that magnetic field can change cell behaviors and activation by affecting the biochemical and/or biological process in aquatic animals. In this study, the effects of variable magnetic fields on biochemical factors of Acipenser ruthenus were investigated. 14 juvenile fish was exposed to magnetic fields with different intensities (3, 5, 15, 25 mt) induced by electrical power supply for 2 months. The results showed a significant difference in growth rate between the control and exposed fish. However, there were significant differences in lysosome level and complement activity between control and exposed fishes.

**WE116** Sublethal effects of copper in early life stages of development of rainbow trout (*Oncorhyncus mykiss*)

S.L. Weeks Santos; B. Morin, P. Gonzalez, University of Bordeaux; J. Cachot, EPOC-Université de Bordeaux

Copper (Cu) is an essential element needed in small quantities for organism metabolism as well as for functioning of some essential enzymes. Due to its fungicidal properties, it has been extensively used for vineyard treatment against plagues resulting in its high emission into soils and aquatic environments. At high concentrations, copper can inhibit growth and interfere in several cellular processes including respiration, synthesis of proteins and cellular division. The aim of this work was to study the possible sublethal effects that copper could cause in the early life-stages of development of rainbow trout fish (*Oncorhynchus mykiss*). Eyed-stage embryos rainbow trout (265 °J) were exposed in semi-static conditions to sublethal concentrations of CuSO4 up to the larval stage (528 °J) under laboratory-controlled conditions. During 23 days, they were exposed to the environmentally-realistic concentration of 2 µg/L Cu2+ and to a 10 fold higher concentration 20 µg/L Cu2+. Several biological and morphological (survival, hatching success, malformation, growth), functional endpoints (swimming activity) and genotoxicity (DNA damage) were studied. Exposure to 20 µg/L Cu2+ resulted in a significant reduction in hatchability (63%) and an increase of half-hatched embryos (25%). Copper had an inhibitory effect on hatching. At the end of the exposure, no significant differences were observed in growth of the larvae exposed to the highest Cu concentration. However, larvae exposed to the weakest Cu concentration resulted with a major growth in comparison to the control. Malformations were recorded in larvae of 492 °J, and the percentage of malformed larval was significantly higher for the conditions of 2 and 20 µg/L Cu2+ (with 53 and 60% of malformations respectively) in comparison to the control (27% of malformation). For both conditions, the skeletal malformations were the most observed. Behavioural study was conducted at the end of the experience by imaging analysis system DanioVision (version 10.0 of Noldus). Larvae exposed to the weakest copper concentration were less mobile, whereas no significant difference were observed between larval distance travelled by the larvae exposed to the strongest concentration of copper. A comet assay was done and no evidence of significant DNA damage was observed for both conditions. This study confirms the toxic effects of copper on early life-stage of rainbow trout, even at the lowest environmentally relevant tested concentration.

**WE117** Zinc or swim: are mechanisms of sub-lethal zinc toxicity conserved in a key southern hemisphere fish species?

N. McRae, University of Canterbury / School of Biological Sciences; S.K. Gaw, University of Canterbury / Chemistry; C.N. Glover, Athabasca University

A. Rhodocetecontaminated southern hemisphere estuary was shown to be highly toxic to fish species. However, very little is known regarding the sensitivity of "non-model" fish, particularly indigenous species in the Southern hemisphere. This study investigated the impacts of zinc, a ubiquitous metal contaminant, on the freshwater fish, inanga (*Galaxias maculatus*). Inanga is widespread throughout the Southern Hemisphere and was homogenously targeted by contamination, and is of significant cultural and economic importance. Inanga also have a number of unusual physiological characteristics, including extensive use of the skin as a transport surface, and an amphidromous life-history that conveys an exceptional ionoregulatory ability. These factors may alter the relationship between metal exposure, accumulation and impact, particularly for metals such as zinc, which have impacts on epithelial processes. To determine mechanisms of sublethal toxicity, fish were exposed to graded, environmentally realistic concentrations of zinc over 96 h (1000, 270, 10, 0.1 µg L−1). Endpoints measured included those relating to ionoregulatory disturbance (whole body calcium and sodium influx), respiratory/metabolic impairment (respirometry) and oxidative stress (catalase activity and lipid peroxidation). Tissue accumulation of zinc was also measured. Results indicated that zinc exposure stimulated catalase activity, but also increased hepatic lipid peroxidation. Effects were also seen on ion transport, with an impairment of calcium influx, consistent with other studied fish, but a stimulation of sodium influx. However, these effects were only observed at the highest exposure concentration (1000 µg L−1). No effects on metabolic rate were observed, and whole body zinc concentration was significantly regulated below the lowest zinc exposure level. Overall, these data suggest some similarities in terms of inanga response to zine relative to model fish, but also some key differences (increase in sodium influx). These data were the first to examine the sensitivity of juvenile galaxiid fish to zinc, information that will be critical to ensuring adequate environmental protection of this important species.

**WE118** Recovery of seahorse (*Hippocampus reidi*) after acute and subchronic exposure to water soluble fraction of diesel oil using a DNA damage approach

E. Cariello Delamarino, Applied Ichthyology Laboratory; L.C. Medeiros, A. Chávez, Univ. de Guavilanes, Universidade de São Paulo / Marine Ecology Laboratory.

The main goal of this study was to investigate the effects of acute (12, 24, 48 and 96h) and subchronic (168 and 360h) exposition to 50 % water soluble fraction (WSF) of diesel oil on the integrity and functionality of DNA in seahorse (*Hippocampus reidi*) (Ginou, 1953). In addition, we investigated the ability of fish to recover (504h without contamination) after termination of exposion and determination of diesel spill impact on fish populations. Financial support: FAPES, UVU.

**WE119** Effects of four substituted phenylamine anti-oxidants (SPAs) in chronic fedhead minnow early life stage tests

J. Bartlett, RMIT University / School of Applied Sciences; R.S. Prosser, Environment Canada / Canada Centre for Inland Waters; A.J. Bartlett, P. Gillis, V. Balakrishnan, Environment Canada / Aquatic Contaminants Research Division

Substituted phenylamine antioxidants (SPAs) are a group of chemicals on Canada’s Domestic Substances List. They are high volume chemicals with a variety of uses, including: dye formulations, photosensitizers, lubricant antioxidants, viscosity improvers, and dispersants. SPAs are considered to be persistent, bioaccumulative, and inherently toxic. They can enter the environment in municipal wastewater effluents or via direct run-off from road surfaces. We investigated the chronic toxicity of four SPAs in a 21-day fathead minnow embryo-larval assay. Embryo-larval fathead minnow were exposed to SPAs in sediment because of SPA’s affinity for organic matter. Reference sediment (2 % organic carbon) was spiked with 125, 250, 500, or 1000 µg/g of each SPA, and left for one month to equilibrate prior to fish exposures. Fathead minnow embryos and larvae were exposed in aquariums with 35 g sediment and 700 mL lab water with sediments and water renewed daily. Glass mesh-bottomed cups prevented direct contact of embryos and larval fish with sediment, and facilitated daily transfer to new exposure beakers. Endpoints were % survival to hatch, hatching success, deformities at hatch, survival till 8 and 16 days post-hatch (dph), and weight, length, and condition factor at 21 dph. All four SPAs in larval survival, with LC50s in the range of 250-350 µg/g sediment. Water and sediment concentrations of SPAs are being measured to verify fish exposure concentrations. Concentrations of SPAs in sediment that affected survival of embryo-larval fish will be compared to measured concentrations of SPAs in biosolids and wastewaters from municipal wastewater treatment facilities in Canada.
WE120 Accumulation and maternal transfer of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) from an oral exposure in medaka fish (Oryzias latipes) M. Gonzalez-Donged, National Institute for Agricultural and Food Research and Technology / of the Environment; G. Carbonell, National Institute for Agricultural and Food Research and Technology / Department of Forest Ecology; E.M. Beltrán, INIA - National Institute for Agricultural and Food Research and Technology / Environment; C. Gonzalez, National Institute for Agricultural and Food Research and Technology; C. García-Muñoz, School of Medicine / Cell Biology; C. Fernández, National Institute for Agricultural and Food Research and Technology / Department of Environment Laboratory for Ecotoxicology

In aquatic species, dietary exposure is the most environmentally realistic exposure route to contaminants with very low water solubility. Previous investigations conducted in our laboratory with post-hatch early developmental stages of medaka (Oryzias latipes) showed the capacity of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) to accumulate progressively during a 40-d oral exposure but without disturbing normal growth. Now, in this study effects assessment on reproductive capacity (i.e., fecundity and fertility) and BDE-47 accumulation were measured in adults and in resultant embryos and larvae from a 40-d maternal and paternal dietary exposure. Sexually mature medakas (165-d old) on a 3:2 male to female ratio were fed for 40 d with a diet containing 1000 mg BDE-47/g or with a control diet without BDE-47. At predefined time points (i.e., after 10, 20, 30 and 40 d since the start of the exposure), males and females were sampled and the eggs laid the same day were collected for a BDE-47 quantitative analysis. In addition, the reproductive capacity was assessed for the resultant eluteroembryos analyzed for BDE-47 content. The BDE-47 detected in embryos and eluteroembryos ranged from 200 to 500 ng/g wet weight from 10 to 40 exposure days, respectively with levels comparable in embryos and eluteroembryos. In the parents, the BDE-47 concentration was comparatively higher in males than females. These differences correlated with the higher lipid content quantified in males. No significant effects on reproductive capacity were observed. This work was made possible by Spanish Government Grants CTM2013-44998-R and CTM2014-52388-R.

WE121 The evaluation of betamethasone as an endocrine disruptor using a fish model J. Vestel, Merck & Co., Inc.

To date, little-to-no data have been collected on synthetic glucocorticosteroids in the environment and their potential endocrine disruption (ED) activity. They are antagonists to estrogen, but are expected to be found in higher concentrations in the environment due to higher therapeutic doses and a larger patient population. Steroid hormones have the potential to interact with the hypothalamic-pituitary-gonadal (HPG) axis and disruptions of this pathway can result in decreased reproduction and/or adverse developmental effects in offspring. Betamethasone, a synthetic glucocorticosteroid, has been on the market in the United States since 1983, and is on the WHO Model List of Essential Medicines. It mimics the action of cortisol and may disrupt the HPG axis. Studying fish for the ED potential of betamethasone is logical, as they could be exposed to pharmaceuticals in waste water treatment plant effluent following normal patient use and treatment. Additionally, fish have a much shorter time to reproduction and overall lifespan, making them ideal for determining lifetime, low-level chronic effects. Environmental modelling was used to estimate betamethasone concentrations in surface water and then environmentally relevant concentrations were then used in a two generation fish full life cycle (FFLC) study with Japanese medaka. Gross endpoints, as well as VTG and gonadotropin effects were evaluated in a tiered fashion.

WE122 Use of molecular and hormonal factors as biomarkers of Bisphenol A in Yarrowian Seabream (Acanthopagrus latus) A. Negintaj, B. Archangi, Khorramshahr University of Marine Science and Technology / Marine Biology; A. Movahedinia, Khorramshahr university of marine science and technology / Marine Biology; A. Safaieh, Khorramshahr university of marine science and technology / Department of Marine Biology Farabi Food Science Research; N. Assadi, Khorramshahr University of Marine Science and Technology

Abstract Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane) is an industrial important chemical that is abundantly used as a primary raw material for the production of plastics and resins. This substance is one of the most well-known endocrine disrupters widely released in aquatic environments mainly through urban and industrial sewage effluents, thereby posing a potential threat to the organisms in the ecosystems. In the present study, effect of BPA on liver DNA integrity, erythrocytic nuclear abnormalities (ENA) stimulus and thyroid hormone balance in male yellowfin seabream (Acanthopagrus latus) were investigated. For this reason, fish received intraperitoneal injections during a period of 2 weeks with 10, 50, 100 and 200 mg/kg and 1 of BPA for 28 days. The results showed a significant increase in fish micronuclei frequency after treatment with bisphenol A. In addition, the rate of liver DNA integrity was tested using the DNA alkaline unwinding assay. Results showed a decline in the rate of liver DNA integrity after 7 and 14 days of BPA exposure in comparison to the control group. In BPA treated fish, hepatic somatic index (HSI) rose in a dose-dependent manner (R2 = 0.96) after 7 days of treatment. Our results indicated significant reduction in plasma triiodothyronine (T3) and thyroxine (T4) in treated fish compared to the controls. The findings of the project revealed that BPA strongly affected DNA and caused considerable breaks in strand of the living molecule. Additionally, ethrocytes are adversely damaged by BPA which is studied as ENA. Hence, it could be concluded that Micronucleus test and DNA staining techniques are suitable methods for molecular biomarker of BPA. Keywords: Acanthopagrus latus, Biomarker, Bisphenol A, Micronucleus test, DNA integrity, Endocrine disrupters, Thyroid hormones

WE123 The Effects of mixtures of androgenic and estrogenic chemicals on adult Murray River Rainbowfish A. Miranda, School of Applied Sciences / School of Applied Sciences; A. Gregg, CSIRO Land and Water; A. Kumar, CSIRO / Center for Environmental Contaminants Research; V. J. Pettigrove, The University of Melbourne / Zoology; D. Nunez-Godda, RMIT University / School of Applied Sciences

When individual organisms and populations are exposed to Endocrine Disrupting Chemicals, it is likely that they are exposed to complex mixtures rather than to single chemicals. Studying the effects of single chemicals may not reflect the multitude of antagonistic or synergistic stimuli to which wildlife are subjected. A common source of both androgenic and estrogenic steroid hormones to the aquatic environment is agricultural runoff from livestock rearing and hydrolysed to 17β-trenbolone and this by epimerization is then converted to 17α-trenbolone. Despite 17β-trenbolone being a potent androgen receptor agonist with a 10 fold higher binding affinity, 17α-trenbolone comprises about 95% of the total excreted product. The aim of this study was to evaluate the effects of 17α-trenbolone alone and in combination with two endocrine disruptors of different modes of action, i.e. 17β-oestradiol (50 ng/L) and Fadrozole (250 μg/L) on adult rainbowfish (Melanotaenia fluviatilis). The relative gene expression of steroid receptors in the liver as well as induction of vitellogenin in the plasma were used to assess potential effects. Results show that the expression of ERα and ERβ were significantly induced after exposures to TBL + E2 at both low (5μg/L) and high concentrations (500 ng/L) of 17α-trenbolone. The ERα expression in the TBL + E2 exposures corresponded with the hepatic VTG mRNA. The expression level of the ARβ was significantly reduced in the liver of M. fluviatilis when exposed to low concentrations of TBL in combination with E2 compared with controls, whereas when combined with high concentrations of TBL there were no significant differences. Vitellogenin induction was twofold lower when fish were exposed to E2 with a high doses of TBL than when E2 was mixed with a low dose of TBL. These findings demonstrate that E2 may have anti androgenic effects, leading to the down regulation of ARβ. In the aquatic environment where mixtures of androgens and estrogens are known to occur, similar counteracting effects are likely to occur.

WE124 Developmental effects of 2 and 6-hydroxycyrene on zebrafish embryos G.3. Muller, U3SC / Biochemistry; G. Diamante, University of California Riverside / Environmental Toxicology; A. Baiu, Universidade Federal de Santa Catarina / Bioquimica; E. Yamamoto, Universidade Federal do Paraná / Cell Biology; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Chryene is one of most persistent polycyclic aromatic hydrocarbons (PAHs) present in crude oil. In the water column, chryene likely undergoes photochemical oxidation potentially forming oxygenated photoproducts such as 2-hydroxychryene (2-OH-chry) and 6-hydroxycyrene (6-OH-chry) in the water column. Previous in vitro studies showed that 6-OH-chry was an estrogen receptor (ER) antagonist, but 2-OH-chry was an ER agonist. Since dysregulation of estrogen pathway can cause adverse fish development, the objectives of this study were to investigate the toxicity of chryene photoproducts on embryonic development. For this purpose, Danio rerio embryos (2 hpt) were treated at several concentrations of 6-OH-chry (0.5-8 μM) and 2-OH-chry (0.5-5.5 μM) to determine percent survival and the occurrence of deformities at 72hpf. Survival after exposure to 2-OH-chry was significantly decreased compared to control group. However, 6-OH-chry significantly decreased survival. The LC50 value for 6-OH-chry was 1.14 μM for a 74h exposure. Significant differences in the occurrence of cardiac abnormalities were also observed. The EC50s for 6-OH-chry and 2-OH-chry were 1.3 and 0.64 μM, respectively. Despite the regio-selective differences in toxicity that was observed, co-exposure to an ER antagonist (IC1 182,780) or G protein-coupled Estrogen Receptor (GPER) antagonist (G-15) did not affect the mortality and deformity percentages. Similarly, mRNA of GPER, or downstream
transcription factors (Hand2; Lrc10) was unaltered by hydroxyl-chrysene exposures. Since cardiac abnormalities due to PAHs are mediated through the Aryl hydrocarbon receptor (AhR), each compound was evaluated using an AhR transactivation bioassay, and neither compound showed AhR activity. Evaluation of embryos post-exposure indicated concentrations of 2-OH-chry, but not 6-OH-chry, in the exposure also caused an increased jagged regio-selective difference in the uptake or metabolism for each compound. Our findings indicate developmental toxicities of hydroxylated chrysenes may not be mediated through AhR or ER, but may be due to differences in embryonic clearance mechanisms.

WE125 ASSESSMENT OF THE RESPONSE ZEBRAFISH EMBRYOS TO ANALGESICS
A. Subramoney-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologico Laboratorio Alejandro Villalobos

The pain medications are products that are consumed in large quantities worldwide. These drugs can cause harmful effects on aquatic organisms because they are designed to have a physiological effect at very low concentrations. The aim of this study was to determine the toxic effect of 6 types of analgesics: Acetylsalicylic acid, Ibuprofen, Diclofenac, Naproxen, Paracetamol and mixture of Naproxen and Paracetamol in zebrafish embryos, to evaluate their sensitivity. Static bioassays were performed with a duration of 48 hours, the embryos were exposed to 6 concentrations of drugs: 100, 75, 50, 25, 12.5 and 6.25 mg L⁻¹ (12 replicates), plus a control without toxic. In the tests LC₅₀ was determined and the degree of lipid peroxidation (TBARS) was evaluated. The toxicity of analgesics was (from highest to lowest toxicity): Diclofenac > Paracetamol > Ibuprofen > mixture Naproxen and Paracetamol > naproxen > Acetylsalicylic Acid. The degree of lipid peroxidation was higher in embryo exposed to Paracetamol (83 nM MDA mg⁻¹) and the lowest in those exposed to acetalisalucid acid (24.3 nM MDA mg⁻¹). Organisms exposed to sublethal concentrations (LC₂₅, LC₅₀) during the recovery period, showed higher degrees of lipid peroxidation to that observed in control group at the start of the test, the majority of the embryos failed to hatch and died between the fourth to the seventh day.

WE126 Chronic exposure to pentachlorophenol alters thyroid hormones in zebrafish
G. Zhao, K. Li, China IWHR

Pentachlorophenol (PCP) is frequently detected in the aquatic environment and has been implicated as an endocrine disruptor in fish. Here, 4-month-old zebrafish (Danio rerio) were exposed to one of four concentrations of PCP (0.1, 1, 9 and 27 µg/L) for 70 days. The effects of PCP exposure on plasma thyroid hormone levels, and the expression levels of selected genes were measured in the brain and liver. PCP exposure at 27 µg/L resulted in elevated plasma thyroxine concentrations in male and female zebrafish, and depressed 3, 3',5'-triiodothyronine concentrations in males only. In both sexes, PCP exposure resulted in decreased mRNA expression levels of thyroid stimulating hormone β-subunit (tshβ) and thyroid hormone receptor (ttr) in the brain, as well as increased liver levels of uridinephosphoglucuronosyltransferase(ugt1ab) and decreased deiodinase 1 (dio1). We also identified several sex-specific effects of PCP exposure, including changes in mRNA levels for deiodinase 2 (dio2), cytosolicuraferrase (sul1 st5), and transhydro(ntr) genes in the liver. Endocrine PCP was found in a dose-dependent manner in water samples which received maternal exposure to PCP. The present study demonstrates that chronic exposure to environmental levels of PCP alter plasma thyroid hormone levels, as well as the expression of genes associated with thyroid hormone signaling and metabolism in the HPT axis and liver, resulting in abnormal zebrafish development.

WE127 Effects of perfluorinated compounds on mitochondrial functions in early life stage of zebrafish (Danio rerio).
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Perfluoralkyl acids (PFAAs) are well known surfactants that are widely used in industrial and consumer products, and are persistent in the environment and biota. Many studies have elucidated their toxicological effects on various targets, including the liver, nervous and endocrine systems, and fetal development. This wide range of toxicological effects may be brought about from damages in the mitochondria, as well as mitochondria-mediated oxidative stress. In this study, we exposed zebrafish embryos to perfluorooctanoic sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluoronanonic acid (PFNA) to investigate potential adverse effects on mitochondrial functions. Zebrafish embryos were exposed to five different concentrations (0, 250, 500, 1000, and 2000 µg/L) of PFOS, PFOA, and PFNA, respectively. Embryos were randomly collected from adult zebrafish and exposed to chemicals from 2hpf to 120hpf. Mortality, haematocrit, and gill oxygen consumption were measured at 120hpf. At 120hpf, the larvae were collected and their gene transcriptions were analyzed. Total of 8 genes involved in electron transport chain (cytb, atp5a1, cox1, and coxIV) and oxidative stress (gpx1, gstm, hmx1, and sod2) were examined. There were no changes in gene expression of larvae exposed to PFOS or PFOA. However, altered expressions were observed for PFNA exposure groups, PFNA, at 1000 µg/L, significantly upregulated transcript levels of mitochondrial genes cytb and atp5a1, and of oxidative stress response genes sod2 and gtx. These results had drawn attention to potential effects low concentrations of PFNA have on various mitochondrial functions. Assessment of damages in the mitochondrial membrane and mitochondrial DNA are under way. This work was supported by NRF- 900-20150033, Republic of Korea.

WE128 Effects of neuroactive pharmaceuticals on gene expression profiles in zebrafish embryos and larvae
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Neuroactive and neurotoxic contaminants present growing concern to fish, both at individual and population levels. Neuroactive pharmaceuticals in particular can modulate molecular neurological pathways and consequently interfere with population-relevant behavior at low concentrations. Ecotoxicological investigations of neurore modulation and neurotoxicity can benefit from molecular toolkits which allow to assess expression profile of a large number of genes. In this study, we investigated the expression of a set of genes in zebrafish Danio rerio early life stages after static exposure to neuroactive pharmaceuticals. Zebrafish embryos and larvae up to 5 days post fertilization (dpf) were exposed to venlafaxine (serotonin norepinephrine uptake inhibitor antidepressant), carbamazepine (voltage-gated sodium channel blocker), and gabapentin (GABA receptor agonist and anticonvulsant) and oxazepam (benzodiazepine derivative anxiolytic) at concentrations in the µg/L range (1 nM - 10 µM). These compounds are relevant aquatic contaminants, and selected concentration ranges are representative of environmental situations. Embryos and larvae were exposed for short (during 24 h) and prolonged (starting at day 0) exposure periods, simulating acute and chronic exposure scenarios. Afterwards, there was the evaluation of gene expression in embryonic (2 to 3 dpf) and larval (5 dpf) stages. Gene expression was quantified in pooled samples (n=24 fish) using Sybr Green based quantitative real-time PCR (qPCR). Target genes consisted of two set of genes, proposed as (i) markers of exposure to neuroactive compounds (e.g. cfs, hsp70 and hsp90) and (ii) markers of neuropeptidism (e.g. hbd, neuro1, neuro2). Reference genes were bactin, elfa and 18s. Exposure concentrations were measured at the beginning and at the end of exposure by LC-MS/MS. Results of this study are discussed regarding their ecotoxicological implications and the relevance for the annotation of adverse outcomes pathways. In addition, molecular endpoints are suggested for application in bioassays or as biomarkers in future ecotoxicological studies.

WE129 Development of an omics-based detection tool to discriminate between endocrine-mediated activity and systemic toxicity of substances.
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A mechanistic identification of the events preceding changes in vitellogenin expression is crucial to differentiate systemic toxicity from endocrine modes-of-action (MoA). An adverse outcome pathway (AOP) framework for endocrine MoAs shows that reduced vitellogenin concentrations lead to reduced fecundity and consequently, to a declining trajectory at the population level. Substances with other MoAs, exerting systemic toxicity, might also result in reduced vitellogenin, and thus lead to an identical adverse outcome (AO), although the molecular initiating event (MIE) is different. An improvement in determination tools is thus needed to ensure a reliable discrimination of endocrine disruptors from chemicals substances with other MoAs in order to avoid regulatory action and a further elongated higher-tier testing. The main aim of this study is to develop a method to discriminate between altered vitellogenin expression as a result of systemic toxicity and endocrine disruption. In the present study, we apply iTRAQ proteomic labeling techniques and qPCR assays to quantify and validate genomic expression changes in zebrafish by means of qualitative analysis of respective proteins and peptides. Gnad samples and samples of other tissues treated with endocrine disrupting substance in comparison to the control samples are analysed by mass spectrometry-based approaches to identify regulated pathway and modifications of protein expression. The observed changes in endocrine-mediated MoAs will be compared with the MoAs arising from samples treated with substance leading to systemic toxicity. These improved approaches will allow to use the iTRAQ based tool for regulatory validation of putative endocrine disruptors.
standard approach due to an identical adverse outcome. QPCR analysis will be performed in order to determine if changes in mRNA expression are indicative for the triggered proteomic response. Candidate genes for this discrimination tool are ifgl1, star and dus genes related to steroid biosynthesis for determination of endocrine disruptor or fish/ba, CYP2E1 and GSH genes regulated due to systemic toxicity. The obtained results will provide a detailed understanding of a mechanistically-based alternative approach for hazard assessment.

**WE130**

**Gene transcription profiles during the first 32 days of zebrafish development: thyroid, steroid, digestive and biotransformation systems**

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The zebrafish has become an important model for ecotoxicology testing. Especially the zebrafish embryo is increasingly used for the development of alternative tests since early life stages of fish are not considered laboratory animals according to EU legislation. In addition to observing apical endpoints such as mortality, growth and reproduction, the focus on mechanistically understanding toxicity is increasing, e.g. in the context of AOP development. This demand is also increasingly supported by regulatory bodies. To better understand toxicological processes in early life stages it is essential to describe the reference state of health, normal development and the same sampling during development: 1.5; 6; 16; 24; 36; 48; 60; 72; 84; 96 and 120 hpf (hours post fertilization), and every two days from 5 until 32 dpf (days post fertilization).

We analysed transcript levels of 40 genes involved in the thyroid, steroid, digestive and biotransformation systems using QPCR. While it is well known that these systems play a key role in the development, toxicological processes, the timing of activation of their specific components is poorly understood. For example, the biotransformation capacity of zebrafish embryos still is a topic of debate, and effects of endocrine disrupters on development have been demonstrated – however without properly described mechanisms. For the thyroid system we studied the enzymes involved in steroid hormone biosynthesis, as well as the steroid receptors. With respect to the thyroid system, we included key regulators of the hypothalamic-pituitary-thyroid axis, thyroid hormone synthesizing and activating machinery, as well as thyroid receptors. We analysed the exact timing of activation of different digestive enzymes (carbohydrates, lipids and proteins) which is especially important for understanding the transition to free-feeding larvae. For the biotransformation system, we studied Phase I (Cytochrome P450) but also Phase II metabolizing enzymes and drug transporters. In addition to providing information on gene activation, our data shed light on potential maternal transfer of specific mRNAs. This library of zebrafish gene transcription profiles is intended to function as a reference both for fundamental developmental studies, as well as for toxicological studies.

**WE131**

**Using Multi-Omics Measurements to Distinguish Contaminant Responses in a Zebrafish Model**

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Environmental monitoring of chemical effects is challenging due to the ever increasing number of chemicals and the complexity of environmental samples. Conventional toxicity testing using whole animal organisms provides critical information on biological responses; however, morphological parameters such as growth and survival may not be sensitive enough to detect adverse effects of sublethal exposures, a scenario which is more ecologically relevant. In comparison, the ability to detect changes at the RNAs, proteins, and/or metabolite levels with techniques such as transcriptomics and metabolomics can generate high-throughput and sensitive early indicators of potential sublethal effects. Recently, zebrafish (Danio rerio) embryo has gained considerable interests through OECD and REACH initiatives as an alternative and equivalent toxicity testing version to the adult stage. As early developmental stages are critical and sensitive to environmental perturbations, the combination of zebrafish larvae and multi-omics technologies can be a powerful tool in characterizing early indicators of chemical insults. In this study we utilized a combined targeted metabolomics and transcriptomic approach to evaluate contaminant toxicity in a zebrafish larvae model. Zebrafish larvae (96 hpf) were exposed to a wide classes of contaminants including pharmaceuticals and personal care products and heavy metals at environmentally relevant concentrations for a 24h period. Quantitative real-time PCR were performed on over 40 target genes covering a wide range of biological systems to assess transcriptomic effects. At the same time, over 217 metabolites, including acylcarboxines, amino acids, glycerophospholipids, hexose, sphingolipids, biogenic amines, fatty acids, and bile acids, were also quantitatively measured by LC/MS. The combination of the cytometabolome. The unique molecular signature response may be used to inform the types of toxicants present in the environment. Furthermore, this integrated OMICS approach can better inform the chemical modes-of-actions and allows a systems biology approach in identifying key trajectory that may lead to adverse health outcomes for exposed animals.

**WE132**

**Toxic effects of sediments contaminated with metals in the zebrafish Danio rerio**

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Heavy metals have as their main source of anthropogenic activities being the sediments one of main reservoirs of these elements, acting as sources of pollution by providing metal to the water column, which constitutes a threat to aquatic biota. Because there are few studies of the effect of sediments contaminated in fish, the aim of this study was to evaluate the toxic effect of sediments contaminated with Cd, Cu and its metals mixture on zebrafish. Bioassays were performed. The organisms were exposed to 3 sediment samples: 2 samples spiked with metals Cd (5 mg L-1) and Cu (2.5 mg L-1) and the third with the mixture of the two metals, for 20 days, plus a control group. At the end of the exposure period 5 organisms were taken at random from each experimental group. Its weight was evaluated and the following biomarkers: lipid peroxidation (MDA levels), and frequency of microscleri. The results show that the rates of increase in weight of the organisms exposed to contaminated sediments, had negative values, indicating that fish lost biomass. Fish exposed to contaminated sediments with Cadmium and the mixture of metals showed higher levels of lipid peroxidation (43.9 and 53.7% respectively). The highest frequency of microscleri (0.075%) was observed in organisms exposed to the mixture of metals. The results of this study indicate that sediments contaminated with metals cause adverse effects on organisms living in the water column.

**WE133**

**Reactive oxygen species (ROS) quantification as an endpoint in mixture toxicity analysis with the zebrafish embryo (Danio rerio)**

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Reactive oxygen species (ROS) serve as signaling molecules and are also a general side product of metabolism. The concentrations of ROS are counterbalanced by a group of antioxidant molecules and enzymes as increased concentrations will damage proteins, DNA and lipids and finally could lead to death of organisms due to oxidative stress. The embryos of the zebrafish are new an established model organism in ecotoxicology serving both as a tool for mode of action analysis and as a fish test alternative for regulatory and monitoring purposes. Some literature exists which already use the embryos or larvae of the zebrafish in immunological research. In the following work the existing published protocols were adapted to the use of the zebrafish embryo in ecotoxicology testing as described by the OECD 236 guideline. The different developmental stages of the embryos and elutriom embrions from 2 to 96 hours post fertilisation (hpf) were used. To monitor ROS production as the relative fluorescence unit increase ranged between 70 and 300 % of control embryos and the test proved to be suitable for the analysis of multiple samples in parallel within a maximum of 6 hours. Results will be presented also in the usage of the ROS-test for mode of action analysis of similar and dissimilar acting substances in mixtures of environmental contaminants.

**WE134**

**Effects of Microcystis aeruginosa and the toxin microcystin-LR on target gene expression profiles and histopathological changes in larval zebrafish Danio rerio**

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Microcystis aeruginosa is a cyanobacterium that can produce the hepatotoxin microcystin-LR (MC-LR) and that is responsible for many harmful algal blooms (HABs) in freshwaters. Bloom of M. aeruginosa frequently occur in eutrophic surface waters, and the presence of the cyanobacterium can become an important problem for ecological and human health. We have found that toxicity
in the zebrafish Danio rerio includes effects that are independent of the toxin. Our objectives are to investigate changes in target gene expression profiles in zebrafish larvae in relation to concentration and duration of exposure to M. aeruginosa and MC-LR, and to investigate the histopathology changes that could happen to the larvae during the exposure. We have cultured a toxic-producing strain and a non-toxic-producing strain of M. aeruginosa to generate a supply of lyophilized cyanobacterial cells characterized for use in toxicity tests. Zebrafish larvae (age 72 h post fertilization) were exposed to lyophilized M. aeruginosa or MC-LR for 96h at sub-lethal concentrations (5, 25, 100, 200, 400 µg MC-LR/L). Also, another set of zebrafish larvae were exposed to MC-LR or M. aeruginosa at concentrations of 250 µg MC-LR/L, and the larvae were sampled after different exposure durations (4, 24, 48, 72, 96 h). Survival of larvae was recorded, histopathology conducted, and changes in expression of target genes were investigated by quantitative PCR (Q-PCR) for the following genes: catalase (CAT), superoxide dismutase 1 (SOD1), glutathione peroxidase (Gpx), glutathione-S-transferase (GST), and cytochrome P450 (CYP). We anticipate that these results will enhance understanding of the molecular responses and the histopathological changes in fish during the progression of exposure to a HAB caused by M. aeruginosa. Key words: Microcystis aeruginosa, microcystin-LR, Gene expression, Zebrafish.

WE135 Detection of cholinesterase inhibitors in water samples from Novi Sad (Serbia) using effect-directed analysis

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Organophosphates and carbamates are commonly used in agriculture, residential landscaping, public recreation areas and in public health pest control programs. The main known mode of action is the inhibition of cholinesterases (ChE) and the subsequent increase of acetylcholine in the synaptic cleft. For many of these substances the structure and fate is well characterized and they are routinely monitored in order to detect their concentrations in the ecosystems. The presence of organophosphates and carbamates in the environment can cause several problems and distress in the aquatic life such as malformations, damage to the nervous system and reproduction problems. Despite the clear mode of action (MoA), many factors could influence the results of an in vivo test of environmental samples during the analysis of ACHE activity: mixture effects, developmental delay, malformations and the presence of other compounds with different MoA (e.g. dioxin-like compounds) are only some of the factors that could influence the bioassay. The complex environmental mixture could mask the effect or lead to false positive results. However, effect directed analysis (EDA) can identify masking effects, unravel the presence and identity of primarily causative chemicals by combining chromatographic fractionation, chemical analysis and biotesting. In this study, water samples of the river Danube (Novi Sad, Republic of Serbia) were collected using large volume SPE device with a polymeric sorbent. Chemical analysis with LC/HRMS showed presence of organophosphates in the parent sample of untreated wastewater. The sample was successively fractionated by HPLC semi preparative C18 column and 26 fractions were collected. Preliminary results are showing a clear inhibition of ACHE (20% compared to negative control) in only one out of 26 fractions. This study is trying to elucidate the contribution of the use of ACHE enzyme activity inhibition of substance groups like organophosphates and carbamates in the frame of in vivo testing of environmental (mixture) samples and EDA studies with Danio rerio embryos.

WE136 Effect-directed analysis of atmospheric particulate matter

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Atmospheric particulate matter (PM) is a recognized risk factor for the global burden of disease in human populations and a widespread threat to all living organisms. PM is primarily emitted from combustion processes and comprises a significant fraction of organic compounds, such as polycyclic aromatic hydrocarbons (PAHs). If specific tools could be developed to promptly discriminate the toxic potential of PM, based on the mode of action of its constituents, rather than on PM mass and BaP concentrations which are currently used in air quality legislation, a more straightforward approach to air quality management could be accomplished. Therefore, our main objective was to investigate the toxicity of the organic fraction of atmospheric PM and to provide a compatible and predictive assay. We developed a test to be easily marketed and the preferred mode of toxicity exposure to this fraction. To tackle this objective, a general strategy was adopted, in which a yeast-based assay for dioxin-like activity (AhR-RYA), and the zebrafish embryotoxicity assay (ZET assay) followed by molecular tools, were used to evaluate biological effects elicited PM (PM<1 µm) organic extracts from the city center of Barcelona. This study also clarified whether the effects observed can be further assigned to one of the secondary PM organic components. We performed receptor-based and the SOM (Screening of Organometals) assay on different time points (day vs. night, working days vs. weekends) translate into distinctive effects of PM constituents. The work developed lead to the major conclusion that the AhR-RYA and the ZET are valuable assays for effect-directed analysis of ambient PM, also allowing to study the mode of action of PM organic fraction. Integrated results from different surveys from diverse sampling points support the physiological strongly depend on: particle composition, particle size distribution, and, ultimately, the emission sources of PM. On the contrary, total PM mass and BaP concentrations appeared as poor predictors of potentially adverse biological effects. Therefore, effect-directed air quality assessment would likely represent a major improvement on PM toxicity evaluations. The authors acknowledge the CTM project (CTM2014-51985-R), and the Portuguese Foundation for Science and Technology for the doctoral grant of S.R. Mesquita (SRH/BDE/80710/2011) funded by the Program POPH-QREN through the Portuguese Ministry of Education and Science and the European Social Fund, and support through project PEst-C/MAR/LA0015/2013.

WE137 Development of a refined zebrafish feeding trial

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Fish feeding trials are important for investigating foodborne ecotoxicological exposure as well as for evaluating food safety. Although a dietary biomagnification fish test exists, a standardized fish oral toxicity test is currently not available. Safety of food components, additives and contaminants is generally evaluated using rat feeding trials to assess (re) toxicity. The development of a refined zebrafish feeding trial would advance existing testing strategies by reducing the cost, by replacing the use of mammals/birds by a lower vertebrate species, and by facilitating reproductive and multigenerational studies. When testing food, substitution of part of the food by the component under evaluation is often required, possibly interfering with nutritional requirements. Therefore, the extent of component substitution which can be processed by the zebrafish metabolism (maximum tolerable percentage) should be assessed prior to the component evaluation trial. Since we will conduct a feeding trial with GM maize later on in our study, we defined the maximum tolerable percentage of maize. First, we formulated experimental fish feeds based on the composition of commercial fish feeds. Maize was introduced in the feed by a stepwise substitution of a wheat component (25% of the whole feed). We compared 6 experimental feeds to three different commercial feeds in a one month feeding trial, during which we investigated the following endpoints: condition factor and growth, hepatosomatic and gonadosomatic index (HSI and GSI), reproduction, energy budget and feed digestibility. The growth of fish fed with 25% wheat or 25% maize were slightly decreased by at least one half of the maximum feeding percentage of maize. Feed digestibility analysis showed a decrease in carbohydrate uptake when fish were fed with an increasing percentage of maize substitution. Based on these results, we selected a 15% maize substitution for conducting the GM maize feeding trial. This selection was based on two criteria: (1) the biological limits of the maize component in the zebrafish feed should be respected, (2) the amount of maize substitution should allow us to observe effects of GM maize if there are any. We suggest that our approach of determining component maize substitution rates before carrying out feeding trials could be a valuable asset when evaluating food safety, as well as for investigating dietary ecotoxicological exposure of fish.

WE138 Comparison of Different Feeding Diets to Improve the Performance of Higher-Tier Fish Tests

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For the testing of endocrine functions of substances, the demand for higher-tier/multi-generation fish tests like the OECD304 constantly grows. So far, fish is usually investigated with one complete generation cycle of the F1 generation. The first two to three weeks post hatch is the most sensitive and critical phase. Feeding during this period of time is crucial for the development of fry and therefore the successful performance of the tests. Since higher-tier fish tests are time consuming and complex, feeding of fish should be kept as simple as possible. Therefore, artificial feeds are almost exclusively used for this purpose. The choice of food. However, our results showed that artificial foods were not sufficient for
the sole feeding of fry. The Early Life Stage Test (ELS; OECD 210) is a 35-day-test which exactly covers the critical first life stages of fish and is therefore greatly suitable to investigate development of fry. In order to investigate the optimal diet for fry in our higher-tier fish tests, we performed several ELS tests according to OECD guideline 210 with the Zebra fish Danio rerio in a flowthrough system. We evaluated different diets consisting of commercial micro flake and liquid food fry, hole egg suspension, different living brine shrimp species and decapsulated brine shrimp, rotifers and combinations of these. After 35 days survival, growth and weight were compared. The tests showed that Zebra fish fry was too small to feed on most artificial foods. Further treatment like grinding was necessary increasing time for preparation. Our results indicated a significant preference for natural diets like brine shrimp and rotifers with most survival rates higher than 90 %. The poster will compare the results of the different diets and give hints on which diet may be preferred in higher-tier fish tests. Furthermore, the existence valid criteria of standardized higher-tier fish tests will critically be reviewed in comparison to our findings.

WE139

 Xenobiotic metabolism in alligator gar (Atractosteus spatula): A comparison with rainbow trout (Oncorhynchus mykiss) 

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Metabolic stability determines the extent to which xenobiotics are biodegraded. One of our interests is pharmaceutical compounds, which are chemicals of emerging concern in aquatic environments. Information on the chemical metabolism can be used to determine bioaccumulation potential or BCF (Bioconcentration factor) that is used for regulatory purposes in REACH (Registration, Evaluation, Authorization and Restriction of Chemicals). Under this initiative, chemicals are regulated and must be tested for PBT (Persistence, Bioaccumulation and Toxicity). The trout in vitro metabolism assay is a new in vitro methodology to test for bioaccumulation in the PBT assessment. This assay is undergoing validation through the OECD (OECD Project 3.13) and is gaining acceptance among regulatory agencies. The in vitro trout metabolism assay decreases the number of animals used for testing, testing costs and time required to obtain results. Despite of the advances in this and other methodologies, we must evaluate in vitro metabolism assays across a variety of species to better understand interspecies variation and differences in how species function under different or environmentally relevant conditions such as dissolved oxygen concentrations and temperatures. The objective of this research was to compare in vitro metabolism in the liver S9 fraction of alligator gar to rainbow trout as our reference species. Alligator gar is a unique ancient fish that provides a valuable contrasting model to rainbow trout. Alligator gar live at higher temperatures and lower dissolved oxygen levels than rainbow trout. Differences may be found in how these two species do or do not metabolize xenobiotics. Laboratory or hatchery raised alligator gar and rainbow trout were maintained at 23°C and 13°C, respectively. Fish were acclimated to laboratory conditions for at least one-month prior to sacrifice. Fish were euthanized and livers from alligator gar and rainbow trout were perfused with a saline buffer (HBSS) at pH 7.8. After each perfusion, liver fractions were assayed for the ability to metabolize alligator gar or rainbow trout (Johanning et al., 2012). Phase I and II enzyme activities for both species were compared. Thus far, EROD and GST activities appear to be comparable. Experiments are ongoing to further compare metabolism by these enzymes and metabolism of pharmaceuticals to explore evolutionary strategies and adaptations at the physiological level.

WE140

Endocrine disruptor risk evaluation in vivo with transgenic zebrafish larvae indicating glucocorticoid signaling activity

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Glucocorticoids (GCs) are steroid hormones with important functions in metabolism and stress response. Synthetic GCs have immuno-suppressive, and anti-inflammatory properties and are widely prescribed for the treatment of diseases such as asthma and rheumatoid arthritis. These drugs are released into the environment due to incomplete removal by waste water treatment plants, and remarkably high concentrations were detected in receiving rivers. Acting as endocrine disruptors, these and other compounds may interfere with the hormonal system of wildlife, calling for in vivo tests measuring GC activity. Currently available test systems are centered on in vitro reporter gene bioassays with transgenic cell cultures. In contrast, bioassays with zebrafish larvae enable risk assessment in the whole, living organism. Our Glucocorticoid Responsive In vivo Zebrafish Luciferase Activity (GRIZLY) assay allows us to investigate activation or inhibition of GC signaling in vivo, taking into account effects such as metabolism of compounds or organ specific effects. The assay is based on a transgenic line (GRE:Luc) carrying a luciferase reporter gene under control of four GC response elements (4xGRE). Comparison with a zebrafish cell line carrying the same reporter construct helps to pinpoint indirect or metabolism-related in vivo effects of a sample. The GRIZLY assay identified known activators of GC signaling, including the environmental contaminants dexamethasone, prednisolone and prednisone, in a chemical library of pharmaceutical compounds. It also identified oestradiol, which stimulates endogenous cortisol production and, therefore, was active only in the larval assay. When screening for inhibition of GC signaling, we again found compounds active in vitro and in vivo as well as numerous substances only active in vivo. Importantly, the test can equally be modified to specifically target compounds interfering with endogenous GC bioavailability. Strikingly, not only compounds not specifically targeted in interfering with GC signaling were identified by the larval assays. Deeper investigations of their underlying modes of action are in process. These results point out that interference with GC signaling and biosynthesis needs more attention in environmental risk assessment of compounds, and that in vivo testing is indispensable for this. Our test system covers these needs, allowing rapid whole-organism testing with high sensitivity and specificity.

WE141

Linking gene induction to physiological effects using transgenic medaka

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Gonadal development in medaka (Oryzias latipes) is dependent on the synergy between oestrogens and androgens. Any disruption in steroid hormone levels can cause the induction of ovo-tests, a clear indicator of sex reversed germ cells. To determine the sensitive window for hormonally induced sex reversal in medaka, we developed a novel 42sp50-GFP, ChHgF-GFP transgenic medaka line. This line allows the identification of ovo-tests by fluorescence (GFP) present in developing oocytes and in parallel allows quantification of oestrogen axis activity via GFP in the liver. Germinal transformation resulted in a stable line exhibiting strong GFP signal in the ovaries and increased sensitivity in response to oestrogens. To identify the sensitive developmental period to exogenous agents, fry were exposed to 24 hour pulses of oestradiol (E2; 500g/L), dihydrotestosterone (DHT; 2.5g/L or 500g/L) or flutamide (5mg/L) at various time points between day post fertilisation (dpf) 1 and 12. Evaluation of phenotype followed by genotyping at dpf 16, revealed sensitivity to E2 between dpf 1-5 and two periods of increased susceptibility to DHT or flutamide between dpf 0-1 and dpf 5-8. No phenotypic sex reversal was detected after the exposure to DHT or E2 on dpf 11 or dpf 12 respectively. We show the relative sensitivity of early developmental stages to the induction of ovo-tests in XY and XX medaka and that our newly developed 42sp50-GFP, ChHgF-GFP medaka line is a suitable model for studying this phenomenon.

WE142

Synthetic progestins disrupt the glial cell specific-brain aromatase expression in the developing brain of fish

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Few studies have addressed the biological effects of natural and synthetic ligands of the nuclear progesterone receptor (nPR) on fish development and reproduction, resulting in a significant paucity of data that would allow in vivo comparisons of these compounds on aquatic organisms. Yet progesterone plays key roles in many vertebrates and recent data showed that some progestins are present in the aquatic environment at concentrations that can impair reproduction. In light of this context, we studied the effects of progesterone and a panel of synthetic nPR ligands on the estrogen-receptor (ER)-signaling pathway by investigating the expression of the zebrafish brain aromatase cyp19a1b gene using zebrafish-specific mechanism-based in vitro and in vivo assays. Twenty-four nPR ligands were screened on transgenic cyp19a1b-GFP zebrafish embryos to assess their potential estrogenic effect. Progesterone and its isomer dydrogesterone as well as mifeprone and all trans-progesterone-derived progestins had no effect on GFP expression. However, all progestins derived from 19-nortestosterone induced the cyp19a1b expression in an ER- and concentration-dependent manner with EC50 ranging from the low nM to the µM range. These results show that progestins are estrogenic to fish and that early-life exposure of fish to 19-nortestosterone derived progestins may disrupt the estrogenic pathway by blocking the brain. The 19-nortestosterone derived progestins levonorgestrel and norethindrone were further tested in U251-MG cells transfected with any of the three zEBF ER subtypes zERα, zERβ1, or zERβ2. Progesterone had no effect on luciferase activity with either of the zER subtypes. Norethindrone and levonorgestrel both induced luciferase activity that was blocked by co-exposing the cells with 17β-estradiol. An ER competition assay further showed that these compounds were unable to bind to and activate ER except at very high concentrations (10-7M), suggesting they require metabolic activation prior to eliciting estrogenic activity. This study highlights the relevance of using zebrafish-specific screening assays to characterize endocrine disrupting properties of emerging contaminants and demonstrates that 19-nortestosterone derived progestins are pro-estrogenic compounds targeting radial glial cells inducing cyp19a1b expression in the
developing brain of fish. Given that ER signaling is one of the very first to emerge during early-life stage development, the consequences should be further investigated.

**WE143**: Nonylphenol impairs development of the olfactory bulb by altering projection networks of estrogen responsive cells in the forebrain

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We have established an estrogen biosensor transgenic zebrafish (ERE-TG fish) that detects tissue responses to estrogenic chemicals via expression of fluorescent reporter proteins. Using the ERE-TG fish, we have observed that specific subsets of cells in the forebrain are responsive to estrogen, however, the role(s) of estrogen signalling in development and function(s) of these cells are not known. We observed two types of estrogen responsive cells in the forebrain cells from the early onset of neurogenesis in the ERE-TG embryo, one localised in olfactory bulb with complex projection networks and another in telencephalon showing a characteristic polar morphology with a radial process. These estrogen responsive cells were not immunoreactive for neuronal markers (acetylated tubulin and HuC), but expressed a neural progenitor marker Sox2, suggesting specific roles of estrogen signalling in the neural progenitor development/function(s). We investigated the spatiotemporal relationships between olfactory sensory neurons and estrogen responsive olfactory progenitor cells, applying fluorescent in situ hybridization for olfactory bulb neuron specific markers (lhx2 and tbx21) and immunohistochemistry against the ERE reporter (mCherry). Estrogen responsive olfactory cells were localised at spatially distinct regions from those of lhx2 or tbx21 positive cells but their projections innervated directionally towards lhx2 and tbx21 expressing cells, suggesting the estrogen responsive olfactory bulb cells may play role(s) the development/function(s) of lhx2 and tbx21 positive olfactory sensory neurons. Interestingly, exposure to an endocrine disrupting chemical (nonylphenol, NP) markedly inhibited the establishment of projection networks of the estrogen responsive olfactory cells, which showed shorter projections and ectopic distributions of the cell bodies. NP did not alter the spatiotemporal expression of lhx2 or tbx21 but impaired the innervation of estrogen responding olfactory cells to Lhox2 and tbx21 positive cells. NP also reduced significantly the number of estrogen responsive cells in the forebrain. Taken together, these findings indicate estrogen responsive cells in the forebrain possess fundamental roles in olfactory bulb development and the environmental oestrogen present in the blood may affect brain development during embryogenesis by impairing development of estrogen responsive olfactory cells and their spatiotemporal networks.

**Wildlife ecotoxicology: from food chain exposure to population effects (P)**

**WE144**: Effects of Persistent Organic Pollutants on Vitamin A Levels in Faroese Pilot Whales

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Long-finned pilot whales (Globicephala melas) are highly exposed to persistent organic pollutants (POPs), such as those classified as organohalogen contaminants (OHCs). POPs are known to interfere with the regulation and homeostasis of retinoids (vitamin A and its metabolites). Retinoids are lipophilic molecules essential for vision, growth, reproduction, immune function and cellular division and differentiation in mammals. Disruption of retinoid homeostasis can thus lead to impairment of these functions in mammals. OHCs and their metabolites can interfere with retinoids by different mechanisms including the disruption of vitamin A metabolism. The CYP26 enzyme is involved in the metabolism of retinoids by catalysing the oxidation of retinoic acid to polar metabolites. Effects of POP exposure on vitamin A in pilot whales were presently studied by analysing correlations between the concentrations of selected OHCs (PCBs, OCs and PBDEs) and retinoids in plasma and liver and cyp26 mRNA expression in the liver of the pilot whales. The results showed that cyp26a1 mRNA expression, but not cyp26b1 expression, was inversely correlated to all analysed POPs (r = -0.543, p < 0.05). The higher retinol levels with increasing POP concentrations in plasma could indicate increased mobilisation of retinol, although the effect was not detected in the liver stores.

**WE145**: Contamination of farmland bird’s eggs by present and past plant protection products - the grey partridge as a case study

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Farmland birds are in decline in Europe. The contamination of their eggs by present and past plant protection products (PPPs) is poorly documented despite a potential to adversely impact their breeding performance. In this context, 139 eggs of 52 grey partridge Perdix perdix clutches were analysed using GC/MS-MS and LC/MS-MS screenings ca. 500 compounds at a LoQ of 0.01 mg/kg. A total of 15 different compounds were detected in 24 clutches. Nine of them are currently used by farmers as fungicides (Difenconazole, Tebuconazole, Cypromeconazole, Fenpropidion, and Prochloraz), insecticides (lambda-Cyhalothrin and Thiamethoxam/Clodiathionine), and herbicides (Bromoxynil and Difluilencan). Mixtures were less detected than expected by potential exposure. We could associate a contamination and a probable exposition (field treatment) in several cases. Some older PPPs were also detected (Fipronil (+sulfone), HCH (a,b,d), Difenylamline, Heptachlor (=epoxide), DDT (Sisomeres)), as well as PCBs. Concentrations ranged between <0.01 and 0.05 mg/kg, but reached 0.067, 0.11, and 0.34 mg/kg in three cases. Our results testify a contamination of females and their eggs to PPPs as soil pollutants or their residues, banned in France since 2006, even decades, that persistently contaminate the environment. We discuss the possible routes of exposure, the probability to detect a contamination in the eggs, and the prevalence of such a contamination by means of multicriteria analysis. Unfortunately, although the wealth of information collected and analysed, it remains difficult to conclude about effects on egg / embryo characteristics yet, and additional investigations are needed. Lastly, we share our experience from our case study to document unintentional effects of PPPs on the breeding success of wild birds in the real world. This work was supported by the research programme Pesticides funded by the French Ministries in charge of Ecology and Agriculture with a financial support from ONEMA. Funds issue from the fees on diffuse pollution.

**WE146**: Exposure of Red kite nestlings to chemicals and consequences on health

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The Red kite is a European endemic raptor listed as “Near-Threatened” by the IUCN. Poisoning by pesticides is considered one of the major threats for kites and they can be exposed to metals/loid(s), and/or POPs. Within the framework of the French National Action Plan, some breeding populations are monitored, several tens to hundreds of chicks being ringed each year since 2006. This offers an opportunity for blood sampling in order to assess exposure to chemicals. Here, we present the results from a monitoring done on the Red kite in France during springtime 2013 and 2015. Blood was sampled from 121 chicks in 2013, metal(loid)s, rodenticides, PAHs and OCPs were measured and related to body condition and breeding success. In 2015, analyses on different health indicators are currently in progress on 50 blood samples and results will be presented at the congress.

**WE147**: Emerging chemicals in birds of prey from Spain and Norway: exposure and effects

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In 2014, the international research project NewRap (field tested in Svalbard, Norway) was launched (funded by the Norwegian Research Council and the Norwegian University of Science and Technology). In this project, we investigate the exposure and effects of emerging chemicals in birds of prey from three regions (Murcia, southern Spain; Trøndelag, mid Norway; Troms, northern Norway). The goshawk (Accipiter gentilis) was selected for this study as three breeding populations of the species (albicilla) in only the two Norwegian locations. Both blood and body feathers were obtained from the chicks in the nest when they were 3-7 weeks old. The chemicals of interest include novel brominated flame retardants (nBFRs), organophosphate flame retardants (OPFRs) and per- and polyfluoroalkyl substances (PFASs). In addition, metals and legacy chemicals, i.e. persistent organic pollutants (POPs), were included as well. To this end, we have developed and used a comprehensive and carefully conducted algal growth inhibition experiments with a defined endpoint (e.g. the EC10) instead of the NOEC in a long-term study, recently EU regulation 283/2013 introduced the ECx in pesticide hazard assessment, with the capacity factors were extrapolated to 100% water (kow). The affinity of surfactants (kow) was calculated using the AstraZeneca Hygienic Interaction liquid chromatography (HILIC), and ion exchange) with a liquid chromatographic method. Retention times of surfactants were measured and sorption and bioaccumulation of different surfactant classes. To this end we aimed to develop a comprehensive sorption of non-ionic and anionic surfactants are therefore required. Our research is focused on developing and using a defined point estimate (e.g. the EC10) instead of the NOEC in long-term risk assessments of chemicals, excluding alcohols and acids) are divided between shake flask and slow-stir conditions. In summary, the use of increasing POP concentrations in liver and cyp26 mRNA expressions in the liver of the pilots showed that cyp26a1 mRNA expression, but not cyp26b1 expression, was inversely correlated to all analysed POPs (r = -0.543, p < 0.05). The higher retinol levels with increasing POP concentrations in plasma could indicate increased mobilisation of retinol, although the effect was not detected in the liver stores.
relation to habitat and dietary preferences (using stable isotope analysis), differences in latitude, and to evaluate the interactive effects of the pollutant mixtures on different biochemical, immunological and endocrine parameters. The first results of the sampling campaign in 2015 indicate that the PFAS exposure in goshawks from Murcia are lower than in Norwegian chicks, with up to 10 times higher values in the liver stores of linear PFOS than in the background level (DE-1:1 ng/ml in Murcia; 4.9-30 ng/ml in Trondelag; 0.72-24 ng/ml in Troms). Regarding the white-tailed eagle, linear and branched PFOS were the most prominent PFAS (linPFOS: 6.0-32 ng/ml in Trondelag).

WE148
Age-dependent changes in oxidative stress biomarkers and essential elements in blood of red deer
Changes in the concentration of the essential elements in the blood of animals throughout their life may be indicative of periods of greatest vulnerability to deficiencies and associated diseases. Here we have studied the changes in antioxidant biomarkers and essential elements in the blood of females of farmed red deer along their life. These changes are discussed in relationship with the incidence of enzootic ataxia, white muscle disease and reproductive failure in farmed deer. Blood samples of female red deer (n=132) were analysed to determine the concentrations of essential elements with roles as cofactors in antioxidant enzymes (GPx and SOD). Moreover the activity of three antioxidant enzymes and antioxidant vitamines (retinol and α-tocopherol) was measured in the same blood samples. In addition, samples of plants, soil and water from the same study area were analysed to verify possible interactions of trace elements in the diet of the studied deer. Levels of Zn and Mn in blood decreased with age, whereas Se and Cu levels showed an increase during the first year of age and then stabilized at slightly lower levels the following years. Farmed deer showed deficiency levels of Cu during most of their life and, at a lesser extent, were also Se-deficient. The activity of GPx increased along the life of deer, while a decreasing trend was found for SOD. In the case of vitamins, retinol increased during the first year of age and tocopherol peaked at 3-4 years of age. Positive correlations were observed between the blood levels of Se and GPx activity and between levels of Zn/Cu/Mn and SOD activity. These results indicate that trace element deficiencies described above (especially Cu) may have an effect on the antioxidant capacity (especially SOD activity) of the animals at adult stages, when reproduction effort may increase antioxidant requirements. Cu deficiency in farmed deer could be related to low levels of Cu in soil of the study area or to elevated levels or sulphates in water that could lead to the formation of non-bioavailable forms of Cu in the digestive tract of deer. In summary, the use of enzymatic and non-enzymatic antioxidants as effect biomarkers of the exposure to pollutants should be always considered taking into account the age of the animals and the levels of essential elements acting as antioxidant cofactors.

An in silico modelling perspective to advance hazard assessment of aquatic ecotoxicology (P)

WE149
Linking algal growth inhibition to chemical activity: A tool for identifying endocrine disruption
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Aquatic toxicity data are most often expressed on a concentration basis (e.g., EC50 in mg/L). Whereas the data are useful within regulatory risk assessment, a given EC50 value offers no direct information on whether the compound exerts baseline toxicity or excess toxicity. Chemical activity (a) is a medium-independent exposure parameter that quantifies the energetic level, and not the concentration, of an organic compound relative to its energetic level in pure liquid [0-1, unitless]. Several studies have linked toxicity to chemical activity, and baseline toxicity (narcosis) for neutral hydrophobic organic compounds has been shown to initiate in the narrow chemical activity range of 0.01 to 0.01. These findings suggest EC50 values for baseline toxicity to be at or above 1% of liquid saturation, and EC50 values well below this limit do thus indicate excess toxicity. In the present study, published toxicity data were expressed on a chemical activity basis in order to identify excess toxicity. More specifically, algal growth inhibition was linked to chemical activity using two approaches: (1) algal toxicity data were plotted relative to a regression for the (sub-cooled) liquid solubility, which served as a y-axis reference for chemical activity of unity and (2) ratios of algal EC50 values and (sub-cooled) liquid solubilities were determined, since the this ratio essentially equals the effective chemical activity (Ec50). In both approaches, compounds exerting toxicity well below the limit of 1% of liquid saturation (a=0.01) were identified as compounds exerting excess toxicity and investigated further. Also, the two approaches were compared on both a methodological and scientific level. In the present study, toxicity data came from several comprehensive and carefully conducted algal growth inhibition experiments covering a wide range of solid and liquid organic compounds, covering several expected modes of action and also several algal species. This presentation shows how the chemical activity concept can be used to compare and combine toxicity data across compounds and species in order to characterise toxicity – and further how the concept can be used in environmental risk assessment.

WE150
High-accuracy water solubility determination using log KOW from different methods
P. Bicheng, P. Thomas, KREATIS
Work was carried out to determine the relative accuracy of several experimental methods used to determine log KOW values: HPLC (OECD 117); shake flask (OECD 107) and slow-stir (OECD 123). In order to do this log KOW values were plotted against water solubility values. It was found that the method for log KOW determination directly influenced the relationships found between log KOW and water solubility. Water solubility is a parameter which is typically measured with a shake flask method (OECD 105) or improved using a slow-stirring method (derived from OECD 123 for log KOW determination) when water solubility is low. When log KOW data for a group of compounds (e.g. oxygenated substances excluding alcohols and acids) are divided between shake flask and slow-stir methods and compared to results from the HPLC method, goodness-of-fit correlation are significantly different. Water solubility values correlate better with log KOW values determined using shake flask or slow-stirring methods. However HPLC method overestimates log KOW with an average error of 0.43 log units. HPLC cannot be used as a reliable method to measure log KOW with accuracy and this is also the reason why HPLC data should be excluded as a data source during development of High-Accuracy QSR models.

WE151
Analysis of sorption of non-ionic and anionic surfactants to different stationary phases
J. Hammer, University of Utrecht / IRAS; J. Haftka, P. Scherpenisse, J. Hermens, Utrecht University / Institute for Risk Assessment Sciences; P. de Voogt, University of Amsterdam / IBED
Anionic and non-ionic surfactants are high-production volume chemicals that are found in many consumer products and consequently also in the environment as organic contaminants. Octanol-water partition coefficients (Kow) are often used in QSAR studies as a measure of hydrophobicity to predict the environmental fate and exposure of neutral organic compounds. However, Kow is not meaningful for surface-active compounds because sorption to environmental matrices is not only driven by their hydrophobicity, but also by specific interactions with the sorbent (hydrogen-bonding and electrostatic interactions). Furthermore, experimental Kow values of both non-ionic and anionic surfactants are difficult to determine because of their surface active properties and ability to emulsify the octanol-water system. Alternative approaches to quantify and predict the environmental behaviour of surfactants are therefore required. Our research is focused on developing and testing new approaches and parameters that can be applied in models to predict sorption and bioaccumulation of different surfactant classes. To this end we studied the interactions between surfactants and different stationary phases (C18, Hydrophilic Interfacial liquid chromatography (HILIC), and ion exchange) with a liquid chromatographic method. Retention times of surfactants were measured and capacity factors were extrapolated to 100% water (k′w). The affinity of surfactants for the C18 phase increased with carbon chain length for both anionic and non-ionic surfactants. Fragment values (log Dk) were calculated by multiple regression analysis of log k′w values for all surfactants. These values showed that hydrocarbon and fluorocarbon moieties contributed most to hydrophobicity while anionic head groups such as carboxylate, sulfonate, and sulfate groups contributed much less to hydrophobicity. All fragment values determined for the C18 phase could be interpreted through identification of interactions between surfactants and the aqueous phase (hydrogen-bond acceptors/donors, charged head groups) and are a good measure for the hydrophobicity of surfactants. Capacity factors for all stationary phases together may be applied as parameter in predictive models for sorption to specific environmental phases (e.g., membrane liquids or organic matter).

WE152
Reliability of BMD and ECX calculations required by Regulation EU 283/2013 for bird and mammal reproduction studies
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The ECx is a key endpoint for the long-term risk assessments of chemicals, including pesticides. Aquatic ecotoxicologists have long been in favor of using a defined point estimate (e.g. the EC50) instead of the NOEC in longterm risk assessment. For avian and mammalian studies however, reliability has always been more on NOAELs as determined with statistical significance testing. Only recently EU regulation 283/2013 introduced the ECx in pesticide hazard identification and risk assessment. All ecotoxicologists have gained influence through understanding the need for using ECx endpoints for birds and mammals. ECx endpoints are much better suited than NOAEL or LOAEL.
also for combined assessments, e.g., in mixture toxicity evaluations, or for geometric mean calculations in case data are available from multiple studies or additional species testing. However, studies conducted, or evaluated, for bird and wild mammal longterm risk assessments are different from guideline studies in other ecotoxicological areas, with a comparatively high number of endpoints, but generated in test conditions particularly only at three dose levels plus control. Therefore, ECx evaluation of such studies needs careful consideration. EU 283/2013 stresses the importance of reliable endpoints for risk assessment; this applies, of course, also to the calculation of ECx values. Currently, different approaches are used for calculation of ECx for birds and mammal studies, which are based on different principles and have a different historical background. Benchmark dose (BMD, equivalent to ECx) calculations are one of the newer approaches, developed for human toxicology and based on the precautionary principle. The key principle of BMDs is that different functions are compared and finally a conservative one is chosen to determine the BMD and its confidence intervals. In ecotoxicology, conventionally only a few (or even a single) dose-response functions are used and different methods are used to select the most adequate one. The selection of dose-response curves in ecotoxicology often follows other principles than the BMD approach. In the present study benefits and drawbacks of the calculation methods are compared and a new approach is proposed for calculation of robust and reliable ECx values. The proposed approach integrates the benefits of existing methods and provides endpoints that comply with the reliability criteria and is based on the OECD guidance for statistics in the analysis of ecotoxicity data.

**WE153**
A unique Q SAR model to determine microorganism toxicity in activated sewage sludge

P. Thomas, J. Oses, P. Bicheler, KREATiS

Toxicity of microorganisms, usually following the Activated Sludge Respiration Inhibition Test (ASRT) – OECD guideline 209 is a required endpoint in fulfillment of Annex VI REACH dossiers i.e. substances produced in quantities >10 TPA. Physicochemical properties (like adsorption to sludge or volatility) as well as the lack of obligation for analytical quantification makes it difficult to perform this test for some substances and interpretation can be ambiguous. In order to replace this kind of experimentation a High-Quality QSAR model has been developed. This work has been carried out as a part of the DAMIER project, a French funded project for the development and the use of High Accuracy QSAR models for REACH compliance. Therefore the model meets the five OECD principles (OECD, 2004) and has been validated to provide accurate EC50 values for non-polar narcotic substances. The algorithm is based on a simple linear relationship which links toxicity to activated sludge microorganisms to sub-cooled liquid solubility. It is based on the concept that chemical activity expressed through water solubility can explain aquatic toxicity as has previously been shown for fish, invertebrates and algae (Mackay et al., 2009; Thomas et al., 2015). This is the first model in ecotoxicology able to predict such an endpoint for regulation known to the authors.

**Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (P)**

**WE154**
A DEB analysis of responses to baseline toxicants in C. dubia and D. magna

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Mechanistic effects models are gaining interest in the scientific community and in regulatory settings. These models have the potential to facilitate species extrapolation, thereby decreasing the need for toxicity testing for risk assessment. They also provide an opportunity to quantify the complexities of multiple interacting stressors on environmental scenarios. Dynamic energy budget (DEB) theory represents a unifying framework for assessing the mechanisms that drive toxicant effects on life history traits. We report here on the progress of two case studies for an example chemical tested on two species of Cladocerans, Ceriodaphnia dubia and Daphnia magna. Over 70% of the ingredients in home and personal care products are considered baseline toxicants. We use phenol as a model baseline toxicant. A dynamic energy budget model was calibrated for each species, and differences in parameters and physiological mode of action are discussed. This work also explores how DEB based modelling can incorporate environmental factors such as food availability and temperature into risk assessment. In conclusion, the present study demonstrates the potential utility of DEB based models for species extrapolation and chemical risk assessment in an AOP framework.

**WE155**
Calculation of exposure-specific species sensitivity distributions by toxico kinetic-toxicodynamic modelling for the insecticide compounds imidacloprid, chlorpyrifos, and lambda-cyhalothrin

D.M. Buijert-de Gelder, Wageningen University / Aquatic Ecology and Water Quality Management; A. Fecks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management; Species sensitivity distributions (SSDs) are statistical distributions describing the variation among different species for a particular compound or mixture. An SSD can be made by fitting a statistical distribution through the results of acute or chronic toxicity tests for different species. SSDs can be used in environmental risk assessment of chemicals in two different ways, either for defining Environment Quality Criteria (EQC), such as the often used HC5 concentration that is thought to be protective for 5% of all species in a community, or to estimate the fraction of potential affected species (PAF). In this work, we used toxico kinetic-toxicodynamic (TK/TD) models that were already parameterised for the insecticide compounds imidacloprid, chlorpyrifos, and lambda-cyhalothrin to generate model-based toxicity values for a series of time-variable exposure scenarios. Exposures contained different time-variable exposures, or chronic exposures over prolonged times, up to 100 days. SSDs were then generated based on these toxicity values, and could be compared with experiment-based SSD. The aim of this study is to assess how protective SSDs based on acute toxicity experiments are in the light of environmentally relevant time-variable exposure concentrations. Model-based HC5 and HC20 values were compared with respective values from experiment-based SSDs for chlorpyrifos, and hardly any differences could be identified, hence the method could be considered to work. Current results indicate that only small differences in HC5 values for the investigated compounds are visible for the different exposure profiles. This depends on the TKTD characteristics of the chemical, the type of TK/TD model used and the length and type of the time-variable exposure regimes. Further results will investigate also HC50 values which are supposed to show larger differences.

**WE156**
Application of ToxicoKinetic-Toxicodynamic Modelling of Effects of Chlorpyrifos-Methyl on the Survival of Aquatic Macroinvertebrates for Environmental Risk Assessment

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In this study, toxico kinetic/toxicodynamic (TK/TD) models were calibrated, validated, and used for predictions for analysing effects of chlorpyrifos-methyl (CPF-M) on the survival of the mayfly, Cloeon dipterum, the crustacean, Gammarus pulex, and the dipteran, Chironomus riparius. Model calibration was performed based on data from laboratory toxicity studies conducted with the three species and CPF-M, by fitting the model parameters to the data and calculating their confidence limits. The parameterised models were validated with independent data on the toxicity of chlorpyrifos (CPF), because there were no appropriate datasets for testing available for CPF-M. The calibrated and as far as possible validated model parameters were used to predict effects of concentration time series selected from 133 FOCUS Surface Water Step 4 exposure scenarios on the survival of the three investigated invertebrate species. The ultimate aim was to check that if CPF-M is applied according to the proposed application scenarios, then exposure only leads to negligible levels of individual mortality for the three sensitive macroinvertebrate species in edge-of-field water bodies. For C. dipterum, the least sensitive of the three investigated species, deterministic and probabilistic analyses indicated that more than the 10-fold of the predicted environmental concentrations would still only lead to negligible effects (mortality< 10%). For the second-most sensitive species, G. pulex, deterministic analyses indicated that for moderately increased concentration levels, i.e. up to the 4 fold of the predicted environmental exposure, non-negligible effects can be expected. For the most sensitive species C. riparius, all analyses gave indications that predicted environmental concentration levels would lead to non-negligible effects on survival. It must be critically remarked, however, that as a result of model calibration overestimation of mortality is likely for concentrations larger than 0.2 µg/L, and that quality and quantity of validation data for Cripriarius did not meet the requirements. Hence, development of Water Quality Management Standards to show the potential screening large numbers of exposure scenarios using TK/TD modelling, and how TK/TD models can be used to evaluate realistic time-variable exposure more extensively than by toxicity-exposure ratios alone. The results show that the toxicity potential of an exposure scenario is not governed by the maximum concentration alone.

**WE157**
Quantification of uncertainty in toxico kinetic-toxicodynamic model predictions

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extrapolation, thereby decreasing the need for toxicity testing for risk assessment. These models have the potential to facilitate species such an endpoint for regulation known to the authors.

Ambiguous. In order to replace this kind of experimentation a High-Accuracy approach integrates the benefits of existing methods and provides endpoints that fulfill the need for different risk assessments. We anticipate that TK/TD model predictions as reflected by the size of the uncertainty bands of survival dynamics plots of dose-response curves. Deterministic model predictions using best-fit parameters are in this way augmented with information about uncertainty of model predictions. This supports the evaluation of such model predictions for consideration in the regulatory environmental risk assessment of chemicals.

Variability in life stage sensitivity is a matter of toxicokinetics A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental Social and Spatial Change; F. Gabsi, Rifcon GmbH; A. Zenker, University of Appl. Sc. Northwestern Switzerland / Institute of Ecoprevention; T. Preuss, Bayer CropScience / Environmental Modelling Aquatic effect assessment uses results from short-term laboratory experiments in which it is purposely attempted to keep constant exposure conditions and to minimize inter-individual variability, mainly in the body size or age of the tested animals. In contrast, natural populations are in most species size- or age-structured. Body size is an important demographic attribute, not only because it determines population properties such as abundance and density but also controls the main ecological processes regulating population dynamics such as density-dependence and size-selective predation. Furthermore, individuals in the same population exhibit different sensitivities to the same exposure conditions, depending on their life stage, which means that toxicity may be under or overestimated based on the standard toxicity tests. In this study, we investigated to what extent intraspecies variability in sensitivity can be explained by differential toxicokinetics, i.e. uptake and elimination of the chemical. Furthermore, we tested toxicokinetic-toxicodynamic models of different complexities, including body size scaling approaches, for their ability to represent lethal effects observed for Daphnia magna exposed to triphenyltin. To assess the consequences of these approaches at the population level, we simulated different scenarios using an individual-based model and confronted predictions with population data from previously performed TK/TD models. The idea is to improve the importance of considering population demography in toxicokinetics and toxicodynamics for understanding and predicting potential chemical impacts at higher levels of biological organization.

Ecological scenarios for the assessment of chemical effects on stream communities: a mechanistic modelling approach A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental Social and Spatial Change; S. Classen, Research Institute gaiac; A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; K. Strauss, Research Institute gaiac; M. Hammers-Wirtz, Research Institute gaiac

The ecological risk assessment of chemicals aims at quantifying the likelihood of adverse ecological effects posed on populations and the communities they comprise. Effects caused by the exposure of organisms to toxicants can however to a large extent be understood as consequences of behaviors and interactions of organisms with consequences for individual life history, population responses and community dynamics. In the past, these aspects have often been ignored in ecotoxicology and the calculation of adverse effect concentration, which classically focuses on toxicant concentration responses while keeping the environmental conditions as constant as possible. We present the conceptual design of a mechanistic simulation model which aims at the integration of toxicokinetic-ecological scenarios from adverse effect risk to the ecological conditions. This individual-based community model will integrate stream typologies as formulated within water framework directive, functional trait data bases, spatial explicit movement behavior, dynamic energy budgets triggering live history processes and toxicokinetic-toxicodynamic models linking exposure and effects in a dynamic manner. We will increase our understanding of how chemicals act on multiple interacting species and provide a basis for bringing more ecological realism into the ecological risk assessment of chemicals.
ability of the model and the uncertainties will be assessed for both TK parameters taking into account fish species differences and prediction of toxicity parameters. 

WE162 Using mechanistic effect modelling to justify the TWA approach in aquatic risk assessment
T. Preuss, Bayer CropScience / Environmental Modelling; A. Solga, Bayer CropScience AG; V. Ducrot, Bayer CropScience AG / Environmental Safety 

Ecotoxicology; G. Goerlitz, Bayer CropScience AG / Environmental Safety; E. Bruns, Bayer CropScience AG / BCS D ETX. Ecotoxicology 

Risk assessments based on laboratory toxicity tests performed under constant exposure conditions may overestimate potential risks if the exposure concentration of the experiment is compared for the PEC_{exp} for short term exposure situation. To use PEC_{TWA} for the aquatic risk assessment several criteria have to be assessed following the EFSA Aquatic Guidance document: reciprocity and onset of effects as well as latency of effects. Most of these criteria cannot be assessed based on standard studies alone. On the one hand several endpoints are only measured at the end of the experiment. On the other hand effects are often found only in a few concentrations. Therefore mechanistic effect modelling especially toxikokinetics-toxidynamics modelling is already recommended in the EFSA Aquatic Guidance document to test these questions. In this poster we will demonstrate case studies in which we used mechanistic effect modelling (namely GUTS and IDamP) to justify the TWA approach compound and species specific. Therefore the models were calibrated to the experiment triggering the risk assessment, if possible the preditive power of the models were tested on a different dataset (model validation). In the last step the models were used to investigate the questions necessary to justify the TWA approach according to the EFSA Aquatic guidance document.

WE163 Landscape context in bee risk assessment - a way to a realistic worst case environmental scenario
T. Preuss, Bayer CropScience / Environmental Modelling; A. Jeltzsch, University of Potsdam; M. Miles, Bayer CropScience UK / Environmental Safety; T. Schad, Bayer CropScience / Environmental Modelling 

The western honey bee (Apis mellifera) is considered one of the most important pollinators concerning food crops. No sole underlying cause for increasing winter colony loss has been found so far, although there is consensus that the issue is of multicausal nature, and that multiple stressors, in addition to pesticides especially landscape characteristics and parasites play an important role. In the present work a modified version of the BEEHAVE model has been used to understand the effects of pesticides on a single bee hive under different ecological conditions. A pesticide module was added to simulate the effects of pesticides on foragers and larvae due to nectar and contact exposure. Virtual experiments are conducted to figure out how landscape context, weather, beekeeper practice and Varrhoa mites affect the outcome of a pesticide risk assessment. Virtual experiments were conducted on different levels of complexity starting with two artificial patches over simplified scenarios up to realistic landscapes. The model approach allows to adress directly the specific protection goals, namely colony survival, colony size (number of bees in a colony) and honey production. We tested in virtual experiments different combinations of beekeeping practice, weather and landscape features on bee colony health with and without the impact of a synthetic insecticide. This kind of analysis could not be conducted experimentally showing the strength of simulation models like BEEHAVE for environmental risk assessment. As defined in the EFSA Scientific Opinion on Good modelling an environmental scenario is a combination of agronomic, abiotic and biotic parameters which forms a realistic worst case situation. We will demonstrate that if setting up ecological scenarios the baseline against which the pesticide is tested has to be clear and this is not straightforward. To exclude unrealistic scenarios we suggest introducing the following constraint (boundary condition) for a realistic worst case scenario for bee risk assessment: honey harvest of at least 20 kg per hive should be possible within each year otherwise the bee colony would not be able to survive. As the colonies are modelled based on simple scenarios with realistic landscapes shows that realistic scenario selection can be possible, but a minimal complexity is necessary for a realistic worst case environmental scenario.

WE164 Colony level impact of sublethal pesticide effects on honeybees: a simulation study using BEEHAVE
P. Thorbek, Syngenta / Environmental Safety; P. Campbell, Syngenta / Environmental Safety; H. Thompson, Syngenta Ltd / Environmental Safety 

The research on neonicotinoids and honeybees has changed focus from direct mortality to sublethal effects such as reduced foraging and reproduction. Sublethal effects on brood care. Most of these tests have been performed in the lab using methods such as the proboscis extension reflex or histology, which would be challenging to translate into quantifiable effects on the individual’s or colony’s performance under field conditions. Here we use a published honeybee model, BEEHAVE (1), to study sublethal effects which cannot be assessed based on simple scenarios with realistic landscapes shows that realistic scenario selection can be possible, but a minimal complexity is necessary for a realistic worst case environmental scenario. 

WE165 Modelling the effect of bioturbation on pesticide exposure of beethive Chironomus riparius populations
L. Wipfler, H. Bergstedt, Alterra Wageningen UR; H. Baveco, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; W. Beltman, Alterra Wageningen UR; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group 

Intraventricular sediments from the western honey bee (Apis mellifera) are assessed concerning their potential to affect honeybee health. We propose that sediments are not only a barrier to the accumulation of pesticides, but may also be affected by chemical exposure but ERA does not incorporate aspects of behaviour would add further realism to risk assessment. Population modelling is employed to extrapolate from individual effects and biotic parameters which forms a realistic worst case situation. We will demonstrate that if setting up ecological scenarios the baseline against which the pesticide is tested has to be clear and this is not straightforward. To exclude unrealistic scenarios we suggest introducing the following constraint (boundary condition) for a realistic worst case scenario for bee risk assessment: honey harvest of at least 20 kg per hive should be possible within each year otherwise the bee colony would not be able to survive. As the colonies are modelled based on simple scenarios with realistic landscapes shows that realistic scenario selection can be possible, but a minimal complexity is necessary for a realistic worst case environmental scenario.

WE166 Individual-based simulation models of multi-species systems under the impact of chemicals: Complex dynamics can lead to changes in population recovery times and regimes
P. van den Brink, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; W. Beltman, Alterra Wageningen UR; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group 

There is a tendency to include ever more realism in models used for prospective environmental risk-assessment. Incorporating species interactions in multi-species models, may lead to emergent ‘complex’ dynamics and systems with alternative stable states (ASS). With ASS sudden shifts to alternative state may occur, following disturbances. In a previous study, we analysed complex dynamics in individual-based models (IBMs), comparing IBM simulations with analyses of a strategic model. We found that stress could change the stability regime of multi-species models and trigger transitions to ASS. As an example of competing species the aquatic macro-invertebrates Asellus aquaticus and Gammarus pulex were chosen; the classical Lotka-Volterra competition model (LVM) served as the thoroughly understood two-component model of predator-prey dynamics. The potential of ASS in multi-species systems emerged as one of the important roles
of models. With the same models, we perform additional analyses aimed at further understanding the consequences for risk assessment. Firstly, we identify what might go wrong with estimates of recovery times when these are based on a single-species models, when in the actual, real, system, competitive interactions occur. Secondly, we show how much of the rich behaviour of the system we would overlook if we address only the interactions that are evident on the scale of the region in parameter space associated with coexistence. Results show that an observer of the dynamics of *Ae. ludens* only, will encounter a bewildering variety of behaviour, ranging from fast and slow recovery to quick extinction. This variety in dynamics is logically related to the settings chosen for the competitor and easily explained from bifurcation diagrams. We conclude that when competition is playing a role, it needs to be addressed in population models, as it will have a large impact on recovery times. Recovery times depend on characteristics of target and competitor species. When the multi-species system has ASS, this is an additional complicating factor: the relative abundance after a stress event may lead to regime shifting, and recovery of the target species will never take place. Considering dynamics only for the coexistence case will ignore an important part of the potential behaviour of a competition system, and thus many possible alternative outcomes for recovery times will not be accounted for. From the results, we formulate a set of recommendations.

**WE167**

**CPFISH: A new approach for evaluation of binary data in many-to-one control vs. treatment setups**

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Fisher’s exact test for pairwise comparisons of proportions is widespread. However, when sequential application induces alpha inflation, Bonferroni correction or Bonferroni-Holm correction of p-values are often used to overcome this issue. For detection of significant effects in binary control vs. treatment setups proportion data are often evaluated by using multiple statistical tests, e.g. Dunnett t-test. While normal approximation of these data is a minor problem multiple t-tests suffer from variance heterogeneity. Applying arcsin-square root transformation variance homogeneity is provided. However, normal approximation is corrupted. To avoid data transformations the Cochran-Armitage-test has been proposed to investigate trends in proportions. The test statistic used is approximately normal. Thus, critical values, p-values and derived significant effects lack of statistical accurateness, too. Other tests based on approximate normal distribution are the maximum efficiency robust test, MAX3, the constrained likelihood ratio test and the GMS. In times of high computational power we can account for alpha inflation by using the closure principle (CP), instead. There is no need for adjustment of p-values. We propose to apply the extended version of Fisher's exact test (FISH) for 2 x s contingency tables in coherence with the CP using R statistic software. The FISH neither makes use of data transformations nor of approximate distributions. Using the real binomial distribution of the data CPFISH does not suffer from the drawbacks induced by normal approximation and heterogeneous variances. By combining CP and FISH the CPFISH is derived. The applicability of CPFISH is shown by many real and fictitious data sets. Finally, we compare CPFISH to the well-established approaches of exact test to prove the coherence with arcsin-square root transformation and Fisher’s exact test in coherence with adjusted p-values using Bonferroni and Bonferroni-Holm correction.

**WE168**

**Species Sensitivity Distributions with Censored Values**

J.W. Green, DuPont / Applied Statistics Group

Species Sensitivity Distributions (SSDs) are increasingly requested by regulatory authorities, especially in the EU, as part of product registration submissions. There is a growing concern, implying that recovery of treated species will never take place, and that the real-system requirements and acceptable methods of fitting and interpreting SSDs. The primary issue to be discussed is the handling of censored values (e.g., EC50=100 ppm). It has become common in some EU registrations to reject species data for SSD purposes if the data do not conform to the log-normal distribution. There is no scientific basis for this requirement and its use does not serve regulatory conservatism. Indeed, exclusion or improper treatment of censored values tends to increase HC5 estimates. Another issue is forcing all data to fit a single distribution, such as log-normal. It is shown that this too can produce significant bias. The treatment of censored data in SSD fitting has not received as much attention as it deserves, given that right-censored data is common in toxicity studies and occasionally, left-censored data are found. The latter are common in monitoring data, but not common in toxicity studies. Some recent regulatory guidance suggests discarding censored values in fitting an SSD. (e.g., EFSA 2014; EFSA 2015; Schmitz et al. 2015) or using them only under strict conditions (EFSA 2013). There is no scientific basis for avoiding censored values or for treating ends as uncensored. There have long been known mathematically correct ways to include censored values in fitting a distribution. Ignoring censored values means the distribution being analyzed is truncated and there are well known differences between truncated log-normal and log-normal distributions. The bias introduced by eliminating censored values will be demonstrated, both through datasets used in product registrations, computer simulations, and mathematics. A well-known mathematically correct approach to including censored values will be described. Force fitting by a standard distribution or rejecting data that do not conform to this distribution, and exclusion or improper treatment of censored data will bias the HC5 and HC5LB estimates. Mathematically sound and tractable methods for selecting distributions and handling censored values are known and available in validated software. There is no good reason for ignoring sound science in fitting or interpreting SSDs.

**WE169**

**Dynamics and recovery of a sediment exposed Chironomus riparius population: A modelling approach**

N. Diepens, Wageningen University; W. Beltman, Alterra Wageningen UR; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; H. Baveco, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Group

To assess risks of sediment-bound contaminants, larger temporal and spatial scales have to be addressed than can be covered in laboratory tests. Although models can address these scales, they usually lack the coupling between chemical fate in the sediment, toxicokinetic-toxicodynamic processes in individuals and the propagation of individual-level effects to the population. We developed a population model that includes all these processes and assesses the importance of chemical uptake routes on damage and recovery of a *Chironomus riparius* population after pulsed chlorpyrifos exposure. We show that particle ingestion is an important additional exposure pathway affecting *C. riparius* population dynamics and recovery. Neglect of particle ingestion underestimates damage and recovery times, which implies that risks of sediment chemicals are underestimated. Additional scenario studies showed the importance of selecting the biologically relevant sediment layer and the use of long term data output.

**WE170**

**An individual-based model of the three-spined stickleback: incorporating effects on breeding behaviour into the assessment of endocrine disrupting chemicals.**

K. Mintram, University of Exeter / Biocenoses; P. Thorbek, Syngenta / Environmental Safety; S.K. Maynard, Syngenta / Environmental Safety; A. Brown, Exeter University / Biosciences; C. Tyler, Biosciences College of Life and Environmental Sciences

Population modelling is employed to extrapolate from individual effects (including behavioural effects) to population-level effects in the environmental risk assessment (ERA) of chemicals. Population models that incorporate ecological processes such as density dependent competition, individual variability and aspects of behaviour would add further realism to risk assessment. Reproduction in some fish species involves complex breeding behaviours that can be affected by chemical exposure but ERA does not incorporate aspects of behaviour into regulatory testing. In the three-spined stickleback (*Gasterosteus aculeatus*) it has been shown that nest building, courtship displays and parental care, may be disrupted by exposure to multiple toxic endocrine disrupting chemicals (EDCs), consequently affecting population recruitment. Here, we present an individual-based model (IBM) for the three-spined stickleback with the purpose to simulate realistic scenarios for the chronic exposure effects of EDCs. The three spined stickleback is widespread geographically, and potentially sensitive to chronic exposure to EDCs that mimic sex hormones given its complex breeding strategy, low fecundity and the provision of high level of parental care. Density dependent growth and mortality and individual breeding behaviours are key parameters within the model. The IBM has been structured using a series of sub-models, based on empirical data obtained from published literature. The poster will present a full description of the model with some preliminary testing, and illustrate its potential application within ERA.

**WE171**

**Tribolium castaneum under toxic stress: mechanistic effect model for a holometabolous insect.**

A. Barzeg, Ctbg / Dept of Theoretical Biology; J.L. Maino, University of Melbourne / School of Biosciences; T. Jager, DEBox Research; A. Bednarska, Polish Academy of Sciences / Institute of Nature Conservation

Several bioenergetics models for animal species are available to address questions about organism functioning under stressed conditions. However, a limited number of such models exists for holometabolous insects, mainly due to difficulties in capturing the complexity of their life cycle. These organisms are characterized by several metabolically distinct developmental stages, *i.e.*, egg, larva, pupa and adult. We have developed a bioenergetics model to describe the entire life cycle of the red flour beetle *Tribolium castaneum* (*Coleoptera: Tenebrionidae*) under toxic stress. Experiments in which individuals were exposed to copper concentrations over 90 days were conducted. The obtained experimental data on individual
survival, growth, development and reproduction over time were used to calibrate the model. The model shows good performance at describing the life cycle of an individual organism. Although the beetles undergo a complex developmental path, our model captures well the pattern of toxic effects such as impaired development or reproduction. We demonstrate how conducting one integrative analysis, based on one common molecular and organismal endpoint, can provide mechanistic insight into the energy budget of the red flour beetle under toxic stress. Finally, aiming at being generic for holometabolous insects, this model has a potential to address issues relevant for chemical risk assessment of regulatory important organisms such as bees or non-target arthropods. The project was financed by the National Science Centre project HARMONIA (No. 201206/M/ NZ/00137).

Quantitative in vitro to in vivo extrapolation (QIVIVE):
Advances in tools to quantify exposure (dose)-response relationships and use in risk assessment (P)

WE172 How representative are rate liver cytochrome P450s for possible endocrine disruptors identification? An in vitro azoles screening study.
E. Apazoglou, University of Coepnhagen / Plant and Environmental Sciences; N. Cederroen, M. Gottardt, University of Copenhagen / Plant and Environmental Sciences
Endocrine disruptors are a group of chemicals that interfere with the endocrine system and are an emerging health concern. In this study we focus on the endocrine disrupting chemicals that inhibit the enzyme aromatase (CYP19) in the steroidogenesis pathways, therefore creating imbalance in the vertebrates' hormone systems. CYP19 belongs to a larger group of enzymes called cytochrome P450 monoxygenases, which are preserved across different species. They are heme-containing metalloenzymes that catalyze hydroxylation and oxidation on the metabolism of thousands of endogenous and exogenous compounds. Azole antifungal agents are designed to inhibit 14α-demethylase in the fungal ergosterol biosynthesis and therefore they act as inhibitors for other non-target cytochromes as well. In this study, 21 different azoles were tested in vitro on rat liver microsomes (ECOD) activity and compared with previous studies on CYP19, in terms of their inhibition potential. The rat liver microsomes were used to represent the cytochrome P450s enzymes and there was a positive correlation (imidazoles a.o.34, R² = 0.70 and triazoles a.o.68, R² = 0.52) between P450s and CYP19 50% inhibitory concentrations (IC50) suggesting that azoles were stronger inhibitors of P450s as compared to CYP19. Imidazoles appeared to be more potent than triazoles for both P450 and CYP19 inhibition. The structure and the lipophilicity of the compound play an important role for the affinity of the inhibitor for the enzymatic binding pocket. It was shown that inhibition, measured as IC50, increased with increased lipophilicity of the compound. For measuring P450 activity 7-ethoxyconuarnin was used as a standard substrate because of its low selectivity. Testing different substrates such as testosterone can possibly be more relevant to compare to CYP19 activity. The results of this study are encouraging on further studying the possibility to use rat liver microsomes as representative for human CYP19 and as a screening tool for possible endocrine disruptors.

WE173 Critical Membrane Concentrations of Amines in In Vitro and In Vivo Fish Bioassays
s_droge, Utrecht University / BRAS; J. Hermens, N. Kramer, Utrecht University / Institute for Risk Assessment Sciences
Neutral organic contaminants are assumed to be nontoxic specifically at the baseline critical membrane burden (CMB) of ~100 mmol/kg lipid. Assuming a contaminant’s octanol-water partition coefficient (logP) is equal to its membrane affinity (logKmw), the specificity of the toxic mode of action (or ‘excess toxicity’) can be estimated, where CMB in mmol/kg lipid = median lethal concentration (LC₅₀) in mmol/L x logKmw in L/kg lipids. This assumption needs to be refined for contaminants that are predominantly present in their ionic form in toxicity assays as the CMB has only been determined for a large set of neutral contaminants. This is an important data gap in environmental risk assessment because the majority of pharmaceuticals and a substantial fraction of industrial chemicals are highly ionizable. The aim of this study was two fold: 1) to define the CMB for strong bases (pK₉ > 9) using existing in vivo fish acute toxicity data from the US EPA Fathead Minnow Database, and 2) to determine whether this determination could be replicated in an in vitro toxicity assay with a fish cell line (RTGill-W1). Membrane affinities for a series of amines listed in the US EPA’s Fathead Minnow Acute Toxicity Database were determined using immobilized artificial membrane chromatography (IAM-HPLC), which allows for a direct interpretation of the critical membrane burden (CMB) in acutely exposed fish for these cationic chemicals. Accordingly, the CMB of thirteen amines with a neutralization mode of action (MOA) was found to be around a value of a range of 7.6±5 mmol/kg lipid, more than an order of magnitude lower than the baseline CMB for neutral chemicals. For the neurotoxic amines, the CMB of amphetamine was similar to those of the “unsure MOA” amines, while strychnine and nicotine showed an “excess amine toxicity” of a factor of 10, with CMB’s of 0.6 mmol/kg lipid. RTGill-W1 cells were exposed for 48h to the abovementioned amines and cytotoxicity was assessed using the Alamar Blue assay. Test media were sampled before and after exposure and amine concentrations were analytically measured to define actual exposure concentrations. Unlike for neutral organic chemicals, in vitro median effect concentrations (EC₅₀) for the tested amines were at least twofold higher than fish acute LC₅₀ values. This suggests that the specific mechanisms by which amines accumulate or cause toxicity in vivo are not replicated in vitro.

Application of a coordinated OMICS research program and data into regulatory frameworks: case-studies and perspectives (P)

WE174 Developing an enhanced experimental work-flow for maximising the use of ‘omics data within the Adverse Outcome Pathway framework
N. Kool, University of Birmingham / Biosciences College of Life and Environmental Sciences; E. Butler, Unilever / Safety and Environmental Assurance Centre SEAC; S. Gutsell, Unilever / SEAC; J. Zhou, N. Shi, S. He, University of Birmingham / Computer Science; K.G. Sappington, U.S. EPA / Office of Pesticide Programs; A. Biales, U.S. EPA / National Exposure Research Laboratory; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; J. Colbourne, University of Birmingham / Biosciences College of Life and Environmental Sciences; M.R. Viant, University of Birmingham / School of Biosciences; G. Holmes, Unilever Research / Safety and Environmental Assurance Centre Developments in ‘omics technologies have opened new possibilities for assessing the functional and biochemical impact of xenobiotics on organisms. Feature selection tools are increasingly being used to discover responses within the ‘omics datasets that may be predictive of adverse outcomes. These molecular responses can help to define the ‘key events’ (KEs) underpinning the toxicity pathway within an Adverse Outcome Pathway (AOP) framework. KEs are deviations from a healthy state, through a stress response, that may predict an adverse outcome and therefore indicative of a chemical’s potential health hazards. Here we investigate the potential of multi-omics technologies to discover KEs of specific acting chemicals and baseline narcotics. The first phase of this work has been the development of a proposed work-flow of how to utilise the non-targeted, high-throughput datasets, data triage and data aggregation towards this into an enhanced AOP driven risk assessment approach. The second phase has been to start developing comparative multi-omics approaches as part of an integrated (weight of evidence) approach in combination with available in silico or in vitro data to support the categorisation of chemicals by their KEs based upon their molecular and biochemical responses in Daphnia and algae. We aim to achieve this by elucidating the relevant KEs of specifically acting chemicals and baseline narcotics and measuring the strength of the association with the subsequent adverse outcome. The use of organisms that span two trophic levels could enable qualitative assessment of the predictive capability of the KE(s) both within and across species, improving the prediction utilities for set species and in setting exposure thresholds in environmental risk assessment. To identify target homology and further enhance cross species applicability, molecular target sequence analysis tools; such as the US-EPA developed SeqAPASS (Sequence Alignment to Predict Across-Species Susceptibility), will be utilised. Sampling at high temporal resolution (spanning the acute and chronic experimental stages) for these assessments will provide reproducible toxicological effects. The use of multi-omics networks is an opportunity to complete the puzzle by gene expression and metabolite profiling. By detecting the point of departure from a healthy state, we aim to use acute exposure scenarios to predict chronic outcomes, ultimately streamlining the experimental design to capture just the KEs making the work flow rapid and cost-effective.

WE175 Transcriprome network analysis of toxicity pathways in aquatic organisms
D. Coman Schmid, Eawag Swiss Federal Institute of Aquatic Science and Technology; S. Pillai, Eawag Swiss Federal Institute of Aquatic Sciences; K. Schirmer, Eawag Swiss Federal Institute of Aquatic Sciences; S. Zapunic, Eawag Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology Network analysis is based on the guilt-by-association principle and it is a powerful method for identifying patterns within many types of data (e.g. social, medical, biological, environmental etc.). Aiming to expand our understanding of toxicity pathways in aquatic organisms, we developed nertoxx – an integrative systems biology workflow, which infers mutual information co-expression networks from large collections of transcripome data in several organisms (e.g. green algae, zebrafish). Although the biological processes for some chemical stressors have been characterized, most toxicity pathways and their mechanisms of action at the cellular level are still unknown. Integrative bioinformatics and statistical analyses of large and diverse collections of omics datasets have great potential in revealing the mechanisms of cellular response of aquatic organisms to chemical stressors. Specifically, gene co-expression network analyses can identify hubs and modules of genes with similar expression patterns, indicative for structured, modular toxicity response characteristic to certain chemical stressors. nertoxx uses both
in-house generated omics datasets as well as publicly available data, summing up to more than 650 transcriptome samples in Cladonodulina reinhardtii. An entropy based mutual information metric is then used to infer statistically significant co-expression relationships between gene products (i.e. transcripts). The resulting network is visualized as a graph (nodes represent genes and edges show co-expression relationships between genes) and various network statistics are employed to identify interesting patterns such as hub genes (frequently essential genes) and highly interconnected modules (indicators for groups of genes involved in the same cellular process and/or metabolic pathway). Our vision is to use new tools to identify models of toxicity pathways responses and to advance from single mechanism of action to toxicity pathway-based understanding of chemical effects on the transcriptome of aquatic organisms.

WE176
Ecological case studies using chemical, target and outcome-based approaches to AOP development.
While formal guidance documentation exists for describing Adverse Outcome Pathways (AOP), development strategies are lacking. Here, different approaches for developing AOPs are considered, including: chemical-, target-, and outcome-based. The chemical-based approach to AOP construction is common. Often, a chemical with a well-studied mode of action is used to identify key events and propose relevant biomarkers. An alternative, the knowledgebase approach (aopkb.org) is AOP 4: Ecycosynone (EcR) activation leading to mortality in Daphnia magna, which is based on the action of the EcR agonists 20-hydroxyecdysone, ponasterone A, and tebufenozide. Prioritization efforts can also drive chemical-based AOP approaches. For example, an AOP describing potential fluoroxetine metabolism in aquatic species (NAFP) was developed using data from the Adverse Outcome Knowledgebase which are not yet available for the water bodies with regard to sustainable development.

WE178
Fish acute toxicity data -water partition coefficient (logP) is equal to its membrane demethylase in the fungal ergosterol -baseline critical membrane burden (CMB) of ~100 mmol/kg lipid. Assuming a -water partition coefficient (logP) is equal to its membrane demethylase in the fungal ergosterol -baseline critical membrane burden (CMB) of ~100 mmol/kg lipid. Assuming a

WE179
Constitution of a reference transcriptomic resource for the ctenophore Euytemora affinis and differential expression between sexes exposed to acetone.
E. LEGRAND; J. Forget-Leray, A. Duflot, S. Olivier, C. Boulange-Lecomte, SEBIO UNIVERSITY OF LE HAVRE / UMRI SEBIO
Copepods represent the most abundant group of aquatic invertebrates which includes freshwater and marine species. Among them, the calanoid copepod Eurytemora affinis is widely represented in the northern hemisphere estuaries and has become a species of interest in ecotoxicology. Like other non-target organisms, E. affinis could be exposed to a wide range of chemicals. In this context, we investigated the transcriptome of E. affinis in order to (i) constitute a benchmark to identify adverse responses in the different species exposed to acetone. We first identified and characterized an AOP for E. affinis exposed to solvent acetone as a base for future chemical exposure. After 3 days of stalling, a first sample of 500 copepods (including males, females and young stages) was realized. Then, a solvent (acetone, 0.005% w/v) exposure was performed. After 48h of exposure, 400 males and 400 females were sorted and frozen for further analyses. RNA from non-exposed and exposed organisms were respectively sequenced by 454 GS FLX and Illumina®. E. affinis transcriptome was functionally analyzed using the KEGG pathway database. The differential expression analysis was performed from the acetone samples. Differentially expressed genes (DEG) were investigated after applying a threshold of PPDE (Percentage Probability Differentially Expressed) of 0.95 and a log2FoldChange ≥ 2. A total of 1,651,761 reads were pyrosequenced. De novo assembly resulted in 16,903,584 bases divided in 19,721 transcripts with a mean length of 857 bp. About half of them (9,729) were annotated. 64% of the Blast results were provided by arthropod sequences including crustaceans and insects. 6,446 sequences were identified in shared pathways. Among these, the category "cellular processes", "environmental information processing", "human diseases", "organismal systems", "genetic information processing" and "metabolism" were illustrated. Moreover, 1,786 DEG were identified between sexes after acetone exposure highlighted specific sex patterns. 1,072 genes were expressed in males whereas 714 genes were expressed in females.

WE180
Sorting the signals: meta-analysis of transcriptome data of zebrafish embryos after chemical exposure.
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New Technologies; R. Altenburger, UFC Centre for Environmental Research / Bioanalytical Ecotoxicology: W. Bushch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology

Adverse effects of chemicals on cells and organisms are in many cases either preceded by or result in gene-expression changes (Nuwaysir et al., 1999). This makes gene-expression analysis a valuable tool in toxicological research, e.g. for future bioassay or diagnostic tool for biomonitoring. There are several advantages in comparison to established tools, e.g. the non-targeted approach, allowing assessment of several modes-of-action at the same time, or the possibility to combine assessment of adverse effects with identification of effect-drivers, which could also be helpful in the establishment of adverse outcome pathways (AOPs). During recent years several studies analysed global gene expression in Danio rerio embryos after exposure to chemicals (Williams et al., 2014). Many of those studies show specific gene-expression changes in zebrafish embryos after exposure to chemicals and claim to offer a proof of concept of the suitability of microarray analysis as potential bioassay. To put the so far published results into context, and analyze the quality and comparability between the studies, we performed a meta-analysis of published transcriptome data. The analysis combined the data of 33 published studies using different chemicals, concentrations and exposure times. The analysis revealed a large heterogeneity in gene expression profiles. In contrary to our expectations no gene could be identified as regulated across all conditions. For sure, heterogeneity can be partly explained by differing experimental designs and the lack of time- and dose resolved data. Since comparability is one major requirement for any OMIC-technology on the way to usability in risk assessment, we propose an experimental design for transcriptomics with time and concentration resolved measurements. B. Ecke, B. Ritter, H. Sprungt, J. Barrett, J. C., & Afshar, a. a. (1999). Microarrays and toxicology: the advent of toxicogenomics. Molecular Carcinogenesis, 24(3), 153–9. Williams, T. D., Mirbahai, L., & Chipman, J. K. (2014). The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. Briefings in Functional Genomics, 13(2), 157–71.

**WEI81**

The phytotoxic family of pharmacologically relevant freshwater organisms J. MAZZITELLI, Centre Universitaire Jean-François Champollion, Albi, France / UMCR CNRS; E. BONNAFE, J. Malougyres, Centre Universitaire Jean-François Champollion Albi France; F. Geret, Centre Université Jean-François Champollion Albi France / UMCR CNRS

Aquatic organisms are frequently exposed to a wide diversity of chemical compounds suspended or absorbed on substrates. These compounds such as pharmaceuticals are released into aquatic environment from either industrial or domestic effluent. Most of chemicals, especially psychotropic pharmaceuticals, are very persistent even after biotic and abiotic treatments in environment and waste water treatment plant (WWTP). Despite of the important occurrence of detection, few study concern the aquatic environmental impact. Among the aquatic biodiversity, the molluscs, broadly studied for their role in the food chain (primary consumer), but also because of their low celerity. Radix bathica is a freshwater mollusc present in many French rivers, allowing easy sampling of this snail for eggs collection. The flatworms including Schmidtea polychroa, belong to benthic invertebrate and are also studied as ecotoxicological model due to their regeneration ability and secondly for their role in food web chain (species consuming a specific organ). The objective of this study was to expose the toxicity of four psychotropic drugs from four different therapeutic classes and three chemical families. Oxazepam (anxiolytic, benzodiazepine), carbamazepine (antiepileptic, benzodiazepine), cymamazin (neuroleptic, phenothiazin) and sertraline (antidepressant, Inhibitor Selective of Reuptake of Serotonin (SSRI)) have been selected because of their frequencies and their presence in the French WWTP effluents. Pharmaceuticals toxicity was evaluated on R. bathica embryos by hatching success and hatching delay on a concentration range from environmental concentration to 100µg/L. For instance, these indicators showed an effect from 1µg/L to 100µg/L for oxazepam. A differential RNASeq method and an RT-qPCR microarray analysis were performed to search the potential effect of this studied chemical. The differential expression analysis revealed some genes differentially expressed between the two treatments. This geneset included ribosomal proteins and RNA polymerases as well as histones and methyl-binding proteins. Despite the recent developments in molecular technologies, little attention has implied the possibility that modifications might be inherited across generations. The epigenetic and evolutionary effects of pollutants: new systems are required.

**WEI81**

A Combined Targeted/-non-Targeted Metabolomics approach using UHPLC-Oribtrap- MS: Method validation and ecotoxicological applications A. Ribiensdottir, Stockholm University / Department of Environmental Sciences and Analytical Chemistry ACESO, M. Abeg, ITM Stockholm University / Department of Environmental Sciences and Analytical Chemistry ACESO; J. Bajdencik, Stockholm University / Environmental Science and Analytical Chemistry

Characterization of the naturally occurring small molecules in a biological sample (e.g. the metabolome) has shown great promise in ecotoxicology for characterizing chemical modes-of-action, fingerprinting toxicant exposure, or redefining low dose effects levels. Metabolomics can be carried out using either a targeted approach, involving a full complement of standards, or non-targeted approaches in which analysis is carried out in open scan and individual chemical peaks are identified through mass spectral library/unmatching. Advantages of the former include generation of quantitative data and improvements in accuracy and precision, while the latter offers the possibility of discovering novel biomarkers. Few have attempted to combine these approaches in order to quantify known metabolites and unknown compounds, allowing for a re-dimensioning of exposure. We report on the development of a novel targeted/non-targeted metabolomics approach using ultra-performance liquid chromatography-high resolution (Orbitrap) mass spectrometry. The method facilitates targeted, quantitative analysis of 20 amino acids, 10 non-proteinogenic amines, 7 biogenic amines, 7 TCA-intermediates, and 10 ion channels and neurotransmitters in multiple reaction monitoring mode, while simultaneously collecting high resolution (R=100,000), accurate mass (<1ppm) data in open scan (m/z100–1000) to facilitate novel biomarker ID. Three methods were developed to maximize metabolite coverage: The first 2 methods employed C18 (1.8µm, 2.1x100mm) and HILIC (1.7µm,2.1x100mm) stationary phases with trifluoroacetic acid and acetonitrile mobile phases for determination of non-lipid metabolites. The third method utilized flow injection-MS/HRMS with isotopic overlap deconvolution for determination of >150 lipids (including carnitines, glycerophospholipid and sphingomyelins). The benefits of HRMS for FI-MS/MS-based lipidomics were assessed by comparing results with those obtained using the identical method run on a quadrupole mass spectrometer. Non-targeted data for all analyses were processed using TracMass2 and MetaboAnalyst. This method is among the first to combine a combined targeted/non targeted approach to obtain quantitative information on known metabolites, while offering the possibility of novel biomarker ID.

**WEI83**

Metabolite formation from 3-methylindole by hepatic microsomes from carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) during phase I metabolism V. Burkin, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses Vodnany Czech Republic; V. Zlabek, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; B. Bisseter-Paira, Product Quality Program, IRTA-Monells; S. Sakalli, University of South Bohemia Ceske Budejovice / Faculty of Fisheries and Protection of Waters; G. Zamaratskaia, Swedish University of Agricultural Sciences / Department of Food Science

Emission from intensive livestock can affect health of living organisms. The aquatic environments are exposed to chemicals from livestock excreta and discharge in sewage systems. In this study, we investigated the proteomic responses of the widespread copepod Eurytemora affinis and the freshwater cladoceran Daphnia magnav of 3-hydroxy-3-methylindole (3MI) and indole-3-carbinol (I3C), while carp produces only 3MI. The rate of 3MI production was similar in both species at both ages. The rates of 3MI production were higher in carp than in E. affinis and D. magna. The results of this study indicate that 3MI is not a target metabolite for fish. However, current knowledge on 3MI metabolism in fish is limited and the outcome of these results imply further studies are required.

**WEI83**

Application of transgenic zebrafish in adverse outcome pathway analysis: a case study on bisphenol A A. Brown, Exeter University / Biosciences; L. Gunnarsson, University of Exeter / Biosciences; J. Green, University of Exeter / College of Life and Environmental Sciences; S. Mourabit; m. hetheridge, Exeter University / Biosciences; R. Currie, Symgenga; C. Tyler, Biosciences College of Life and Environmental Sciences Mammal drama, or suspicion of non-targeted hormonergic receptors, and/or to affect hormone metabolism, and some of these molecular initiating events (MIEs) may lead to adverse effects on a wide range of developmental processes and physiological functions. However, only a small fraction of chemicals have been investigated for endocrine disrupting effects in invertebrates. Although the toxicological evaluation and the indication of the potential endocrine activity of chemicals, reliable in vivo systems are required.

292 SETAC Europe 26th Annual Meeting Abstract Book
to assess for adverse effects. Transgenic (TG) animal models (e.g. zebrafish) provide in vivo systems, in which MIEs triggering adverse outcome pathways (AOPs) can be identified and quantified by fluorescence protein reporters linked to specific receptors or enzymes. Spatial resolution of MIEs at the organ and tissue levels in larval TG zebrafish facilitates targeted assessment of downstream molecular events and subsequent physiological and functional responses. Here we present a case study on embryo-larval TG zebrafish exposed to the estrogenic plasticiser bisphenol A (BPA) previously reported to affect brain development and function at or below aqueous exposure concentrations (230 µg/L) leading to tissue concentrations (10 µg/kg), which are equivalent to mean human placental concentrations measured in amniotic fluid for doses for bottle fed infants. Using TG(ERE-Casper) zebrafish, which combine a reporter system for indicating Estrogen Response Element activation throughout the body in a translucent Casper phenotype, we show that BPA (100, 1000 µg/L) and its metabolite MBP (0.25, 2.5, 25 µg/L) target the heart valves, with consistent ERE expression at 4 and 14 days post fertilisation (dpf). Via assessments of differential gene expression (using RNA-Seq) in micro-dissected heart tissues obtained from treated and control fish at 4 and 14 dpf, and a series of cardiovascular and other functional endpoints (e.g. heart beat rate, blood flow, critical swimming speed at 14 dpf), we attempt to define AOPs for the two test compounds. References: 1) TEDX 2015.

WE185
Proteomic analysis of the estuarine copepod, Eurytemora affinis, in response to endocrine disruptors
Aquatic ecosystems constitute the chemicals’ final destination. One of the chemicals’ families targeted by the EU Water Framework Directive is pesticides due to their endocrine disruption (ED) potential. Nowadays, ED effects of chemicals are well documented in vertebrates whereas they represent 95% of the wild fauna. In this context, we investigated the proteomic responses of the widespread copepod Eurytemora affinis to model pesticide exposures, in order to (i) improve the knowledge on their effects in crustaceans and (ii) evaluate the potential of proteomic analysis to develop molecular biomarkers of endocrine disruption. After 3 days of stalling in the laboratory, copepods were divided into three 10-liter aquariums. Copepods were exposed to sublethal concentrations of pyriproxyfen (PXF, 10µg/L) and chlordione (CLD, 10µg/L) separately and in binary mixture (PXF, CLD: 2.5 µg/L; 3.75µg/L respectively). A solvent (acetone 0.005% v/v) control was used. After 48h of exposure, males and females were sorted and frozen for further analyses. After extraction, proteins were identified by HPLC coupled to mass spectrometry (GC-MS-MS). Analyses were performed for both genders for each contamination in order to identify sex-related responses. 1192 proteins were identified using the GC-MS-MS procedure. The cluster analysis highlighted two groups corresponding to gender. Male proteins were more impacted by PXF and CLD respectively. Finally, 76 proteins were differentially detected between conditions including 25 proteins under-expressed and 51 over-expressed. Proteins were involved in several processes such as energetic metabolism and defence. We have focused our investigation on proteins involved in reproduction, growth and development in response to EDs. For example, proteins involved in reproduction like vasa or thiodorexin were under-regulated in male copepods highlighting a potential disruption of gametogenesis. Furthermore, muscle activity with actin and myosin were under-regulated in male copepods, suggesting an impact on locomotion.

WE186
Screening of pesticides, alone and in binary mixtures, for ecysteoid hormone agonist and antagonist activity using the Drosophila cell-based (SZ) reporter assay
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Aquatic ecosystems constitute the chemicals’ final destination. One of the chemicals’ families targeted by the EU Water Framework Directive is pesticides due to their endocrine disruption (ED) potential. Nowadays, ED effects of chemicals are well documented in vertebrates and the Organization for Economic Co-operation and Development (OECD) recommends the use of human receptor assays for the screening of ED chemicals. Few data are available on the ED effects of pesticides on invertebrates. We present current efforts to analyze the transmission of estrogenic hormones to non-irradiated progeny to investigate the potential agonist and antagonist effect on the ecysteoid receptor for five pesticides known as endocrine disruptors, alone and in a binary mixture using an in vitro test. In this project we used chlordione (CLD), vinclozolin (VZ), tributyltin (TBT), pyriproxyfen (PXF) and methoprene (MP), as well as invertebrate models of endocrine disruptors. Of each product a range of different concentrations (from 1.5 x 10^-1 to 3 x 10^-8 M) was tested for investigating their agonist/antagonist potential. Drosophila S2 cells were firstly transfected with the pERE-GFP-ERE-Luc construct composed of ecysteoid hormone response elements and the reporter luciferase gene. Then the agonist and antagonist activities were tested in 96-well microplates. No agonist activity was observed for any compound alone. Cell toxicity appeared for the highest concentrations. An antagonism was detected for PXF and MP. As juvenile hormone agonist, interaction with the ecysteoid receptor could be expected. Moreover, CLD – known as an estrogen receptor agonist – may present a weak antagonist activity. TBT did not act as an antagonist of the ecysteoid receptor. No agonism was observed for any binary mixture and toxicity appeared for the highest concentration. All the binary mixtures showed an antagonist activity. However, interaction between chemicals could induce a response which could not be specific to the ecysteoid receptor. In conclusion, the cell-based reporter assay for screening ecysteoid receptor agonists/antagonists highlighted antagonist activity for both vertebrate and invertebrate models of endocrine disruptors.

Epigenetic and evolutionary effects of pollutants: new challenges for long-term ERA (P)

WE187
Changes in DNA methylation in response to cyanobacteria in the model crustacean Daphnia
J. Asselmann, Ghent University / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; D. De Coninck, Ghent University / Laboratory of Environmental Toxicology Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; K. De Schampheleere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit
Despite the recent developments in molecular technologies, little attention has been given in environmental toxicology to epigenetic modifications and their potential consequences for environmental risk assessment. We focused on the crustacean Daphnia whose well-known ecology and phenotypic plasticity offer unique opportunities to enhance our understanding of epigenetic mechanisms and their importance in environmental challenges. We characterized the methylation pattern in Daphnia in response to a rich diet and a diet contaminated with the toxic cyanobacterium Microcystis aeruginosa, a common harmful algal bloom known to have detrimental effects on higher organisms. We used bisulfite sequencing to identify methylated cytosines in genomic features. Methylation was primarily targeted to ecogenic regions while introns and intergenic regions were generally devoid of methylation. We observed that despite the low methylation level in Daphnia, some genes are preferably methylated with methylation levels higher than 50% while others have no methylation at all. Roughly 300 genes were differentially methylated between the two treatments. This genest included ribosomal proteins and RNA polymerases as well as histones and methyl-binding proteins suggesting a functional role for DNA methylation in gene regulation. Overall, the results suggest that DNA methylation may play a significant role in environmental stress responses in Daphnia.

WE188
Transgenerational effects of a chronic external gamma irradiation on Daphnia magna: epigenetic modification and inheritance
M. Trijau, IRSN/PRP-ENV/LECO / PRPENVSERISLECO; F. Alonzo, C. Abad-Guillerm, IRSN
Aquatic and terrestrial ecosystems are exposed to radionuclides through planned discharges or accidental releases linked with the nuclear industry activities. Over the past decade, ecosystems protection against ionizing radiation has become a growing public, regulatory and scientific concern. Nowadays, ecological risk assessment for ionizing radiation is still based for a vast majority on studies investigating effects under acute doses and short exposure time, far from realistic environmental conditions in which natural biota are actually exposed. Recently, studies in daphnids at chronic low doses have been conducted over several generations in order to increase our understanding of the long-term effects of low doses of gamma radiation on the transmission of underlying molecular mechanisms. In this context, radio-induced epigenetic modifications have never been investigated in invertebrate species although it was shown that ionizing radiation can induce changes in DNA methylation patterns and gene expression. Epigenetic changes imply the possibility that modifications might be inherited across generations. The present study (still in progress) examines the potential transmission of epigenetic alterations from gamma irradiated daphnids to their non-irradiated progeny up to...
the third generation (F1, F2, F3). Using the cladoceran crustacean Daphnia magna as a model organism, our objectives are threefold: (i) to examine whether environmentally relevant doses of gamma radiation (0.01 and 3.54 mGy h⁻¹) received by parents will induce effects on survival, growth and reproduction over non-exposed generations, (ii) to test possible epigenetic modifications caused by gamma radiation and its transmission to non-exposed generations, (iii) to compare responses to chronic gamma radiation between the molecular and organism levels.

WE189 Crossing scales: From genes to the phenotype and back in ecotoxicological extrapolation for genetic and epigenetic effects - the next frontier

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Propriety Science (PS) relies on methods and methodologies that provide tools to extrapolate findings across varying scales. Scales can include organisms, environments, space and time. In Ecotoxicology and Climate Change Science, the issue of extrapolation presents a pivotal challenge to assess the risks of chemicals and other stressors in the environment. Evolution acts on scales different to the genetic and epigenetic effects of toxicaants. As a consequence, extrapolation is a formidable temporal problem that may determine specification, defined as in Hubbell (2001), and furthermore disrupt the genetic diversification triggers. Moreover, assessing the risk for chemicals from the environment remains a debatable target of modern approaches to risk assessment. In our poster we introduce the Daphnopolator, a model that allows transiency of temporal and spatial boundaries to assess risks on selected scales by the user from genes to ecosystems. We demonstrate the feasibility of our approach for an emerging contaminant, next generation sequencing (NGS) and transcriptomics (RNA-Seq), especially in complex ecosystems. The predictive toolbox of the model is based on closed-form solutions of the elementary stochastic differential equations (ESDE), as well simulation techniques including Levy jump process, variance reduction techniques, and pseudo-regression approaches with Martingale stochastic processes (with non-normalized semi-Gaussian regressors). Calibration data originates from population genetic studies in the wild with collembolans under exposure from their offspring. In fact, there are already standardized test procedures aimed to one generation, thus disregarding those potential detrimental effects that may occur across generations. These changes might result in altered responses to environmental challenges, such as nanoparticles, and might be manifested as phenotypic effects in the offspring. Here we described the activities that are currently being conducted on different problems, scales, compounds and calibration data.

WE190 Development of an OECD two-generations reproduction test in Daphnia magna.

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Most eco-toxicological test guidelines only evaluate the effects observed within one generation, thus disregarding those potential detrimental effects that may appear across generations. The toxicological potential of contaminants may either decrease, increase, or remain unchanged. Reproductive toxicity in mammals monitors the effects on the parental generation as well as on their offspring. In fact, there are already standardized test procedures aimed to assess multigenerational effects for mammals and fish, but there are no equivalent tests for invertebrates, despite that their short life-cycles offer to cost-effectively monitor multigenerational effects of toxic substances in individuals and populations. Here we described the activities that are currently being conducted on behalf of the SETAC EVOCENERATE Working Group to set up protocol for a two generation Daphnia magna reproduction test and to test the performance of that protocol across up to 20 different labs from Europe, America and Asia. Indeed this is one of the largest conducted intercalibration tests ever conducted using the OECD 2011 guideline. We aimed to develop a two generation test with only few amendments of the already existing guidelines. The few modifications included to start the second generation with the brood females collected from reproducing females from the first generation. We selected pyrethron butoxide as a test chemical based on previous studies that showed that this compound was the best candidate among other two compounds. To minimize interlab variability all laboratories used the same stock solutions, which were prepared by one of the partners and send it the other ones. The results obtained in this intercalibration test will be discussed in this poster.

WE191 Maternal nanoparticle treatment induces multi-generational effects in the nematode Caenorhabditis elegans

S. Kim, Konkuk University; J. Moon, Konkuk University; Y. Ahn, Konkuk University / Department of Environmental Health Science

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MetaOMiCs in ecotoxicology: evaluation of alterations in the structure and functions of ecosystems (P)

C. Fajardo; G. Costa, Universidad Complutense de Madrid; S. Sanchez-Fortuz, M. Nande, UCM; M. Martín, Universidad Complutense Madrid

Advances in environmental biotechnology have allowed the characterisation of environmental risk. The forecasted growth in the manufacture and use of these nanomaterials makes it likely that increases in environmental exposure to ENMs will occur, whereas the toxicological effects of nanomaterials on environmental biota, soil health and functionality remains mostly unknown. A microbial ecotoxicological approach based on the response of soil microbial communities offers the opportunity to evaluate the impact of nanomaterials on natural populations, and it leads consequently to a better environmental risk assessment. Recent innovative breakthroughs in genotypic profiling, ultrafast genome pyrosequencing, metagenomics, metatranscriptomics, metaproteomics and metabolomics along with bioinformatics tools have provided crucial insights in studies of microbial communities and their mechanisms to cope with environmental pollutants. Likewise, molecular microbial biomarkers or bioreporters have been developed for in situ detection/ monitoring of environmental pollution. Structural Biomarkers: Multiple group-specific rRNA probes targeting prokaryotic and eukaryotic microbial taxa can be used in a FISH experiment for simultaneous phylogenetic classification of physiologically active microbial populations in an environmental sample. Metagenomics based methods have been useful to determine novel gene families and or microbes involved in biomediation of xenobiotics. Availability of whole genome sequences from several environmental microorganisms has been useful to determine the gene pool of enzymes involved in the microbial response to anthropogenic pollutants. Functional Biomarkers: Transcriptomic or metatranscriptomic tools are used to gain functional in-sights into the activities of environmental microbial communities by studying their mRNA transcriptional profiles. Proteomics based investigations have been useful in the identification of key proteins involved in the physiological response of microorganisms when exposed to anthropogenic pollutants (e.g. ENMs). Here we show results of the application of some of these molecular techniques in linking the structure and function relationships of microbial communities and for biological sensing of pollution at ENMs contaminated sites.
Reprotoxic effects of microcystin-LR and microcystin-producing Microcystis extract on adult medaka fish
Q. QIAO, UMR; B. MARIE, CNRS / MNHN MCAM, Muséum National d'Histoire Naturelle, Paris / UMR
The massive cyanobacterial blooms threaten human health as well as any living organisms present in waters. The especially due to the production of natural toxic components, so-called cyanotoxins. One of the most characterized cyanotoxins are the microcystins (MCs) that can cause a severe hepatotoxicity. In recent years, the potential reproductive toxicity of MCs has been also explored by several laboratories. The chronic exposure to MCs may threaten human reproductive health via transmission of MCs-containing water to fish. In the present work, we investigated the reproductive effects of MCs and MC-producing Microcystis extract on adult medaka. Medaka were exposed to pure MC-LR 1.5 μg L⁻¹ and MC-producing Microcystis extract with 5 μg L⁻¹ of equivalent MC-LR for 28 days. Various reproductive parameters including the fecundity, the fertilization success and the egg hatchability were examined during the exposure, revealing negative effects induced by MC-LR. In addition, sex hormones and vitellogenin levels in plasma were also affected by the exposure. Histological observations indicate that hepatocyes present glycogen storage loss together with cellular damages. Transcriptome profiling of liver was performed using Illumina RNA-seq and around 5M unique reads per library were mapped to the reference genome of medaka. Differential expression analysis identified over 100 up-regulated genes from 5 μg L⁻¹ MC-LR and MC-producing Microcystis extract treatments and gene ontology enrichment analysis reveals that response to stimulus, metabolic process and transcription regulator are highly affected in both females and males. RT-qPCR has been used to investigate the transcriptional level of selected genes in the liver. Furthermore, proteomics profiling of liver by using iTRAQ indicates that a set of proteins involved with lipid metabolism, amino acid metabolism, generation of energy and choriogenin/vitellogenin syntheses were statistically significantly different from those of controls. A sex-specific response was implied from both transcriptomics and proteomics profiling. The outcomes obtained through the joint analysis will advance our understanding of the reproductive toxicity of microcystins and its manifestations. Indeed this is one of the largest conducted intercalibration tests ever conducted with or without pure culture inoculants. Growth of co-cultured species members is followed by microscopy and enables a global overview of potentially positive effects of the inoculant on growth of the members in the soil on specific carbon substrates. In addition, the “interactome” study allows detecting possible species pairs, negative or positive, that can be recovered and identified in detail. The resulting knowledge not only provides ample data in designing functional synthetic communities but also construct new avenues for “synthetic ecology.”

WE194 Microbial community molecular analysis and sequencing linked to mixtures of chemical compounds
In Europe, the good quality of surface waters is established under the Water Framework Directive (WFD) based either on the chemical monitoring or on the ecological status. There is a growing concern about the combined action of pollution and mixtures of related genes. Furthermore, pollutants affecting the composition of aquatic microbial communities might be related to the presence of new xenobiotics and emerging pollutants. Emerging contaminants (e.g. PFOS; PFOA) are already known to affect the composition of aquatic microbial communities. Here we investigate the long-term effect of the bactericide triclosan (TCS) on the 16S and 18S gene richness, diversity and composition of marine periphyton communities. A long-term (17 days) flow-through microcosm experiment with constant TCS exposures of 0 – 316 nM was performed. DNA was extracted using the FastDNA spin kit (initially developed for for soil communities) and we performed multiplexed amplicon sequencing using the Illumina platform. In total 313,855 16S and 176,566 18S sequences were retrieved and the sequences were clustered into 1789 and 509 Operational Taxonomic Units (OTUs), respectively. The prokaryote part of the communities was dominated by the phyla Proteobacteria, Bacteroidetes and Planctomycetes, whereas the eukaryote part of the communities was dominated by the green algae Chroococcales and Bacillariophyta. TCS exposure resulted in a decrease of 16S richness and diversity, and a changed 16S composition, at an exposure of 31.6 nM and above. In the concentration interval of 1 – 10 nM more subtle changes in the overall 16S composition were detected. For Proteobacteria and Bacteroidetes, the richness and diversity followed a bi-phasic concentration response curve with a clear decrease between 3.16 nM and 31.6 nM, but with a much smaller decrease at 316 nM. This might be an indirect effect from a changed 18S composition at 316 nM. Unfortunately, we do not have 18S data for the exposure interval 10 – 100 nM and hence cannot compare the sensitivity of pro- and eu-karyotes. In general, the 18S composition was more variable than the 16S composition. Still, the TCS exposure resulted in a decrease of the 18S richness and diversity, and a changed 18S composition, at an exposure of 316 nM. Based on richness and diversity, the eukaryote taxa Virdiplantae was sensitive whereas Stramenopiles was insensitive to TCS. In this study we demonstrate that triclosan has adverse effects on microbial community composition and that amplicon sequencing is a suitable method to describe taxion-changed changes in community structure to a reasonable cost.

WE197 Importance of Preserving Environmental Risk Assessment (ERA) of Personal Care Products
In Europe, the good quality of surface waters is established under the Water Framework Directive (WFD) based either on the chemical monitoring or on the ecological status. There is a growing concern about the combined action of pollution and mixtures of related genes. Furthermore, pollutants affecting the composition of aquatic microbial communities might be related to the presence of new xenobiotics and emerging pollutants. Emerging contaminants (e.g. PFOS; PFOA) are already known to affect the composition of aquatic microbial communities. Here we investigate the long-term effect of the bactericide triclosan (TCS) on the 16S and 18S gene richness, diversity and composition of marine periphyton communities. A long-term (17 days) flow-through microcosm experiment with constant TCS exposures of 0 – 316 nM was performed. DNA was extracted using the FastDNA spin kit (initially developed for for soil communities) and we performed multiplexed amplicon sequencing using the Illumina platform. In total 313,855 16S and 176,566 18S sequences were retrieved and the sequences were clustered into 1789 and 509 Operational Taxonomic Units (OTUS), respectively. The prokaryote part of the communities was dominated by the phyla Proteobacteria, Bacteroidetes and Planctomycetes, whereas the eukaryote part of the communities was dominated by the green algae Chroococcales and Bacillariophyta. TCS exposure resulted in a decrease of 16S richness and diversity, and a changed 16S composition, at an exposure of 31.6 nM and above. In the concentration interval of 1 – 10 nM more subtle changes in the overall 16S composition were detected. For Proteobacteria and Bacteroidetes, the richness and diversity followed a bi-phasic concentration response curve with a clear decrease between 3.16 nM and 31.6 nM, but with a much smaller decrease at 316 nM. This might be an indirect effect from a changed 18S composition at 316 nM. Unfortunately, we do not have 18S data for the exposure interval 10 – 100 nM and hence cannot compare the sensitivity of pro- and eu-karyotes. In general, the 18S composition was more variable than the 16S composition. Still, the TCS exposure resulted in a decrease of the 18S richness and diversity, and a changed 18S composition, at an exposure of 316 nM. Based on richness and diversity, the eukaryote taxa Virdiplantae was sensitive whereas Stramenopiles was insensitive to TCS. In this study we demonstrate that triclosan has adverse effects on microbial community composition and that amplicon sequencing is a suitable method to describe taxion-changed changes in community structure to a reasonable cost.

Challenges in Environmental Assessment of Cosmetics and Personal Care Products (P)
S. Kim, Konkuk University; J. Moon, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science
Modern DNA sequencing platforms have an enormous throughput at a lower cost than ever before. The possibility to multiplex and barcode PCR amplified gene fragments, and sequence them in the same run can further lower the cost per sequencing depth. This approach, amplicon sequencing, has great potential to detect adverse changes in the structure of toxicant-exposed microbial communities. Here we investigate the long-term effect of the bactericide triclosan (TCS) on the 16S and 18S gene richness, diversity and composition of marine periphyton communities. A long-term (17 days) flow-through microcosm experiment with constant TCS exposures of 0 – 316 nM was performed. DNA was extracted using the FastDNA spin kit (initially developed for for soil communities) and we performed multiplexed amplicon sequencing using the Illumina platform. In total 313,855 16S and 176,566 18S sequences were retrieved and the sequences were clustered into 1789 and 509 Operational Taxonomic Units (OTUS), respectively. The prokaryote part of the communities was dominated by the phyla Proteobacteria, Bacteroidetes and Planctomycetes, whereas the eukaryote part of the communities was dominated by the green algae Chroococcales and Bacillariophyta. TCS exposure resulted in a decrease of 16S richness and diversity, and a changed 16S composition, at an exposure of 31.6 nM and above. In the concentration interval of 1 – 10 nM more subtle changes in the overall 16S composition were detected. For Proteobacteria and Bacteroidetes, the richness and diversity followed a bi-phasic concentration response curve with a clear decrease between 3.16 nM and 31.6 nM, but with a much smaller decrease at 316 nM. This might be an indirect effect from a changed 18S composition at 316 nM. Unfortunately, we do not have 18S data for the exposure interval 10 – 100 nM and hence cannot compare the sensitivity of pro- and eu-karyotes. In general, the 18S composition was more variable than the 16S composition. Still, the TCS exposure resulted in a decrease of the 18S richness and diversity, and a changed 18S composition, at an exposure of 316 nM. Based on richness and diversity, the eukaryote taxa Virdiplantae was sensitive whereas Stramenopiles was insensitive to TCS. In this study we demonstrate that triclosan has adverse effects on microbial community composition and that amplicon sequencing is a suitable method to describe taxion-changed changes in community structure to a reasonable cost.

SETAC Europe 26th Annual Meeting Abstract Book
Within such an assessment, environmental occurrence, fate and ecological effects are assessed via standard test guidelines, such as those prescribed by the OECD (Organization for Economic Cooperation and Development), or by high quality non-standard experiments. Testing strategies are often tiered, meaning that a number of screening level or modeling methods (such as QSARs, or quantitative structure-activity relationships) can be used to prioritize materials for further, more in depth testing. This approach is cost effective and reduces unnecessary animal testing. The result is the generation of a PEC (predicted environmental concentration) and a PNEC (predicted no effects concentration). \( \text{'n} \text{Next, a risk characterization ratio (RCR) is derived by dividing the PEC by the PNEC value.} \]

The result can be reported in a tiered format: if the ratio is calculated then a material is likely to pose an acceptable environmental risk. Conversely, if an RCR \( > 1 \), then an unacceptable environmental risk will be posed by the material. \( \text{'n} \text{ERA is widely prescribed by a number of major local regulatory bodies and frameworks. These include the USEPA, USFDA, ECHA and VICH. A large number of regulatory and functionally diverse materials have therefore been assessed by risk-based methods, or are currently being assessed in this manner. As such, ERA represents a widely used, scientifically relevant and reliable means of determining materials which may be of environmental concern.} \]

\( \text{'n} \text{Despite the availability and proven utility of ERA methods in identifying materials of potential concern, a number of regulatory and legislative schemes in the US and Europe have abandoned an ERA-based approach when assessing environmental impacts of consumer chemicals. In particular, hazard and exposure data are often considered in isolation. This therefore results in regulatory and legislative decisions being taken without a full comprehension of how a material may impact a specific environment. Moreover, alternative compounds, which are then applied instead of materials that can sometimes pose more of an environmental risk. These are called regrettable substitutions. \( \text{'n} \text{This poster will discuss the benefits of ERA compared with only considering hazard or exposure. We will also use examples of regrettable substitutions to highlight instances for which comparative ERAs may have avoided these from occurring.} \]

WE198 PBT Behavior and Aquatic Ecotoxicity of Personal Care Products: QSAR modeling and prioritization

P. Gramaté, S. Cassani, A. Sangiovanni, University of Insouvia / DsTA

Several ingredients in Personal Care Products (PCPs) are now of recognized environmental concern for their increasing presence in aquatic compartments. These ingredients have heterogeneous chemical structures and very different properties, but for the majority of them persistence and toxicity data are lacking. Moreover, the determination of all the dangerous properties, required by REACH and Cosmetics Directive, is a long and difficult task. Due to the high variety of these chemicals and the big number of endpoint points that should be studied, it is important to have tools to highlight the most harmful compounds, focusing the experiments only on these. In this study we propose tools for prioritizing the most dangerous ingredients in PCPs, thus reducing the costs and the animal tests as required by the new Regulations. More than 500 PCPs (fragrances, parabens, most dangerous ingredients in PCPs, thus reducing the costs and the animal tests important to have tools useful to highlight the most harmful compounds, focusing

WE199 Legal gaps in the European chemical legislation impede environmental risk assessment and risk management of personal care products

U. Klaschka, University of Applied Sciences

Some features in the REACH- and the CLP-Regulation seem to be designed especially for products like personal care products with the intention to improve protection of the environment and humans health. However, a closer look reveals several exception provisions with the effect that these rules need not be applied to cosmetics. Cosmetics are special. But are the exceptions in the European chemical legislation justifiable? A comparison of the REACH- and the CLP-Regulation with the Cosmetics Regulation reveals that the Cosmetics Regulation cannot compensate for these gaps. Several studies show that this situation impedes a decent hazard and risk assessment of personal care products and ingredients.

WE200 Natural substances in personal care products

U. Klaschka, University of Applied Sciences

Natural personal care products enjoy great public acceptance today. Is this bonus justified? Natural substances in personal care products are used in increasing amounts. Most of them are complex mixtures of natural compounds belonging to various chemical substance classes. A large number is classified as dangerous for human health and the environment and many constituents are known for their potent physiological effects. A market study revealed the big variety of natural substances used in personal care products. It also showed that many dangerous natural substances are indeed used today. However, the most frequently employed natural substances are not classified as dangerous, such as Xanthan gum, Simmondsia chinensis oil, Helianthus annus seed oil or Butyrospermum parkii butter. (Etho)Toxicity of multi-constituent substances like natural substances is caused by the constituents. Some constituents of the natural substances found are chemically identical to industrially synthesized chemicals. In cases where the constituents are known, the classification as dangerous for the aquatic environment of the natural substance can be calculated following the procedure of the CLP regulation for mixtures. There is no scientific reason why natural ingredients in personal care products should in general be regarded as less hazardous compared to synthetic ingredients.
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.

WE203 Comprehensive review of several surfactants in the marine environment: fate and toxicity
M. Jackson, Shell Health / Shell Health Risk Science Team; C.V. Eadshorth, Shell International / Shell Health; D. Schowanek, Procter & Gamble / Brussels Innovation Center; T. Delfosse, The Procter & Gamble Company / Product Safety Regulatory Affairs; A. Riddle, Ecospain; N. Budgen, AstraZeneca
Numerous environmental risk assessments of surfactants in the freshwater compartment have been developed (HPV, i.e. HERA, OECD REACH, etc), which can serve as a basis for the marine compartment through extrapolation of freshwater data to marine data. However, risk assessment of surfactants in the marine environment has not been systematically investigated. To investigate the extent and quality of available data from marine studies (water and sediment), ERA3M (Environmental Risk AAssessment and Management) commissioned a compilation of marine exposure and effects data for a number of current and historical surfactants (alcohol ethoxylates (AE), alkyl sulphates (AS), alcohol ethoxy sulphates (AES), linear alkylbenzene sulphonates (LAS), and ditallow dimethyl ammonium chloride (DTDMAC)). Industry sources and the literature identified, evaluated and reported a variety of biodegradation and ecotoxicological studies. Marine biodegradation studies reported half lives in the range of 2-28 days (AE), 0.3-20 days (AS), 1-50 days (AES), and 0.3-45 days (LAS) (no biodegradation studies were found for DTDMAC). A wide variation in the number of published marine ecotoxicological studies was found for each of the surfactant classes. AS and LAS have been studied most, and AE, AES and DTDMAC the least. Marine invertebrates are the most studied class of organism in both acute and chronic toxicological assessments, whereas plants and fish are comparatively much less studied, particularly in terms of chronic assessments. Based on data ranges and mean toxicities from the studies reviewed, it was concluded that the toxicity of the five surfactants, from highest to lowest is: LAS > AS > AE > DTDMAC > AES. Acute toxicity (for algae, invertebrates and fish) was shown to be generally equivasive across the board. Where it was possible to compare marine and freshwater ecotoxicological values, marine data ranges generally fell within typical freshwater data ranges. Marine bioconcentration data are virtually non-existent, and were only available for LAS and AES. Significant gaps in data were found in the marine ecotoxicology assessments for all surfactant classes.

WE204 Fate and effect of sediment-associated triclosan in a freshwater microcosm setup: the relative importance of bioaccumulation and biotransformation
R. Windfeld, Roskilde University / ENSPAC; I. Roessink, Alterra / ERA team; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Dept of Environmental Science; H. Selck, Roskilde University / Dept Environmental Social and Spatial Change
Aquatic environments are increasingly exposed to household and personal care products (HPCPs) from their extensive use in urbanized areas. The environmental fate of these chemicals and their impact on benthic communities in receiving waters is not completely understood. This study aims to examine the fate of sediment-associated triclosan (TCS) as well as its toxic impact on the benthic community in a fresh water microcosm system. To investigate toxicity of TCS, a community of benthic invertebrates was exposed for 60 days to a high and a low concentration of TCS. The community consisted of a range of organisms, (Gammarnus pules, Tubifex tubifex, Lumbriculus variegatus, Asellus aquaticus and Caenis horaria). The impact on the community was recorded as decreasing organism abundance. Benthic systems containing T. tubifex and G. pules were employed to examine the fate of TCS during 30 and 60 days, respectively. The biotransformation capacity of these organisms was assessed by measuring the concentration of TCS and selected metabolites in overlying water, sediment and organism tissue after the two exposure intervals, respectively. The results will be discussed in the context of how bioaccumulation and biotransformation of the products will affect the environmental fate of these compounds

WE205 LogP: a classic endpoint for ERA, but what happens when the main part of the test substance is a resin-like insoluble, volatile, mostly solid, complex mixture and constituents are unknown?
B. CASENAVE, CEHTRA SAS; S. LEGAY, FCBA; R. SAMERSA, CEHTRA SAS; P. Thomas, CEHTRA SAS / Ecotoxicology and Risk Assessment Unit, the REACH and the CLP under several substances are considered: monococonstituents, monococonstituents & UVCBs. Across these substance types several families always present a challenge to test especially in physico-chemistry, ecotoxicology and environment. One of these challenging groups is fragrances. In Fragrance chemicals fall under multiple categories, they can be natural, synthetic, monococonstituent, monococonstituent or considered as UVCBs. One group of fragrances that falls under the title of monococonstituent/UVCB are known to be most difficult to assess: Essential oils. Essential oils (EO) are complex mixtures, with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. Some of these substances are even more complex: gums, resinsoids and concretes, extract from natural raw material like essential oils. They are as complex as EO but their composition is mostly unknown and their physical state leads to further difficulties for physico-chemical and ecotoxicity testing: they are (mostly) solid(ish), extremely viscous resins, with a frozen honey-like texture. However, some of these substances still require environmental risk assessment under the registration process. Therefore some key PhysicoChemical endpoints are needed, such as water solubility and/or LogP value. It is difficult to determine the LogP value of a substance which is solid(ish) with a composition which is basically unknown except for the fact that the number of constituents amounts to potentially well over a hundred. We discuss the protocol we designed to meet the requirements of the log Kow endpoint following the OECD 123 (Slow Str & HPLC) guideline for one of these substances and how we expect to use this result in the REACH Chemical Safety Report. Keywords: risk, assessment, logp, fragrance

WE206 Ecotoxicity of natural complex mixtures: raw material of plant extracts which are resin-like solids
R. SAMERSA, CEHTRA SAS; N. DELPIT, Laboratoires des Pyrénées et des Landes; P. Thomas, CEHTRA SAS
Under REACH review program several kinds of substances are considered: monococonstituents, monococonstituents, & UVCBs. Across these substance types several families always present a challenge to test especially in ecotoxicology and environment. One of these challenging groups is fragrances. Fragrances chemicals fall under multiple categories, they can be natural, synthetic, monococonstituent, monococonstituent or considered as UVCBs. One group of fragrances that falls under the title of monococonstituent/UVCB are known to be most difficult to assess: Essential oils. Essential oils (EO) are complex mixtures, with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. Some of these substances are even more complex: gums, resinsoids and concretes, sub-categories of essential oils. They are as complex as EO but their composition is mostly unknown and their physical state leads to further difficulties for ecotoxicity testing: they are (mostly) solid(ish), extremely viscous resins, with a frozen honey-like texture. In order to acquire the data required for registration of these substances, we performed a series of OECD 202 (daphniphyte acute studies) using WAF methodology, with a protocol adapted to fit the substances in question, taking into account the particular properties of these substances in an attempt to keep the results robust from a regulatory point of view. We present and discuss these results on their ecotoxicity properties and the conclusion that we can draw from such results. Keywords: fragrance, raw, material, ecotoxicity

WE207 A thermodynamically-based step by step calculation method to assess aquatic toxicity of mixtures
P. Bichere, P. Thomas, KREATIS
From an environmental perspective chemical substances do not act in the same way as pure substances tested in the lab. Many substances, classified as such under REACH are actually mixtures (i.e. Natural Complex Substances, such as essential oils). Therefore LogP value of such a mixture cannot be directly applied to obtain the water is the universal solvent in the environment, aquatic toxicity of mixtures has been of high concern for many years. One recognised way to obtain toxicity data on mixtures was to follow OECD (2000) Guidance No. 23 on difficult substances and mixtures which advocates use of the water-accommodated fraction (WAF) method (i.e. testing the constituents together at specific loading concentrations). Implementation of the WAF method costs time and money. More recently, research has been conducted in order to determine if an additivity approach can be used to predict aquatic toxicity of mixtures of chemicals or whether independent action predominates. Nowadays the favoured hypothesis is the Concentration addition (CA) where toxic units of mixture constituents with same toxic mode of action can be summed (Loewe and Muischeck, 1926; Backhaus et al., 2003). In this work, the chemical activity concept has been used to normalise the relative part of toxicity of each constituent (Mackay et al., 2009; Thomas et al., 2015). Schmidt et al. (2013) and Smith et al. (2013) have both shown that aquatic and terrestrial toxicity can be predicted by using summation constitutive activities of sub-lethal levels of constituents in a mixture comprised of solid P AHs. However unlike solids compounds (Banerjee et al., 1984), liquids partition into each other thus reducing the overall toxicity of the mixture. Therefore the initial rule of CA modified according to the chemical activity has also to be completed by an additional rule which accounts for the availability of activity is overcome from the calculation. Based on thermodynamic liquid-liquid equilibrium, aquatic toxicity can finally be accurately calculated for mixtures of liquid hydrophobic compounds, like essential oils. This method can provide results within a factor of 2 of experimental results while the CLP additivity method results are at least a factor of 10 lower.
Predictive laboratory methodology to assess coral bleaching: application to UV filters
J. Fel, LOréal Research & Innovation, Aulnay-sous-Bois, France / Environmental Research; M. Leonard, IOREAL SA

Increasing ocean temperature and acidification, overfishing, coastal development and pollution are well known stressors on coral reefs. They may induce coral bleaching, a process by which corals lose their symbiotic microalgae (zooxanthellae). Ultimately, corals may die when these stressful environmental conditions last too long. Weakened corals, more susceptible to infectious diseases, show poor resilience from episodic bleaching events. Some studies have reported that certain UV filters (mostly 4-methylbenzylidenecamphor, benzophenones and octylmethoxycinnamate) contained in sunscreens lotions and washed off by swimmers, could contribute to coral bleaching. Media took it for granted and suspicion has been extended to all organic UV filters present in sunscreens products. The present study was aimed at clarifying the potential effect that organic UV filters (e.g. Avobenzone, Octinoxate, Terephthylidene-dicamphor sulfonic acid, Silitrizole, etc…) may have on different coral species. Two herbicides (Monuron and Diuron) were used as positive references. First a preliminary laboratory screening test was developed to assess potential adverse effect of short exposure (4h) to elevated concentrations (from 1 to 100 nM) of the compounds. As a subethal endpoint predictive of coral bleaching, chlorophyll photosynthetic efficiency of the symbiotic micro-algae (zooxanthellae) was monitored with PAM (Pulse Amplitude Modulated) fluorimetry on nubbins of hard coral species Seriatopora calicium and Stylophora pistillata. In a second step, coral nubbins of Stylophora pistillata (hard coral) and Turbinaria reniformis (soft coral) were exposed for 5 weeks at low nitrate concentrations in 15 L aquariums, under semi static conditions with weekly solution renewal. A specific analytical methodology was developed, combining automated solid phase extraction with UPLC-UV detection, to monitor the compounds concentrations in sea water and analyze large number of samples. 5 weeks of chronic exposure to these UV filters at concentrations above those reported in natural sea waters, did not induce coral bleaching nor reduce the photosynthetic efficiency of the symbiotic micro-algae.

WE209
ISTREEM 2.0: new enhancements for down-the-drain modeling to support environmental aquatic exposure assessments for cosmetics and personal care products
R. Vamshi, K.E. Kapo, M.L. Sebasky, C.M. Holmes, Waterborne Environmental, Inc.; P. DeLeo, D. Ferrer, American Cleaning Institute

The iSTREEM® model (“in-stream exposure model”), a free and publicly-available web-based model supported by the American Cleaning Institute (istream.org), provides a means to estimate chemical concentrations in effluent, receiving waters, and drinking water intakes (DWI) across the conterminous U.S. as well as a number of watersheds in Canada under mean annual and low-flow (7Q10) conditions. This presentation will discuss recent upgrades made to enhance the model, underlying data, algorithms and presentation of results in the new version. iSTREEM® 2.0 incorporates geographic locations of over 12,000 wastewater treatment plant (WWTP) facilities along more than 300,000 segments of effluent-impacted river reaches, providing a framework to integrate geographic data to assess environmental risk for multiple scenarios of interest. WWTP associated facility level information were derived from the latest available USEPA Clean Watershed Needs Surveys database. A water quality network used by iSTREEM® 2.0 was upgraded to a higher-resolution hydrologic dataset based on the USGS/USEPA NHDP Plus version 2, which includes estimated mean annual and low flow (7Q10) data based on USGS stream gage measurements. Model results are presented in a standardized manner for consistent results communication across all users and are provided in a readily usable format (MS Excel) for easy interpretation and further customization of result presentation. Major assumptions used in constructing the model will be discussed. Recent developments are geared to expand adoption of the model by a wide variety of users as an environmental risk assessment tool across multiple cosmeticity groups (cosmetics, personal care products, pharmaceuticals, food additives, pesticides, etc.) that require internal or regulatory environmental assessments. The discussion will also include a comparison of model results between the prior version of iSTREEM® and latest iSTREEM® 2.0 to examine the impact of recent updates on the national distribution of predicted environmental concentrations.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (P)

WE210
Is nitrate an endocrine active compound in fish?
M.P. Mose, University of Southern Denmark / Department of Biology; K. Kinberg, P. Bjerregaard, H. Holbech, University of Southern Denmark / Department of Biology

Nitrate and nitrite taken up into fish may be reduced to NO which is known to be a signalling compound in the organism contributing to the regulation of i.e. steroid synthesis. Exposure of male rats to nitrate and nitrite results in reduced plasma concentrations of testosterone (also nitrate concentrations around or below the limits for drinking water). Nitrate concentrations in streams may be elevated due to releases from agricultural practices. The effects of nitrate and nitrite on endocrine relevant endpoints were investigated in zebrafish (Danio rerio) and brown trout (Salmo trutta). Zebrafish were exposed to nitrate and nitrite from hatch to sexual maturation (60 d) and sex ratio and vitellogenin concentrations were determined. Juvenile brown trout were exposed in a short-term experiment and the concentrations of vitellogenin were determined. The sex ratio in zebrafish was not affected by exposure to environmentally realistic concentrations of nitrate and nitrite but vitellogenin concentrations were significantly higher in the exposed groups compared to the control; no obvious concentration-response relationship was observed. The outcome of the brown trout experiment is awaiting the final analyses.

WE211
Neuro-endocrine disruption in mollusces - Effects of pharmaceuticals on a serotonin- and dopamine dependent system
H. Holbech, L.B. Sanderhoff, S.P. Waller, K. Pedersen, University of Southern Denmark / Department of Biology

The Mollusca phylum is the second largest animal phylum with around 85,000 registered mollusce species and increasing attention to effects of chemicals on the molluscan endocrine system have been given during the last years. This includes initiation of the development of OECD test guidelines (TG) to assess the effect of chemicals in mollusces. To date no endocrine specific mollusce biomarkers have though been validated and included in draft test guidelines due to lack of knowledge of the endocrine system. Here we investigated the effects of pharmaceuticals targeting serotonin and dopamine in a cost efficient and fast in vivo system using embryos of the freshwater pulmonate gastropod Lymnaea stagnalis (the great pond snail). It is known that serotonin and dopamine are involved in many reproductive processes in mollusces Incl. egg maturation and spawning and that catechol ciliary activity, causing L. stagnalis embryos to rotate in the eggs is serotonin/dopamine regulated. We have developed a system to quantify embryo rotation and present results of exposure to serotonin, dopamine and different anti-depressive pharmaceuticals (e.g. selective serotonin reuptake inhibitors, SSRI) s using the L. stagnalis embryo rotation test system.

WE212
In silico Screening for Potential Endocrine Disruptors
R. Kühne, N. Ost, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Chemistry; L.A. Baumann, University of Bern / Centre for Fish an Wildlife Health; H. Segner, University of Bern / Centre for Fish and Wildlife Health; J. Arning, German Federal Environment Agency UBA / Section IV Chemicals; G. Schüttmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry

Endocrine disrupting activities of chemicals trigger adverse effects on organisms. Endocrine disrupters are suspected to be responsible for a number of diseases as testis and breast cancer, oligosperma of young men, or adipsiosis. Compounds with endocrine activity are labelled as substances of very high concern (SVHC) and are subject of regulations as e.g. under REACH. However, there are so far no internationally harmonised criteria to identify endocrine active chemicals. Furthermore, the endocrine system is rather diverse. Despite of several already developed OECD tests to reveal respective activities, it is still not clear whether they comprise all (eco)toxicologically relevant pathways. Even more, these tests are rather expensive with respect to costs and duration. Consequently, the number of existing data is limited. In silico tools are promising alternatives to avoid testing or at least to allow for the prioritisation of tests by screening larger compound lists. The German Federal Environment Agency holds a prototype of a structural alert model and corresponding software tool for this task. Based on it, the current study aims to significantly extend this model and to make it available as an automated tool in the software system ChemProp (UFZ Department of Ecological Chemistry 2015. ChemProp 6.3. http://www.ufz.de/index.php?en=6738). The envisaged model will provide a decision tree to identify potential endocrine disrupters and their respective modes of action. The paper gives an overview of the approach and reports results for several modes of action with respect to thyroid hormones. Structural rules to identify potential agonists and antagonists for effects on the ligand binding domain (LBD) as well as putative antagonists against the activation function 2 (AF-2) have been established. Furthermore, the opportunity to derive specific rules for enzyme/protein interaction, thyroid receptor interaction, aryl-hydrocarbon-receptor interaction, and other modes of action based on existing data and mechanistic knowledge has been explored. Acknowledgment: This study is financially supported by the German Federal Environmental Agency, FKZ 3714 63 412 0.

WE213
Evaluation of anthropogenic micropolllutants in drinking water: the Tox-Box approach
S. Chudoba, German Environment Agency / Toxicology of Drinking Water and Swimming Pool Water; M. Kramer, Rheinenergie; W. Brack, Helmholtz Centre
for Environmental Research UFZ; Effect/Directed Analysis; E. Rabbow, German Aerospace Centre; M. Knaur, Hydrotechnik GmbH; H. Glatte, Institute for Nutrition; C. Baumstark-Kahn, German Aerospace Center; E. Küster, Helmholtz Centre for Environmental Research, Dept.Bioanalytical Ecotoxicology / Bioanalytical Ecotoxicology; T. Grummt, Federal Environment Agency / Toxicology of Drugs and Swimming Pools; D. Bünning, University of Heidelberg / Centre for Organismal Studies; P. Waldmann, Incosbóté; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis

Anthropogenic micropollutants in drinking water have been a concern for several years. Due to analytical improvements more substances are found in drinking water and their presence has to be evaluated to provide the public with safe drinking water. So far only lengthy test protocols mostly in animals are used. The aim of the joint project Tox-Box is therefore the establishment of a test battery that allows for the evaluation of a substances in terms of possible genotoxicity, neurotoxicity and endocrine disruptive effects using in vitro approaches whenever applicable. Based on the theoretical concept of the Health Related Indicator Value (HRIV), published by the German Federal Environment Agency (Umweltbundesamt, UBA) in 2003 a practical approach was designed. For each toxicological endpoint several tests were chosen, which provide for a fast and reliable risk assessment, once a chemical is found. The test battery for genotoxicity will encompass the Umu test, in addition to Ames test and micronucleus test. The assessment of neurotoxicity will consist of testing for necrosis, apoptosis and oxidative stress by comparing neural cells with liver cells as well as assays specific for neurons like neurite outgrowth and MAP kinases. Moreover, the activity of acetyl choline esterase and the development of lateral limbs in zebra fish (Danio rerio) will be tested. A third set of tests deals with endocrine effects. These are assessed by applying hormone specific reporter gene assays in combination with the H295R assay. When one of these tests is positive, a reproduction assay in Potamogeton crispus potted plants will be performed for further clarification. During the project some 60 chemicals were tested by the different project partners. The test scheme which was developed suggests that it will meet the demands for fast and reliable assessment. It will also be in line with the Tox21 program which aims for an increase in in vitro testing. From a regulatory perspective, genotoxicity is considered the most relevant endpoint. Since genotoxic effects currently result in the lowest cut-off value, no further testing is required, once a substance is considered genotoxic. When no genotoxic effects can be found the assays for neurotoxic and endocrine effects will be carried out. The guideline for testing will be published as a handbook, first in German. An English version is planned for the near future.

WE214 Investigation of (anti)estrogenicity of triclosan using in vitro and in vivo zebrafish bioassays

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Triclosan (TCS) is a wide spectrum antimicrobial agent used in many personal care products (PCPs) that has been detected in surface water and biota. TCS is suspected to be an endocrine disrupting chemical (EDC) and to interact with the estrogen and androgen signaling pathways. However, data available regarding its estrogenic or anti-estrogenic potency provide contradictory information with respect to the hormonality of its action. The few studies published so far on chronic reproductive effects of TCS on fish report ambiguous results, i.e. a male bias sex ratio in Japanese medaka but a vitellogenin induction in male mosquito fish. In this context, our study aimed to investigate whether TCS acts as an agonist or an antagonist of the estrogen receptor (ER) in human (in vitro) and in zebrafish (in vitro and in vivo). For this purpose, we assessed luciferase-dependent ER transactivation in vitro using a human breast cancer patient cell line expressing hER (MELN cells), and three zebrafish (zf) liver cell lines (ZELH) stably transfected with zER (i.e. ZELH-zERα, -zERαβ and -zERβ2). In addition, cypr9a1b gene induction, which is strictly ERα dependent, was measured using co-transfections of TCS had no effect on the E2-induced luciferase activity in either human or zebrafish cells, suggesting that TCS did not elicit any antagonist activity. In vivo, TCS alone did not induce GFP in transgenic zebrafish embryos (EASYZ assay). Our results showed that TCS alone from 3mM to 10µM was unable to induce luciferase activity whatever the in vitro models used. At higher concentrations, cytotoxic effects were observed. Co-exposure of cells with E2 0.1nM (ZELH-zERα, ZELH-zERαβ and MELN) or E2 5nM (ZELH-zERα) and the docking compound cypr9a1b-GFP in P zebrafish embryos showed the potential of a combination of different datatypes to distinguish among the MOAs: as well as assays specific for neurons like neurite outgrowth and MAP kinases. The test scheme which was developed suggests that it will meet the demands for fast and reliable assessment. It will also be in line with the Tox21 program which aims for an increase in in vitro testing. From a regulatory perspective, genotoxicity is considered the most relevant endpoint. Since genotoxic effects currently result in the lowest cut-off value, no further testing is required, once a substance is considered genotoxic. When no genotoxic effects can be found the assays for neurotoxic and endocrine effects will be carried out. The guideline for testing will be published as a handbook, first in German. An English version is planned for the near future.

WE215 Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with endocrine disruptive properties

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Pharmaceutical companies are obligated to perform an environmental risk assessment for each new drug that is launched on the market. The mandatory tests for potential endocrine disrupting (ED) compounds require a lot of test animals, which is not consistent with the 3Rs principle. Therefore, the aim of this study is to develop a zebrafish embryo test, which is not considered an animal test according to the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting modes of action (MOAs). The MOAs that will be studied are aromatase inhibition, estrogen receptor (ER) agonism, estrogen receptor (ER) antagonism, androgen receptor (AR) agonism and antagonism. As a first stage of test development, 1 pharmaceutical per MOA was investigated to map differences among the 5 compounds in zebrafish embryos at the morphological, physiological and molecular level. We are currently exposing transgenic zebrafish embryos of 2 lines to aid in discriminating among the MOAs: SRAG (fluorescent signal after glucocorticoid receptor activation) and 5xERE-GFP (reports estrogen receptor activation). An ontology study is ongoing to determine the gene transcription levels of the receptors, biomarkers and enzymes of the steroidogenesis pathway which will be compared to the gene transcription levels of embryos which were exposed to each of the 5 pharmaceuticals. As a model for ER agonism, 17ß-ethinylestradiol (EE2) was used. We have previously demonstrated that EE2 was used to investigate the ER antagonist. The aim of this study is to develop an in vitro assay to use in zebrafish embryos to detect estrogen receptor antagonism. The use of in vitro assays makes it possible to perform an early risk assessment, which is not consistent with the 3R principle. Therefore, the aim of this study is to develop an in vitro assay to use in zebrafish embryos to detect estrogen receptor antagonism. The use of in vitro assays makes it possible to perform an early risk assessment.

WE216 CYTOCHROME P450 AS POTENTIAL TARGET FOR ENDOCRINE DISRUPTION IN TILAPIAS CAGED IN FOUR RESERVOIRS OF IGUAÇU RIVER

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Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with endocrine disruptive properties

WE217 Butyltin levels and imposex occurrence in Hexaplex trunculus (Gastropoda, Muricidae) from the Northern Adriatic Sea (Italy) after the EU ban

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Imposex (the development of additional male sex organs in females Caenogastropods) is an endocrine disruption effect commonly used to indirectly assess the presence of tributyltin (TBT), a compound used worldwide in industrial and agricultural applications, mostly in antifouling paints, and now ubiquitous in the marine and transitional environments. Organotins were in the
European Union by the EC Regulation 782/2003 and TBT compounds are among the priority hazardous substances according to the European Water Framework Directive 2000/60/EC and its daughter Directive 2008/105/EC. This study aims to assess the butyltin (BT) content in Hexaplex trunculus from the Venice Lagoon and the Northern Adriatic Sea after the EU ban. In 2013-2015, gastropods were collected in four sampling sites in the Venice Lagoon and in the Adriatic Sea to assess their BT (TBT, DBT, MBT) contamination and imposex levels. The stations were chosen among those previously surveyed in the same area, for a temporal comparison. Very low BT concentrations (range: 16-29 ng cation/g d.w.) and imposex levels (range %I: 5-38%; VDSI < 3.3; RPSI < 0.10%) were found with a clear priority to prevent higher BT levels. Quite similar values were shown in lagoon and off-shore samples. TBT levels and VDSI values determined in this study are consistent with those reported in literature for the same species from other geographical areas, with VDSI < 3 at TBT concentrations of 15 ng/g d.w. However, contrary to previous surveys in the Venice Lagoon, imposex was found in male and female gastropods with BT body burden in females. This finding could be explained by the high sensitivity of the species that is known to show an initiation of imposex at concentrations lower than 1 ng Sn/g d.w. (2.44 ngTBT/g). Moreover, also the development of imposex exhibited a different course as compared to that observed before TBT ban, when BT concentrations and imposex levels were higher. Previous studies documented first the development of the incipient penis, followed by the development of vas deferens. In this study, the onset of imposex occurred first with the appearance of the vas deferens and then with the development of the incipient penis. In this regard, it cannot be excluded that other factors may alter the endocrine control of imposex development, such as exposure to heavy metals, changes in environmental conditions and the influence of other endocrine disrupting chemicals.

**WE218**

**An integrative study design for a zebrafish full life cycle toxicity test**

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Fish full life cycle (FLLC) toxicity tests are considered to provide definitive assessments for population relevant endpoints, including those mediated by endocrine mechanisms (i.e. OECD Conceptual Framework level 5). These tests have been conducted with various fish species possessing diverse life history traits, often as part of a global registration package for plant protection product active substances. A life cycle test protocol with limited validation (OECD TG 240) exists for the medaka (Oryzias latipes), but other species such as the zebrafish (Danio rerio) are more commonly used for toxicity testing and research. In recent years, several fish test guidelines have been released, which are targeted to screen for endocrine effects on specific stages of the fish life cycle and have been robustly validated using zebrafish. Herein we describe a study design for a zebrafish full life cycle toxicity test that integrates critical aspects from previously validated guidelines. The exposure starts with freshly fertilized eggs (F0-generation) from a non-exposed population. The effect of the test substance on the survival, growth and sexual development of the F0-generation are evaluated (consistent with OECD TGs 210 and 234). After the onset of consistent spawning behavior (approx. 70 days post hatching), reproduction is assessed as fecundity and fertility (consistent with OECD TG 229). Upon sacrifice, the phenotypic sex of adult F0 fish is confirmed and biomarkers such as vitellogenin and E2 or E1/DHE production are measured (consistent with OECD TG 229 and 234). Fertilization rates produced by the F0 test groups are used to start the F1-generation, which is exposed for about 34 days (until approximately 30 days post hatch), to evaluate effects on hatchability, development, survival and growth of the F1 early life stages (consistent with OECD TG 210). We present data demonstrating successful zebrafish growth, sexual development and reproductive performance and provide a statistical power analysis for apical population relevant endpoints.

**WE219**

**A multi-generation study on the effects of 17ß-dihydroequilin, E1 and E2 in the Potamopyrgus antipodarum (Oniscidea: Hygrotidae)** following a combined protocol based on the OECD 229 and OECD 234 Guidelines

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Two separate studies were conducted to understand the endocrine effects of 17ß-estradiol and Conjugated Estrogens (CE), specifically, estrone (E1) and dihydroequilin (DHE) on the freshwater snail, Potamopyrgus antipodarum (Oniscidea: Hygrotidae). In the multi-generations life-cycle test, medaka were exposed to nominal concentrations of 1.0, 7.5 and 75 ng a.i./L each of E1 and DHE, for total exposure of 2.0, 15 and 150 ng a.i./L in combination, a positive control of 100 ng a.i./L E2 and a negative control. Two additional dosing groups were exposed; one to 1.0 ng a.i./L E1 alone and the other to 1.0 ng a.i./L DHE alone. Following a pre-exposure period of 26 days to identify actively spawning fish, medaka males and 5 females were formed and exposed for 21 days under flow-through conditions to evaluate reproduction of the parenteral generation (F0) as per OECD 229, Fish Short Term Reproduction Assay (FSTRA). Following the FSTRA, an evaluation of sexual development in the F1 generation using eggs from the F0 generation carried through the 153 days of the study was conducted. Surrogate Sexual Development Test (FSDT). Following the 83 day exposure of the F1 generation, most of the treatment groups were terminated in keeping with the FSĐT. However, exposures in the 2.0 and 15.0 ng a.i./L E1/DHE combination doses, as well as the negative control were continued through reproduction (22 days) and hatching of the F2 generation embryos (4 days). A similar study was conducted to evaluate 17ß-estradiol and Conjugated Estrogens (CE) in the Adult Life Cycle Study (ALCS) at 8, 28 and 90.1 ng/L. Survival, growth, reproduction (fecundity and fertility), hatching success, sex ratio and VGT data will be presented for these three endocrine disrupting compounds.

**WE220**

**Is the fungicide tebuconazole toxic for the reproduction of aquatic invertebrates?**

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The triazole fungicide tebuconazole acts via an inhibition of the ergosterol synthesis of fungi, but it is also suspected to interfere with the steroidogenesis pathway. In rats, tebuconazole impairs reproduction by an interference with the endocrine system. Whether tebuconazole shows similar effects in invertebrates is unknown, even though for some invertebrate species effects of endocrine disrupting chemicals have been described. As tebuconazole occurs in surface waters at concentrations up to 590 ng/L (Hesse, Germany), the fungicide may interfere with the reproduction of aquatic invertebrates and therefore may pose a risk for invertebrate populations. In order to investigate the toxicity of tebuconazole, long-term reproduction tests with the snail Potamopyrgus antipodarum (28 days) and the water flea Daphnia magna (21 days) were conducted. The number of embryos and the number of offspring served as endpoints for P. antipodarum and D. magna, respectively. In addition, the formation of male offspring was considered in D. magna. In rats, tebuconazole impairs reproduction by an interference with the endocrine system. Other pesticides, mostly insecticides known as juvenile hormone analogs have shown to induce male formation. The results of the ongoing study will be presented and discussed. In Acknowledgments: This study is funded by the Federal Ministry of Education and Research (BMBF, Germany) within the framework of the project “NiddaMan” (02WRM1367A).
ED potency of the “cocktail” of air contaminants on bioassays integrating interactive effects between compounds. Disruptions of the transcriptional activity depending on nuclear receptors to estrogens, androgens and thyroid hormones were thus studied, since alterations of these endocrine systems were previously reported in animals and humans. In this way, the air contamination from an urban area was compared during the contrasted seasons with this from a forest environment and a suburban area, or with this from indoor living places including three different urban habitats (apartment, office, day nursery). All the ED compounds appeared ubiquitous, as well outdoors as indoors. Except PAHs, they showed indoor concentrations 10 to 80 times higher than the indoor ones. In addition, contrary to a priori ideas based upon the low vapour pressures of semi-volatile compounds, it appears that human exposure via the respiratory tract occurs mainly through the gaseous phase, which is supported by the international literature. These contamination data associated with the known ED nature of the target compound were in accordance with the relative ED potency of the ambient air under different atmospheric phases, seasons or environments, and the nature of the highlighted ED effects: estrogenic, anti-androgenic and thyroid-like. The chronic human exposure to indoor air contamination, namely via the gaseous phase, might induce adverse sanitary events related to the ED potency of that contamination.

**WE223**

**Estimating the carcinogenic and non-carcinogenic effects of persistent organic pollutants in food stuffs using risk assessment indices**

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Chemicals play a part in almost all human activities including medicine and agriculture. However, there is a staggering mismanagement of chemicals in Nigeria, leading to high levels of contamination in various environmental matrices and food stuffs. The increasing presence of these chemical residues in various commonly consumed food products is of great concern, due to potential adverse effects on humans and possible impact on sustainable development. In an attempt to estimate the impact of chemicals on human health in Nigeria, several studies have been carried out by researchers at the Laboratory of Ecotoxicology and Environmental Forensics, University of Benin. Pesticides and polycyclic aromatic hydrocarbons (PAH) have been analyzed in food products using gas chromatography (GC) equipped with electron capture detector (ECD), while the attendant health risk associated with the consumption of POPs in contaminated food stuffs have been determined using relevant risk assessment indices for cancer and non-cancer risk. Results have shown high concentrations of these persistent chemicals in several food stuffs in Nigeria, while human health risk predictions have shown that dietary exposures to POPs, increases the potential for cancer and non-cancer effects in humans after consumption. Therefore, there is the need for a concerted effort by relevant authorities in ensuring that chemical use is regulated while the ban on POPs is enforced.

**WE224**

**Consumption of canned foods in Nigeria: assessing the associated human health risk.**

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Ensuring food safety to protect public health and promote economic development remains a significant challenge in both developing and developed countries. In Nigeria, the changing lifestyle of people has resulted in increased consumption of canned foods which makes it imperative to determine the levels of chemical adhesives to human health risks associated with consumption. To this end, levels of food additives (Nitrite and Nitrate, Sodium Chloride) and heavy metals (Fe, Mn, Zn, Cd, Pb, and As) in canned foods frequently consumed in Nigeria, were assessed to estimate human health risks associated with consumption. Chemical additives and Heavy metals were assessed using Perkin-Elmer spectrophotometer and Atomic Absorption Spectrophotometer (AAS) respectively. Human Health Risk estimates were obtained using standard indices (Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Dietary Exposure (DE)). Results showed that the concentrations of Nitrate, Fe and Cd in all the canned food categories exceeded recommended limit set by the European Union, including potential health risks. Further risk estimation using the RfD approach showed EDI values for Cd in all the canned food categories above the tolerable daily intake, while DE for Fe in canned sweet corn, Fe, Zn and Pb in canned beans/peas had values above recommended limits. THQ values for all the canned foods were less than 1 except canned beans/peas, while HI was above 1 in the canned fish category. This implies that there is risk of non-carcinogenic health effects from the consumption of canned foods as intake levels of some additives depends on the continuous monitoring of food additives and heavy metals in canned foods.

**Pollutant risks to amphibians and reptiles: how much we know and what we need (P)**

**WE225**

**Amphibians and Plant-Protection Products - What Research and Action Is Needed?**

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The majority of Swiss amphibians are at risk. There is a large number of factors that are discussed as possible causes, including plant-protection products (PPP), although to date we have not been able to adequately quantify the impact of the latter. Since the influence on amphibian populations of PPP’s in the form of active ingredients, wetting agents, and product adjuvants has not been studied to any great extent, a workshop was held with over 30 invited experts from authorities, academia, industry, agriculture, environmental NGOs, Swiss amphibian research institutes as well as environmental law. A majority of the participants deemed further research on the influence of plant protection products on amphibians appropriate. Identified research issues were: What is the exposure of amphibians to PPP in natural environments? How great is the risk to which species and developmental stages in what crops from what PPP? What effects on individuals are relevant at the population level? What population models can be used to estimate the effects observed in the laboratory with individuals, at the population level and whether PPP co-formulators have an impact on toxicity? How high is the variability in sensitivity between the various amphibian species and populations? Can entrance criteria be developed in order to identify potentially problematic substances for amphibians for the ecotoxicological risk assessment in the authorisation of PPP’s? What risk-management options are there both inside and outside of the field? How relevant are other factors for the decline in the amphibian population, compared to PPP’s? A further topic of discussion was how to better protect amphibians from PPP’s by reducing their exposure on open ground. Discussed measures were: Avoidance of PPP application during migration in affected areas with the help of prediction models Recommendation for not using specific products Advice and awareness-raising for farmers Advice and awareness-raising for private users in their home gardens Compensation measures (e.g. amphibian-specific ecological compensation areas) Promotion of amphibians via direct payments and/or existing label schemes, e.g. IP-Suisse Reduction of PPP use through alternative plant-protection strategies.

**WE226**

**Incorporating Habitat Variables into a Weight-of-Evidence Analysis of Amphibian Risks in Wetlands**


Weight-of-evidence analyses may include a comparison of ambient chemical concentrations in the environment to environmental guidelines, field survey results, and toxicity test results. Risk assessment of amphibians in wetlands can benefit from also considering bioavailable contaminants, and can have a profound influence on populations and communities. The current study compared metal concentrations in water collected from eight wetlands potentially influenced by industrial emissions, and four reference wetlands, to guidelines and amphibian toxicity data collated from the literature. Amphibian diversity and abundance data for these same wetlands also were collected. Statistical analysis of habitat variables relative to amphibian occupancy and relative abundance was completed for these and approximately 40 other wetlands in the area. Including the habitat evaluation contributed to understanding the potential risks to amphibian populations in wetlands near the facility. The analysis showed that, although concentrations in water exceeded guidelines for several metals/metalloids, concentrations generally, wetting agent, and amphibian toxicity effect levels, Habitat variables and life history characteristics of the amphibians were stronger indicators of population presence and abundance than metal concentrations.

**WE227**

**Evaluation of effects of herbicide products and their active substance on amphibians: Tests of glyophosate exposure on the development of common toad (Bufo bufo)**

J. Sadowski, F. Göge, JKI; A. Fischer, JKI / Vertebrate Research Common toad (Bufo bufo) is a typical species of the European agricultural landscape and potentially threatened by pesticide exposure. Even modern perennials agrochemical use is accompanied by a high exposure risk. However, studies on the influence of pesticide exposure to European amphibian species are rare. For that reason we tested in an acute toxicity study the influence of the herbicide Roundup® PowerFlex and its active ingredient (a.i.) glyphosate in high and environmentally relevant doses on survival and development of Common toad. In the laboratory, eggs were exposed to Roundup® PowerFlex or glyphosate
for 24h or to compare with untreated control groups. Rate of hatching individuals and size, weight and survival rate during time to metamorphosis were determined. The preliminary results showed that high dose treatments led to expected severe effects on tadpole’s development. Environmental relevant doses seemed to have no effects, but more replications would be necessary. Systematic toxicity studies are necessary to assess the risk of European amphibian species due to the lack of suitable and comparable data. Standards for laboratory tests should be developed and single and mixed pesticide exposure scenarios on environmentally relevant level should be done for risk assessment of different species living in the current agricultural landscape.

WE228  
Cadmium but not Lead affects Xenopus laevis fertilization and embryogenesis.  
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Environmental contaminations have significant effects on biodiversity, especially on Amphibians group. This class, containing the highest number of known threatened species, is highly sensitive towards chemical contaminants. Despite toxicity, dominance and persistence in the environment of metal contaminants, effects on amphibians remain poorly documented, except through the FETAX test. But this assay, use to determine lethal and teratogenic concentrations of solutions, runs with few-days old tadpoles. So effects on gametes, external fertilization and embryogenesis are not considered. Whereas these steps are also exposed to environmental contaminations, they're still poorly documented yet. In this context and according to previous results of ours showing that X. laevis oocytes maturation was altered by metal chlorides, we examined how cadmium and lead interact with earlier stages of Xenopus laevis, from fertilization to B-cells zygotes. While lead does not disturb the fertilization rates, cadmium decreases fertilization whatever the male or the female gametes have been pre-exposed. But the fertilization success is more affected when oocytes are the Cd pre-exposed ones. Furthermore, no fertilization are able to reach the stage 2 in this condition. Our results highlight that cadmium altered Xenopus reproduction, affecting fertilization success and embryogenesis, preventing the formation of tadpoles. Analysing earlier stages will procure complementary information to the FETAX test. Moreover oocytes seem to be particularly threatened, and let suggest they could be considered as early and sensitive endpoint (and target) to assess environmental contaminants effects.

WE229  
Effects of environmental stressors on the amphibian' pathogen Saprolegnia australis  
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Saprolegniiasis is the common term used to report oomycete water mold infections. They have been responsible for large economic losses on aquaculture due eggs and fish larvae infection. As well, amphibian natural populations are also strongly affected. The pathogenic agent, water mold infections have been documented and linked to amphibian population declines worldwide. These fungus-like organisms are in their majority saprobes, but are capable of parasitism and may act as opportunistic pathogens (e.g.cutaneous injuries). Besides, effects of water molds on amphibians appear to be influenced by several environmental factors, including temperature, salinity and ultraviolet-B radiation. The present work aimed at understanding the effects that environmental changes may cause on amphibian pathogens. To achieve this objective, the pathogenic agent, Saprolegnia australis, was exposed to different levels of NaCl, seawater and acid mine drainage (AMD). Ecotoxicological assays were carried by exposing the pathogen to PAG culture medium with different NaCl concentrations (ranging from 4.9 mScm\(^{-1}\) to 54.9 mScm\(^{-1}\)), AMD dilutions (with dilutions ranging from 0% to 100%) and seawater (concentrations equivalent to NaCl solutions conductivity and the highest tested concentration being the one corresponding to SW). At the end of the assay, vegetative growth, biomass production, extracellular enzymatic activity and mycelium chemical composition were assessed in S. australis exposed under control and contaminated conditions. Results obtained showed that NaCl and AMD effluent solutions caused no effect on vegetative colony growth exposed to NaCl. However, a significant growth inhibition relatively to the control was observed when the mold was exposed to 0%, 25% and 50% of AMD effluent dilution. For biomass production, results showed that NaCl and SW appears to have similar effect concentration values (EC\(_{50}\) 47.0 mScm\(^{-1}\) and EC\(_{50}\) 41.7 mScm\(^{-1}\)).

Regarding the quantity of biomass produced under effluent exposure, a reduction of 75% was observed at the AMD dilution of 75%. NaCl and AMD effluent also exerted effects on extracellular enzymatic activity. Under NaCl exposure, the water melt increase the gelatin and casein degradation and decrease lipase activity on the highest concentration. In general, effluent decreased the degradation of used substrates.

WE230  
Biomarkers of oxidative stress in lizards from crops with different management intensity  
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Kabulenz Landau / Institute for Environmental Sciences; M. Biaginni, University of Florence / Museo di Storia Naturale Sezione di Zoologia La Speccola; S. Costa, E. Fasola, B. Santos, University of Aveiro / CESAM Departamento de Biologia; I. Lopes, University of Aveiro / Biology Department  CESAM; C. Corti, University of Florence / Museo di Storia Naturale Sezione di Zoologia La Speccola

Reptiles are an understudied group in ecotoxicology and little is known about the effects of pesticides on them. Several studies have shown the presence of some species within crop fields, with the consequent risk of becoming exposed to pesticides, and also that increasing intensity of agricultural management could compromise fitness in several lizard populations. The metabolism of pesticides in these organisms produces reactive oxygen substances (ROS), which are normally neutralized by the antioxidant system. When the production of ROS surpasses the capacity of this system, the non-neutralized ROS may damage different structures of the cells, causing oxidative stress. We compared oxidative stress biomarkers among populations of two species of lizards (Podarcis siculus and P. muralis) inhabiting olive orchards and vineyard with a different degree of management (from organic fields to intensively managed crops) at Montelpaldi Farm (Tuscany, Italy). We did not find any effect of species or gender on biomarkers, but the tail integrity influenced the outcome of glutathione concentrations and body condition.

TBARS, an indicator of lipid peroxidation, increased concentration was associated with increased intensity of management in olive orchards. The enzymatic activity of glutathione peroxidase was reduced with respect to organic fields in olive orchards with an intermediate management intensity but in the more intensively managed, which could indicate a consumption of the enzyme at low pesticide concentrations that would eventually lead to new synthesis under increasing concentrations. However, responses of these two variables were only significant when considering lizards with regenerated tails, but not in animals with intact tails. A possible explanation for this result could be that of an energetic trade-off between the reproductive process and the antioxidant system, however further research is needed to unveil such kind of interaction. Our results highlight the importance of running integrated assessments of population health in order to understand the impacts of agricultural intensity in reptiles. The lack of basic ecotoxicological data and standard test methods for reptiles makes especially interesting this type of integrated field approaches for pesticide risk assessment in reptiles.

Science based strategies for the environmental assessment and management of pharmaceuticals and veterinary medicines (P)

WE231  
TOXICITY EVALUATION OF OXIDATION-TREATED WASTEWATER FROM A PHARMACEUTICAL MANUFACTURING FACILITY  
J.G. Tell, Merck & Company, Inc. / Global Safety the Environment; R. Watts, K. de Bruin, Merck Sharp & Dohme; A. Vlaski, A. Haener, ARCADIS; J. Rijk, M. Migheišien, WIL Research Europe B/V

MDS is currently implementing a multi-year initiative to improve wastewater treatment and implement water sustainability projects at manufacturing sites worldwide. As part of the improvements, a pharmaceutical formulation site is installing new two wastewater treatment plants (WWTPs) for waste streams containing Active Pharmaceutical Ingredients (APIs) from two production areas before discharge to the municipal WWTP. Ozone was identified as a potential Best Available Technology (BAT) for the removal of Active Pharmaceutical Ingredients (API) emissions from manufacturing. In this poster, we outline the required Toxicity Vignettes (TVGs) for the removal for some APIs. Extreme ozone dosages of up to 3000 mg O3/L resulted in extreme toxicity, with 70% of the organisms producing reactive oxygen substances (ROS), which are normally neutralized by the antioxidant system. When the production of ROS surpasses the capacity of this system, the non-neutralized ROS may damage different structures of the cells, causing oxidative stress. We compared oxidative stress biomarkers among populations of two species of lizards (Podarcis siculus and P. muralis) inhabiting olive orchards and vineyard with a different degree of management (from organic fields to intensively managed crops) at Montelpaldi Farm (Tuscany, Italy). We did not find any effect of species or gender on biomarkers, but the tail integrity influenced the outcome of glutathione concentrations and body condition.

TBARS, an indicator of lipid peroxidation, increased concentration was associated with increased intensity of management in olive orchards. The enzymatic activity of glutathione peroxidase was reduced with respect to organic fields in olive orchards with an intermediate management intensity but in the more intensively managed, which could indicate a consumption of the enzyme at low pesticide concentrations that would eventually lead to new synthesis under increasing concentrations. However, responses of these two variables were only significant when considering lizards with regenerated tails, but not in animals with intact tails. A possible explanation for this result could be that of an energetic trade-off between the reproductive process and the antioxidant system, however further research is needed to unveil such kind of interaction. Our results highlight the importance of running integrated assessments of population health in order to understand the impacts of agricultural intensity in reptiles. The lack of basic ecotoxicological data and standard test methods for reptiles makes especially interesting this type of integrated field approaches for pesticide risk assessment in reptiles.

Bench-top testing of ozone treatment was conducted using collected waste stream samples from the two production areas to verify removal of APIs below the required discharge concentrations established for no environmental impact. Based on the tests, an optimal ozone concentration range of 50 mg O3/L at 30 min contact time to 100 mg O3/L at 15 min contact/reaction time. The potential negative impact of ozone treatment on water quality, specifically related to toxicity of the future treated effluent discharges, was addressed in a toxicity testing program. This included the following toxicity analyses for waste streams from the two different discharge points before and after
bench-top ozone treatment: Algae and Daphnia toxicity testing Estrogenic and Androgenic hormonal activity testing Bio-respirometry and 21 days DOC elimination testing. All toxicity, hormonal activity and biodegradability tests indicated the reduction of toxic effects in the tested waste streams after ozone treatment. These results are in conjunction with the observed API removal and confirm ozone as BAT for treatment of these APIs-containing waste streams.

**WE232**

**EFFECTIVE REMOVAL OF ACTIVE PHARMACEUTICAL INGREDIENTS FROM INDUSTRIAL WASTEWATER**

R. Warsz, M. Czarnik & Dobrovolny, Ross, M. DeMarco, ARCADIS; D. Vughs, KWR Watercycle Research Institute

MSD is currently implementing a multi-year initiative to improve wastewater treatment and implement water sustainability projects at manufacturing sites worldwide. As part of the improvements an initial laboratory screening on Best Available Technologies (BATs) for the removal of APIs from wastewater, and ozone is generally reported as an efficient API removal/destruction technology. However, a range of ozone dosages up to 120 mg/L are reported depending on the wastewater matrix. In general, the rule of thumb for ozone (O₃) dosage would be a factor 3 or 4 to 1 per unit of chemical oxygen demand (COD). For industrial wastewaters with a high COD, this would result in very high ozone dosages which can translate into high investment and operating costs. During this study several industrial wastewater streams, with varied compositions including 20 different APIs, were sampled and bench scale tests with ozone were performed. A dosage range of 0.01 to 4.4 mg O₃/mg COD was applied during testing. For each API, a method was developed using Ultra Performance Liquid Chromatography – Mass Spectrometry (UPLC-MS/MS) to analyze the APIs in wastewaters at concentrations as low as 0.1 µg/L. Results showed that good API removal efficiencies could be achieved for wastewater matrix COD levels as high as 5300 mg/L. Counter to the general perception, it was found that dosages of 10 mg-O₃/L yielded as much as 70% removal for some APIs. Extreme ozone dosages of up to 3000 mg O₃/L resulted in 99.999% removal of specific APIs in highly concentrated waste streams. Ozone treatment provided the required removal efficiencies for 18 of the 20 APIs tested. Widespread application of effective API removal from wastewater remains a major challenge. However results from this work show that ozone treatment can be an effective solution for a range of APIs and wastewater matrices. The required dosages for API destruction were lower than initially expected, resulting in a cost effective solution.

**WE233**

**Experience with AstraZeneca’s Environmental Reference Concentration (ERC) Policy - Managing API Emissions for Internal and External Manufacturing Facilities.**

B. Dillon, AstraZeneca / AstraZeneca Global Environment; K. Hutchinson; C. Hargreaves, AstraZeneca UK Ltd / AstraZeneca Global Environment

The issue of Pharmaceuticals in the Environment has received increasing attention in both academic literature and wider media of recent. Whilst Environmental Risk Assessments are required for patient use of new medicines as part of the regulatory submission to health authorities within the EU and USA, there remains a lack of guidance associated with potential impact from Active Pharmaceutical Ingredients (API) from manufacturing. However to date, AstraZeneca’s Environmental Reference Concentration (ERC) concept and its application to manufacturing sites both internally and externally as a tool for the risk management of API discharges to the aquatic environment. The Environmental Reference Concentration is defined as “the average concentration of an API in the receiving surface water environment that, based on current scientific knowledge, would be unlikely to result in any adverse long term effects”. AstraZeneca have previously published our approach to monitoring emissions through the ERC procedure.1 This poster aims to highlight our application and experience of the procedure via both practical and theoretical risk based evaluations. Toxicological data from both internal sites and external Contract Manufacturing Organisation (CMO) suppliers.2 Murray-Smith R J; Coombes V T; Grönlund M H; Waern F; Baird J. A. Integ Environ. Assess. Manag. 2012 Apr;8(2):320-30.

**WE234**

**Toxicity assessments of the selected sulfonamides solutions after ozonation in comparison to oxidation by fungal peroxidase.**

N. Lemańska-Malinowska, Environmental Biotechnology Department; Z. Gajda-Meissner, T.F. Fernandes, Heriot-Watt University / School of Life Sciences Veterinary drugs from the sulfonamides group (SAs), such as sulfamethazine (SMZ) and sulfadiazine (SDZ), are the oldest chemotherapeutic agents used for anti-infectious therapy and due to their bioaccumulation capacity they play an important role. Very good results in the decomposition of these drugs in the aquatic environment were achieved by ozonation and using an oxidation process with unspecific peroxidase from Agrocyes aergeria (AueUPO). Nonetheless, special attention should be paid to the risk of by-products’ formation, which may cause potential toxic effects on the living organisms. Therefore, the aim of this work was to compare the acute toxicity of SMZ and SDZ before and after both processes in a range of trophic levels. For this purpose, toxicity tests on Vibrio fischeri, Pseudokirchneriella subcapitata (CCAP 276/4) and Daphnia magna were conducted, as described below. Toxicity tests on Vibrio fischeri bacteria were carried out using Microtox® analyser according to the Whole Effluent Toxicity (WET) test procedure. The effect of test solutions on the growth of the Pseudokirchneriella subcapitata (CCAP 276/4; green water) was measured as the rate of change of biomass. Daphnia acute toxicity tests were performed according to the OECD guideline 202 as range finding and definitive tests. The 24 h and 48 h EC₅₀ values were calculated using SigmaPlot® software. The results obtained from toxicity tests were compared. No EC₅₀ values could be calculated for the test wares & Doh. SDZ and SMZ caused 50% of algae growth inhibition after 72 h of exposure at concentration 4.95% v/v and 6.54 % v/v respectively. EC₅₀ values after 72 hours were 9.82% v/v and 9.83% v/v. For the freshwater crustacean the EC₅₀ values were in the range 25.98- 33.55 %. It is concluded, that the ozone process significantly decreased toxicity of the samples. For the control ozone oxidation of carboxylic acid of V. paradoxa showed a EC₅₀ equal to 50.69 % for SDZ solution after this process was applied. SDZ and SMZ caused 50% of P. subcapitata growth inhibition after 24 hours of exposure at concentration 5.14% v/v and 4.83 % v/v, respectively. After 72 h exposure the growth of algae was almost completely inhibited. EC₅₀ values for D. magna test were in the range 14.54- 15.37 %. In general, SMZ and SDZ mixtures before and after ozonation are much less toxic when compared to the enzymatic oxidation process.

**WE235**

**Difference of physiological activities of pharmaceuticals of wastewater between UK and Japan**

H. ZHANG; M. Ibara, S. Hanamoto, N. Nakada, Kyoto University; M.D. Juergens, Centre for Ecology and Hydrology; Wallingford; A. Johnson, CEH Wallingford / Wallingford; H. Tanaka, Kyoto University

Pharmaceuticals have been widely detected in water environment at low concentrations. However the toxicity of these compounds remains controversial. In order to investigate their potential risks to human and aquatic species, because they are designed to be biologically active. To better understand this concern, we must know the physiological activity of pharmaceuticals in waters based on their modes of action (MOAs). One of the most important MOAs is interaction with G-protein coupled receptors (GPCRs). Until now, we have detected physiological activities of GPCR-acting pharmaceuticals against some class of GPCRs in secondary effluent (SE) of wastewater treatment plants (WWTPs) in Japan by using in vitro transforming growth factor-α (TGFα) shedding assay. However, the situation in other countries is still unclear. In this study, we measured and compared the physiological activities of GPCR-acting pharmaceuticals in SE of WWTPs in UK and Japan by using in vitro TGFα shedding assay. We measured the antagonistic activities of pharmaceuticals against angiotensin (AT1), dopamine (D2), acetylcholine (M1), adrenergic (β1), and histamine (H1) receptors. Activities detected in waters were quantified as antagonist equivalent quantities (EQs). Concentrations of sulpiride (antagonist for D2 receptor) and metoprolol (antagonist for β1 receptor) were analyzed by LC-MS/MS in parallel. We compared the EQs, as measured by the TGFα shedding assay, with concentrations of corresponding pharmaceuticals, and investigated whether measured antagonistic activities in the wastewater could be explained by these known pharmaceuticals. In addition, in order to investigate more detail how different physiological activities of pharmaceuticals in SEs between UK and Japan, and also between SEs and APIs, we measured these fractions between UK and Japan sample. If the fraction in which highest EQ is explained by these known pharmaceuticals. In addition, in order to investigate more detail how different physiological activities of pharmaceuticals in SEs between UK and Japan, and also between SEs and APIs, we measured these fractions between UK and Japan sample. If the fraction in which highest EQ is explained by these known pharmaceuticals.

**WE236**

**Case Studies Demonstrating the Advancement of Wastewater Management in the Pharmaceutical Industry**

J.G. Tell, Merck & Company, Inc. / Global Safety the environment; D.J. Caldwell, Johnson & Johnson

Across the human health, pharmaceutical industry, we make a vast and diverse portfolio of products, each of which can have environmental risks. Good stewardship includes understanding the potential environmental and social implications and employing life-cycle thinking and tools to minimize any potential environmental risks – from the early stages of product design, to formulation and manufacturing, to the product’s impacts during use and final disposal of residual product. EcoPharmacoStewardship is the framework that applies the widely-accepted principles of product stewardship. The three pillars of the framework are extended environmental risk assessment (eERA), extension of the scientific knowledge base (IMI-iPiE), and manufacturing effluent management, which is the focus of this poster. In 2011, a group of major pharmaceutical companies met to initiate a formal dialogue on the issue of best practices for managing pharmaceutical discharges from site operations. The companies shared their current programs at the time and embraced the concept of a maturity ladder that provides a mechanism for benchmarking across the industry. In 2015, these same companies reconvened to evaluate whether each currently studied company has made any changes and improvements to company-wide programs over time. This poster summarizes...
some of the advances made by the pharmaceutical industry and the area for future work.

WE237 Perspective and challenges for generation of effects and fate data on innovative veterinary API
C. Durou, CETHRA SARL; R. Magnier, A. Bauer, Ceva Santé Animale; B. Journel, CETHRA SARL
An environmental impact assessment (EIA) has to be performed on the active pharmaceutical ingredient (API) for the market authorisation of veterinary medicines. The scientific guidelines are made publicly available by the EMA; among others, the guideline lists the minimum data requirements for the assessment of PBT/vPvB properties and how to conduct an EIA. The generation of fate and effect data is an iterative process. In a first Tier (A), the data requirements include the determination of physical-chemical properties (e.g. water solubility, n-octanol/water partition coefficient, fate properties (adsorption/desorption, route and rate of degradation) and short-term ecotoxicity dataset. In a second Tier (B), higher tier testing is required for fate (e.g. biocentration) and effects (long-term ecotoxicity) are requested. Finally, specific data may be requested on a case-by-case. For any new API, the available dataset on the active may be limited to the intended use of the VPI, pharmacological information and mode of action. While such data are important for the purposes of identifying emission route to environment and for designing an appropriate testing strategy, further information are necessary. Firstly, physico-chemical constitutes a key initial step given their importance as input data for exposure assessment and also as prerequisite for fate and effect data. As an example, behaviour and stability of new API in contact with water or standard aquatic test medium is missing at initiation of test program. Also, due to the mode of action but also in case of continuous emission to environment, long term toxicity studies may be preferred to short term studies. Next to these data gaps, the new API may exhibit one or more difficult to test properties for example, isolation status is a common difficult to test property. Considering the overall, there is a need to order testing, allows flexibility in test program. The more relevant and reliable data are, the more robust is the environmental impact assessment. The purpose of this work is to initially review the data requirement for an EIA for a veterinary API which miss basic data need to define the suitable test method and test strategy.

WE238 Parasiticides - highly used veterinary pharmaceuticals and their impact on the environment
N. Adler, UBA FG IV 2.2 Arzneimittel; I. Ebert, Umweltbundesamt / Pharmaceuticals Department; J. Schoenfeld, Federal Environment Agency Several groups of antiparasitic active substances are marketed and often used in food animals. While parasiticides are highly potent to eliminate endo- and ectoparasites, they have also effects on non-target organisms, such as dungen flies and beetles and water flies. Environmental risk assessments that are submitted during the authorization process of veterinary pharmaceutical products show high risks for non-target organisms. In addition to these risks a parasiticide poses to the environment, some of these active substances are assessed to be hazardous. However, no products containing antiparasitic substances were refused to enter the market, so far. Instead, risk mitigation measures are defined to reduce the risks to an acceptable level. The poster presents risk assessments and the hazard characterization of different parasiticides and it will further discuss the defined risk mitigation measures regarding their suitability as well as the decisions made by regulators.

WE239 Methodology for considering the environmental risks of parasiticides in a risk-benefit assessment
J. Chapman, Environmental Department University of York; A. Boxall, University of York / Environment Department; C. Sinclair, The Food and Environment Research Agency / Centre for Chemical Safety and Stewardship; P. Howley, University of York; G. Jones, The Food and Environment Research Agency As part of the authorization process, veterinary medicinal products (VMPs) require a benefit-risk assessment, which includes consideration of environmental risks. However, the environmental risks are not directly comparable to therapeutic benefits (i.e. efficacy). In this study, we therefore developed an approach for communicating environmental risks of VMPs in a benefit-risk assessment. We investigated the risks 3 parasiticide compounds (ivermectin, moxidectin and fenbendazole) using summaries of product characteristics (SPCs), literature sources, and regulatory guidelines. To facilitate the benefit-risk comparison, a visual decision support matrix was developed and applied to each compound. We found the environmental risk matrix highlighted key tradeoffs and discrepancies in available data and could therefore be a valuable decision support tool. The improvement of methods to consider environmental risks in the benefit-risk assessment will aid decision-makers to select to best option and make the authorization process more transparent.

WE240 Towards an updated ERA for propranolol
D. Leverett, WCA-Environment Ltd; R.J. Brown, G. Merrington, wca consulting; A. Peters, wca; M. Gross, WCA Environment; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; S. Owen, AstraZeneca / Safety Health Environment subteam; K. Hutchinson, Propranolol is a non-selective adrenergic receptor blocker (β-blocker) and is prescribed to treat high blood pressure, cardiac arrhythmias, glaucoma, anxiety and migraines. As a result of its widespread use and pseudo-persistence propranolol is frequently detected in European sewage effluents and surface water. Although an environmental risk assessment is available, there is no guidance from European Medicine Agency (EMA) guidelines exists, and PNEC values for propranolol have previously been published (Murray-Smith et al. 2011), it is recommended that these are reviewed at regular intervals (Holtm et al., 2012; Ågerstrand et al. 2015). A considerable quantity of new environmental effects and monitoring data for propranolol has been published in the scientific literature since the original assessment was conducted. We therefore present an updated ERA for propranolol, also conducted according to EMA guidelines, but taking account of all environmental fate and effects data published in the scientific literature to date. This will include an assessment of the measured environmental concentration (MEC) data for propranolol in both treated sewage treatment plant (STP) effluents and surface freshwaters. These will be compared with the predicted environmental concentrations (PECs) derived based on usage and dose data. Updated freshwater PNEC values for propranolol will also be presented, applying both deterministic and probabilistic derivation approaches. Finally, the MEC and PNEC data will be used to present updated Risk Characterisation Ratios (RCRs) for a series of different freshwater exposure scenarios. The ERA is conducted in two phases, with the overall relevance in Germany and adjacent countries.

WE241 Review of ERA data submitted as part of pharmaceutical marketing authorisations
D. Leverett, WCA-Environment Ltd; R. Marks, M. Gross, WCA Environment; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; K. Hutchinson, According to European Medicine Agency (EMA) guidelines, most marketing authorisation submissions for new pharmaceutical products require an Environmental Risk Assessment (ERA) of the active pharmaceutical ingredient (API) in the product, which should be submitted as part of the application. Exceptions to this requirement include so-called biologics (e.g. proteins, peptides, vitamins) where sufficient justification can be provided on a case-by-case basis that there is likely to be no significant environmental risk, and synthetic APIs where it can be shown that the new product will not lead to an increase in environmental exposure to the API. The ERA is conducted in two phases, with the substances meeting the criteria for further evaluation following a Phase I screening assessment proceeding to Phase II. In Phase II, a set of environmental fate and aquatic toxicity studies are required and a refined risk assessment for any compartments of concern may then need to be conducted in a Phase II, Tier B assessment. For products for which an EU-wide marketing authorisation is sought, the outcomes of each ERA are presented in a European Public Assessment Report (ePAR) which is publically available on the EMA website. However, the level and type of ERA information presented for each product can be extremely variable (especially for those applications submitted before 2006, when the current EMA guidelines were published). There may be no ePAR available at all, and ERA information contained in ePARs can vary from simple statements to a full presentation of hazard and exposure data. In addition, for generic APIs (i.e. those outside of patent) there are often a large number of applications (and therefore ePARs) for the same API. Nevertheless, a potentially large amount of environmental hazard data for APIs is available via these documents, and by cross-referencing the information on the EMA website for different products it may be possible to compile API hazard data, and potentially derive tentative PNEC values. In this poster we will present an analysis of the environment hazard information available on the EMA website for APIs in products for which an EU-wide marketing authorisation has been granted. In particular we will show data in the products for which a Phase II ERA was conducted in the author(s)’ area (if at all possible) the environmental hazard data. We then present an evaluation of the hazard data for each API, and attempt to derive tentative PNECs using this data, according to EMA guidance.

WE242 Science-based Approaches to prioritise the Environmental Risks Posed by Legacy Human Medicinal Products
the authorization process more transparent. The improvement of methods to consider environmental risks in the market, so far. Instead, risk mitigation measures are defined to reduce the risks to during the authorization process of veterinary pharmaceutical products show high Pharmaceuticals Department; J. Schoenfeld, Federal Environment Agency Parasiticides – highly used veterinary pharmaceuticals and their impact on test method and test strategy.

there is a need to order testing, allows flexibility in test program. The more toxicity studies may be preferred to short term studies. Next to these data gaps, the example, behaviour and stability of new API in contact with water or standard physico-chemical constitutes a key initial step given their importance as input data for the purposes of identifying emission route to environment and for designing an innovative veterinary API (adsorption/desorption, route and rate of degradation) and short-term ecotoxicity assessment of PBT/vPvB properties and how to conduct an EIA. The generation of hazard data for each API, and attempt to derive tentative PNECs using this data, that there is likely to be no significant environmental risk, and synthetic APIs outside of patent) there are often a large number of applications (and therefore Exceptions to this requirement include so-called biologics (e.g. proteins, peptides, (API) in the product, which should be submitted as part of the application. D. Leverett, WCA-Environment Ltd; R. Marks, M. Gross, WCA Environment; J. Björlenius B, Breitholtz M, Brunström B, Fick J, Gunnarsson L, Larsson J, Coombe VT, Grönlund MH, Waern F, Baird JA. 2011. Managing emissions of Legacy Human Medicinal Products

WE242 Combined Environmental Risk Assessment for the Antiviral Pharmaceuticals Ganciclovir and Valganciclovir J. Strach, F. Hofmann-La Roche Ltd / Roche Group Safety Health Environmental Protection

An environmental risk assessment (ERA) was conducted for the antiviral, primarily anti-cytomegalovirus active pharmaceutical substances ganciclovir (GCV) and valganciclovir (VGCV). VGCV is the L-value ester of GCV and a prodrug for the latter, formulated for better oral bioavailability; hence, the ERA focusses on GCV. Predicted environmental concentrations (PECs) for GCV were calculated for the relevant environmental compartments sewage treatment, surface waters and sediment, based on documented use data for both GCV and VGCV for Europe. Predicted no-effect concentrations (PNECs) for the same compartments were derived from chronic ecotoxicity tests. The ERA compares PEC with PNEC per compartment in the form of a risk characterisation ratio. Moreover, based on mutagenic and reproductive effects in mammals GCV is a CMR (carcinogenic, mutagenic, reproductive) substance; hence the fish early life stage test was extended to encompass reprotox endpoints. A PBT (persistence, bioaccumulation and high ecotoxicity) assessment complements the PEC/PNEC-based ERA.

WE246 Paradigm shift towards a substance-based environmental risk assessment of pharmaceuticals I. Rönnefahrt, Federal Environment Agency / Section IV Environmental Risk Assessment of Pharmaceuticals; N. Adler, UBA FG IV 2.2 Arzneimittel

Even more than 10 years after implementing the environmental risk assessment (ERA) into the EU pharmaceutical legislation there is still a lack of data on fate and effects of active pharmaceutical substances. The reason for that is quite simple: A review program of ‘legacy’ products, which were approved before the ERA requirement was set, was never envisaged. But even for newly approved pharmaceuticals, behaviors sometimes no full data sets essential for an ERA are available due to various reasons. In the pre-market phase an ERA is required for all new applications. This often leads to duplication of data and of assessments and hence, to a waste of resources. This may also lead to contradictory assessments and even to different risk mitigation measures for medicinal products intended for the same indication. The described ad hoc approach is not sustainable and ultimately, a shift towards a substance-based ERA is necessary. This might be achieved by (1) initiation of a Monograph system on active pharmaceutical substances. The aim of such a monograph system is to collate existing fate and effects data on active substances, generate new data gaps through targeted studies and to agree on the endpoints to be used for ERAs of medicinal products. Once established all authorizations of medicinal products would use the agreed endpoints from the monograph of the respective active substance to perform the ERA of the product. Overall, only a pre-market monograph system fate and effects data of active pharmaceutical substances in conjunction with an effective and efficient ERA system is within the reach of practical and economic standards. The shift to a substance-based ERA would allow a more accurate approximation of the overall health condition of trout. Additional tests with trout embryos and sac-fry stages were based on OECD guideline 212. Recorded endpoints in these tests were mortality, developmental parameters and histological condition. The diclofenac exposure of juveniles led to significantly increased mortality and to behavioural abnormalities already at low concentrations (0.1 µg/L). No such effect was seen for metoprolol. Furthermore, the stress protein level and the histology of liver and heart of trout remained unaltered. However, the erythrocyte to lymphocyte ratio in the heart of metoprolol-exposed brown trout was reduced compared to the control group and biochemical parameters such as creatinine, bile acids and cholesterol were increased. To demonstrate the juvenile trout and fry will complement the study. Overall, our study aims at providing further information on the effects of environmentally relevant concentrations of frequently used pharmaceuticals in a species of high ecological relevance in Germany and adjacent countries.

WE243 QSAR Studies on the Potential Environmental Hazard of Pharmaceuticals A. Sangon, P. Grammatica, University of Insurbia / DISTA Active Pharmaceutical Ingredients (API) have become ubiquitous present in the environment, for this reason in 2006 the European Medicines Evaluation Agency (EMEA) published guidelines for the environmental risk assessment of human pharmaceuticals. Every environmental risk assessment (ERA) requires large amounts of data for each chemical but, unfortunately, information on PBT behaviour and ecotoxicological data are available only for a little percentage of API in literature and databases. We propose the characterization of the intrinsic hazard potential of pharmaceuticals by QSARs development and application. Some pharmaceuticals have been prioritized as potential PBTs by both Insurbia PBT Index in QSRINS and US-EPA PBT Profiler in consensus approach. Then, from literature, we collected and carefully curated datasets for ecotoxicity data of species at different trophic levels (algae, Daphnia, fish) in order to consider a simplified aquatic ecosystem. For each species we developed ad hoc QSAR acute toxicity models, based on PaDEL molecular descriptors using OLS method and Genetic Algorithm for Variable Subset Selection in QSRINS software. All models are robust (R > 0.75, Q2 ext >0.70) and externally validated (CCC > 0.85) on different splitting schemes. The structural Applicability Domain (AD) of the proposed models to pharmaceuticals without experimental data has been verified and demonstrated to be very high with the 74% of chemicals inside the AD for all the toxicity models. Moreover, reliable predictions from different models applied on a set of more than 1000 pharmaceuticals were combined by Principal Component Analysis (PCA) to find an ecotoxicity trend representative of the pharmaceuticals' acute toxicity models on aquatic exposures. This study, a few overall aquatic toxicity index (ATI), has been then modelled by molecular descriptors to obtain a QSAR model useful to highlight, directly from the chemical structure, the pharmaceuticals potentially most hazardous for the environment. This index and the predictions obtained by it could be used to refine prioritization of pharmaceuticals at a central control level as is priori in the rational design of environmentally safer pharmaceuticals. Finally, we propose also interspecies correlation models to extrapolate information from one simpler trophic level (such as Daphnia) to another more complex (Fish), reducing the use of animal tests.

WE244 Effects of the beta-blocker metoprolol and the NSAID diclofenac on the embryonic development and health of brown trout Salmo trutta f. fario H. Schmieg, Tübingen University / Animal Physiological Ecology; S. Schwarz, University of Tuebingen / Animal Physiological Ecology; M. Scheurer, Water Telemetry Centre; H. Dreher, WCA-Environment Ltd; H. Aigner, Institute of Evolution and Ecology Animal Physiological Ecology; R. Triebkorn, University of Tuebingen / Animal Physiological Ecology

The discharge of human and veterinary pharmaceuticals into surface waters via wastewater treatment plants is a growing problem in Central Europe. However, despite the frequent detection of various substances in aquatic ecosystems, knowledge on their effects in biota is still scarce. Available data are based on standard biotests with model test organisms, which often lack ecological relevance. Two of the most frequently used pharmaceuticals and, hence, also frequently detected in surface waters, are the beta-blocker metoprolol and the NSAID (nonsteroidal anti-inflammatory drug) diclofenac. Our present study investigated the effects of metoprolol and diclofenac in brown trout, Salmo trutta f. fario, at concentrations of high and medium relevance for Central Europe. The applied concentrations were 0.1, 1, 10, 100, and 1000 µg/L for metoprolol and 0.1, 0.5, 1, 10, 100 and 200 µg/L diclofenac, thus including several concentrations in ranges reported for German surface waters. Juvenile fish (six month post hatch) were exposed for 28 days. Investigated endpoints included biometric measurements, mortality, and behavioural abnormalities, stress protein level (Hsp70) as a biomarker for proteotoxic stress and degree of lipid peroxidation as a biomarker of oxidative stress. Furthermore, histology of liver, kidney, gill, and heart was assessed reflecting the overall health condition of trout. Additional tests with trout embryos and sac-fry stages were based on OECD guideline 212. Recorded endpoints in these tests were mortality, developmental parameters and histological condition. The diclofenac exposure of juveniles led to significantly increased mortality and to behavioural abnormalities already at low concentrations (0.1 µg/L). No such effect was seen for metoprolol. Furthermore, the stress protein level and the histology of liver and heart of trout remained unaltered. However, the erythrocyte to lymphocyte ratio in the heart of metoprolol-exposed brown trout was reduced compared to the control group and biochemical parameters such as creatinine, bile acids and cholesterol were increased. To demonstrate the juvenile trout and fry will complement the study. Overall, our study aims at providing further information on the effects of environmentally relevant concentrations of frequently used pharmaceuticals in a species of high ecological relevance in Germany and adjacent countries.
Advancements in life cycle impact assessment method development (P)

WE249 Bridging land transformation and occupation impacts with water use in life cycle assessment: linking green water consumption with ecosystem quality impacts in Amazonia

M.J. Lathuilhier, University of British Columbia / IRES; C. Bulle, CIRAIG - ESG - UQAM / Strategy corporate social responsibility; M.S. Johnson, University of British Columbia / Institute for Resource, Environment and Sustainability Department of Earth Ocean and Atmospheric Sciences

The framework for assessing potential impacts of land transformation and occupation (LTO) has provided valuable guidance for quantifying mid-point impacts such as changes in erosion resistance or groundwater recharge. At the same time, the inclusion of water use in life cycle assessment (LCA) has filled important gaps in modeling gaps of potential contaminant transport and quality and quantity on human health and ecosystem quality. We propose to bridge land use with green water consumption (as soil moisture regenerated by precipitation) in a cause-effect chain reflecting changing to the atmospheric water balance through LTO. LTO affects the amount of green water consumed by the land through evapotranspiration, which can reduce precipitation downward (mid-point impact), thereby affecting terrestrial ecosystems which are reliant on soil moisture (end-point impact). The mid-point impact modeling step derives the amount of reduced precipitation present in a river basin based on an internal evaporation recycling ratio. The end-point impact relies on differences in climate potential species richness resulting from reduced precipitation as a step to infer ecosystem damage. We apply the proposed method to Southern Amazonia which has experienced extensive deforestation for pasture and crops and whose agricultural frontier plays an important role in changing the atmospheric water balance of Amazonia. This new framework brings closer together the effects of LTO and water conservation which are often considered separately in LCA, but whose connection is particularly important in arid and semi-arid regions as well as highly seasonal areas where evapotranspiration and precipitation are closely coupled.

WE250 Combined environmental and economical inventory for a LCA and LCQA of an insurance

I. Bosch-Frigola, F. Coca, Universidad San Jorge; J. Val, D. Chinarro, San Jorge University; M. Pino, San Jorge University / Facultad ciencias de la salud

Increasingly, the environmental characteristics of a product are analyzed not only from an environmental point of view but also economically. Therefore, combined LCA (Life Cycle Assessment) and LCQA (Life Cycle Cost Assessment) methodologies have been progressively implemented and regularly applied to industrial activities or products but less put into practice on services activities or products. This study aims to develop a combined methodology between LCI (Life Cycle Inventory) and economical inventory costs applied to the insurance policy in order to perform a LCA-LCCA coupling study. One health insurance policy from an insurance company specialized in health and prevention (DKV Seguros a member of Munich Health) was considered as functional unit. The study includes the activity of the company headquarters located in Zaragoza and 61 offices throughout Spain during 2014. The system boundaries of the study are "cradle to grave" thus incorporating not only the phase of creating insurance (business coaching, marketing and vending), but also the phase of use by insured client. Disposal stage is also contemplated. Furthermore, the inventory includes in detail all transport associated with the activity: the receipt of raw materials, transport of waste to the treatment center, transport marketing activities and the displacement of the insured client to the appropriate medical center. These environmental data have been assembled to economic data, primarily costs associated with identified flows input/output. The costs allocation has been carried out through an ABC system (Activity Based Costing). Data obtained from the combined inventory has allowed to connect the economic information to the environmental information at every stage and provide a basis for diagnosing the environmental impact of an insurance throughout its life cycle, as well as the costs associated with these impacts. This data analysis provided useful results to establish new strategies of prevention in the insurance sector. This work has been possible thanks to the financial support and the systematic data provided by the company DKV

WE251 Freshwater Ecotoxicity as an environmental impact category to guide the selection of chemical-based products

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Affairs for Chemistry

There is increasing interest from regulators to apply cycle life based impact assessment methodologies to assess the environmental performance of chemicals and products and to include freshwater ecotoxicity as an impact category. Examples include initiatives on green chemistry in the US as well as the Grenelle Regulation in the strengths of the French and the Single Market for Green Products Initiative that covers both fast moving consumer goods as well as durable goods (e.g. the Product Environmental Footprint, PEF, pilot projects). A key focus area of such initiatives is the assessment of the environmental impact of chemicals and specifically the application of the USEtox method for measuring freshwater ecotoxicological impacts. The extension of the traditional LCA methodology to include the ecotoxicological impacts of chemicals raises some fundamental issues both methodologically and conceptually with the accepted norm of using risk assessment approach to manage chemicals and ecotoxicity consumer goods, within industry and by regulators. At the same time, the scientific committees of the EU have outlined the need for integration of perspectives. Hence, the ecotoxicological assessment methodology is far from being realistic, i.e. not delivering an accurate approximation of the environmental impact of chemicals. However, there has been no detailed assessment of whether decisions based on a CDV (Critical Dilution Volume, EU Ecolabel key criterion) or USEtox comparative ranking lead to real and meaningful differences in environmental impact. With this in mind, an ECETOC Task Force was assembled to conduct a scientific evaluation of USEtox and similar tools (e.g. CDV) with the goal of establishing their relevance to the real world and to provide guidance on how to interpret their results regarding selection/ranking of chemical based products. In addition, for the USEtox methodology in particular, the influence of LCA best practices on the results of the ecotoxicological impact assessment is studied in order to derive attention to data availability and quality as well as treatment of missing data. Finally, a comparison of the purpose and scope of LCA and risk assessment (RA) is performed in which the potential use of specific methods from RA within LCA is explored. Guidance will be offered on how both kinds of assessment can be used in conjuction or can complement each other. This poster presents the preliminary results of this task force’s activities.

WE252

Lessons from the endogenous integration of life-cycle indicators into national energy models

D. García-Gusano, D. Iribarren, M. Martín-Gamboa, Instituto IMDEA Energía / Systems Analysis Unit; A. Lind, K. Espegren, Institute for Energy Technology (IFE); J. Dufour, Instituto IMDEA Energía / Systems Analysis Unit Governments and decision-makers are increasingly aware of the need for assessing sustainability, which is particularly relevant to the energy sector. In this respect, life-cycle approaches are often used for the evaluation of energy systems. A key challenge is the implementation of the time dimension when using these approaches for the analysis of dynamic systems. The energy sector (both as a whole and at a lower scale) is a dynamic system. In fact, energy policies aim to guide the evolution of this system by defining performance targets e.g. in terms of greenhouse gas emissions. Energy systems modelling supports this type of policymaking process thanks to its suitability to deal with future scenarios and therefore with the time dimension. However, current modelling practices leave out a strong implementation of sustainability indicators in energy models. Within this context, the hybridisation of life-cycle and energy modelling approaches attempts to include both sustainability approaches and their limitations. This work summarises the lessons learnt from the integration of life-cycle indicators into national energy models within the SuReTool project of the EEA/NILS Science and Sustainability programme (006/ABEL-CM-2014). The evolution of power generation in the business-as-usual scenario for Norway and Spain was analysed with a time frame from 2010 to 2050. Furthermore, the damage assessment of the involved technologies was carried out using the IMPACT2002+ method. A number of life-cycle indicators (e.g., climate change, ecosystem quality and human health) were therefore endogenously integrated into the energy models of Norway and Spain. This soft-linking exercise enabled the analysts of the evolution of these life-cycle indicators, thus significantly enriching the interpretation of future energy scenarios. Emphasis is laid on the efforts made to mitigate concerns about double counting of emissions and imbalances in electricity trade processes. Overall, the interaction between life-cycle and energy systems modelling approaches is concluded to be advantageous. Nevertheless, further efforts are still required when it comes to strengthening the linkage between both approaches within a harmonised framework.

WE253

LAND USE LIFE CYCLE IMPACT ASSESSMENT: BRAZILIAN ECOSYSTEM SERVICES REGIONALIZATION

A. Raymundo Pavan, University of São Paulo USP / Departament of Production Engineering

There is a growing recognition that it is crucial to consider land use in the environmental assessment of products, especially those with a majority of their life cycle in biological processes (such as agriculture and forestry). This is an important issue in Brazil, which faces the challenge to overcome the anomaly between production and environmental protection within the general framework of sustainable development. The present work aims to develop characterization factors (CFs) consistent with Life Cycle Assessment that estimate the impact of land use on ecosystem services for Brazil. The methodology involves four steps: i) creation of a spatial model; ii) expert consultation; iii) data collection and iv) CFs calculation. A literature review and characterization models analysis led to the choice of Brandão; Milá Canals, and similar tools (e.g. CDV) with the goal of establishing their relevance to the real world and to provide guidance on how to interpret their results regarding selection/ranking of chemical based products. In addition, for the USEtox methodology in particular, the influence of LCA best practices on the results of the ecotoxicological impact assessment is studied in order to derive attention to data availability and quality as well as treatment of missing data. Finally, a comparison of the purpose and scope of LCA and risk assessment (RA) is performed in which the potential use of specific methods from RA within LCA is explored. Guidance will be offered on how both kinds of assessment can be used in conjunction or can complement each other. This poster presents the preliminary results of this task force’s activities.

WE254

Linking water pollution to impacts on water resources

C. Pradines, IRSTEIA Montpellier / UMR ITAP ELSA; M. Núñez, National Research Institute of Science and Technology for Environment and Agriculture Irsee, P. Roux, National Research Institute of Science and Technology for Environment and Agriculture Irsee / UMR ITAP ELSA; G. Junqua, Ecole des Mines d’Alès / LGEI; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - Irsee / UMR ITAP Life Cycle Assessment (LCA) of water consumption considers impacts on the Area of Protection (AoP) “Resources”, depending on the regeneration time. However, there is no method that links water pollution to the AoP resources. Because of the increasing concern over the state of water resources available for future generation, this study aims to answer if there is a link between water pollution and impacts on water resources. This talk proposes a definition of freshwater resource as part of the AoP resources and why it will be relevant to consider impacts on that AoP for certain types of pollution. A key element to clarify this debate is the concept of water functionality. The pollution in present time has potential impacts on human health and ecosystems, but beyond a certain time period persistent substances may rather contribute to potential impacts on water resources (i.e. their functionality) as it becomes increasingly uncertain to predict how the resource will be used in the future. Thus, the functionality loss due to pollution may be considered as damage to the water resource. In order to consider that link in LCIA we propose a new water use impact pathway which can be integrated into existing impact categories. Results proposed take into account that it is crucial not to double-count the impacts which are already assessed on human health and ecosystem quality. For that purpose, and to fit well with the definition given to the water resource, two phenomena are considered: the persistence of pollutants in a water body and the pollution of a water resource currently unused.

WE255

Towards a new midpoint indicator for including noise impacts from mobility in life cycle assessment

R. Meyer, LIST / Environmental Research and Innovation; E. Benetto, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation; C. Lavandier, Université de Cergy-Pontoise / MRTre Recent studies have shown that noise emissions from mobility have a significant impact on the exposed population’s health. The inclusion of noise in Life Cycle Assessment (LCA) has become a necessity as noise may represent the major contributor to Human Health when the LCIA and Potential impact assessment is studied, giving attention to data availability and quality as well as treatment of missing data. Finally, a comparison of the purpose and scope of LCA and risk assessment (RA) is performed in which the potential use of specific methods from RA within LCA is explored. Guidance will be offered on how both kinds of assessment can be used in conjunction or can complement each other. This poster presents the preliminary results of this task force’s activities.
allow linking the inventory in vkm to a change in the population exposure. Later on, the change will then be linked to an impact on Human Health (expressed in DALYs), allowing a systematic integration of noise impact in standard LCA.

**WE256 Pollinators in LCA: relevance and challenges**

E. Crema, University of Milano Bicocca; S. Salg, European Commission - Joint Research Centre / Sustainability Assessment unit

Human activities are threatening biodiversity at an unprecedented scale and pace, thus potentially affecting also the provision of critical ecosystem services, including insect pollinators. Insect pollinators play an essential functional role in terrestrial ecosystems, supporting ecological stability and food security worldwide. Therefore, assessing impact on pollinators is fundamental in any effort aiming at enhancing the environmental sustainability of human production and consumption, especially in the agri-food supply chains. Different drivers are leading to pollinator populations’ decline and a growing need for a supply chain-oriented assessment of the occurrence of pressure and impacts on pollinators is needed. However, current methodologies assessing impact along supply chains, such as life cycle assessment (LCA), miss to assess impact on pollinators. In fact, none of the existing life cycle impact assessment (LCIA) models effectively account for pollinators. Some LCA models have mentioned pollination, but none has presented key drivers of impact and a proposal for integrating pollinators as target group for biodiversity protection within an LCA framework. In order to devise a pathway towards the inclusion of impact on pollinators in LCIA, we conducted a literature review of environmental and anthropogenic pressures acting on insect pollinators, potentially threatening pollination services. Based on the evidence in literature, we identified and described eight potential impact drivers, primarily deriving from industrial development and intensive agricultural practice: 1) intensified land use as a result of uncontrolled expansion of urban areas and modern agricultural practices; 2) use of pesticides; 3) presence of invasive alien plants; 4) competition with invasive alien pollinator species; 5) global and local climate change; 6) spread of pests and pathogens; 7) electromagnetic pollution; and 8) genetically modified crops. To account for these drivers in LCIA, there are specific modeling needs. Hence, the current study provide recommendation on how future research should be oriented to improve the current models and how novel indicators should be developed in order to cover the existing conceptual and methodological gaps.

**WE257 Effect factors for terrestrial acidification in Brazil**

N.C. Mendes, Technical University of Denmark; A. Laurent, Technical University of Denmark / DTU Management Engineering Division for Quantitative Sustainability Assessment; M. Haischkind, DTU Management Engineering / DTU Management Engineering

To support the increased use of existing Life Cycle Impact Assessment (LCIA) methodologies across the world, new methodological elements have been developed towards spatially resolved impact assessment. Spatially resolved methods could better capture the differences of regional environmental conditions, which is an essential approach considering countries like Brazil, with high biodiversity. Previous studies have assessed the impacts of terrestrial acidification from the estimations of the potential losses of vascular plants species richness as a result of exposure to acidic substances for 13 biomes, with 2409 species addressed for whole world. In this context this work aims to provide a spatially-differentiated effect factors (EF) for terrestrial acidification in Brazil and support the development of spatially-differentiated characterization factors for Brazil. In order to maintain compatibility with existing LCIA methods the effect factors were developed using the framework adopted by LC-Impact and Impact World+ methods. Soil pH was used as an indicator of soil acidity to predict plant occurrences. From the number of plant species occurring at each 0.1 pH unit relationship responses of species richness and soil pH were developed. The species richness in each ecoregion were transformed into an empirical potentially not occurring fraction, which is a zero-to-one measure used to represent the presence or absence of species. The database consists of 3465 records of plants occurrences in Brazil, represented by 33167 species, indicating that this is a comprehensive study. Maps of soil pH in Brazil were extracted at 1-km resolution and pH values were extracted for the depth range of 0-30cm. For each ecoregion, species richness was plotted against soil pH and the exposure-response curves for acidification described the behavior of plant species in a certain region when it is exposed to acidic conditions. From these curves it was possible to derive the effect factors for terrestrial acidification. The results of this work show that spatial differentiation is meaningful when it is possible to combine fine spatial resolutions and highly representative data and this approach can be applied for other impact categories and regions, and contribute to the development of spatial differentiated LCIA methodologies.

**WE258 A regionalised characterisation factor for freshwater and marine eutrophication applied at the territorial scale: method and application in an agricultural context**

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Agriculture has impacts on the environment that range from local (eutrophication) and regional (acidification) to global (climate change) scales. To help local stakeholders manage agricultural activities, environmental impacts should be assessed at the territorial level by taking into account its work to provide a region-specific parameter. Characterisation factors are the link between these scales and they have been shown to be one of the most used and powerful techniques in the LCA. Several characterisation factors have been developed to assess environmental impacts at the regional scale. However, these factors have been developed for specific regions and they may not be applicable to other regions. For this reason, a new characterisation factor for freshwater and marine eutrophication has been developed for the French region of the Loire Valley. The methodology developed for this study was based on a spatially resolved inventory of nutrient emissions and an analysis of the receiving environment. The results of this study showed that the regional characterisation factor for eutrophication is higher than the national factor and that the impact of eutrophication in the Loire Valley is higher than in other regions. The methodology developed in this study can be applied to other regions in France and possibly other countries. The methodology can also be used to assess the impact of eutrophication in other regions and to identify regions where the impact is higher than in other regions.

**WE259 Spatial variation of secondary inorganic PM2.5 exposure and human health impact: a case study on milk production**

O. Jolliet, University of Michigan; K. Stylianou, University of Michigan - School of Public Health / Environmental Health Sciences; C. Tessum, J. Marshall, University of Minnesota / Civil Environmental and Geo Engineering; J. Hill, University of Minnesota / Bioproducts and Biosystems Engineering

Purpose: Secondary PM2.5 human health impacts in life cycle assessment (LCA) are currently based on linear and simplified assumptions that may lead to a potential double counting. The aim of this study is to provide spatial intake fractions (FI) for secondary inorganic PM2.5 for the U.S. and apply them to a case study that investigates environmental and nutritional effects associated with increased milk consumption. Methods: Urinary emissions of NH3, NOx, SO2, and PM2.5 are modelled in the Intervention Model for Air Pollution (IMAP) in three U.S. locations: Wisconsin (WI), New Jersey (NJ), and New York (NY). FIs by precursor are characterized for each emission location and are then applied to a case study investigating the environmental and nutritional health effects linked to adding one milk serving produced in WI, NJ, and NY. Environmental health impacts are assessed by calculating the changes in human cancer and non-cancer risk from exposure to environmental pollutants compared to the national baseline. The dietary health impacts are assessed by calculating the changes in human health risk due to exposure to dietary pollutants associated with milk consumption such as colorectal cancer, stroke, and prostate cancer using the Combined Nutritional and Environmental Life Cycle Assessment (CONE-LCA) framework. Results and Discussion: Intake fractions ranking is consistent between locations with PM2.5 having the highest FIs, followed by NH3, SO2, and NOx. Differences in exposure per precursor between locations due to main factors of influence. NJ shows the highest FIs followed by WI and NJ. For example, the NJ estimate for NH3 is about an order of magnitude higher than that in WI and about 15 times higher than that in NY, reflecting the higher population density. The relative ratios for NH3, NOx, and SO2 FIs varies between locations; the ratios between locations are in the range from 18in New Jersey (abundant NOx and SO2, but limited NH3) to 1.7 in Wisconsin (abundant NH3, but limited NOx and SO2). Finally, adding one serving of milk to the average U.S. diet leads to an overall health benefit due to nutritional benefits if production was to occur in NY or WI while this benefit is considerably reduced when milk is produced in NJ due to higher NOx and SO2 emissions. Conclusion: Preliminary results support a spatial variation of secondary PM2.5 exposure in the U.S. and suggest an overestimation of health effects in regions with high NH3 emissions or underestimation in regions limited in NH3 from current estimates. PM and dairy related exposures and impacts are substantially greater if emissions occur in in highly populated regions limited in NH3.
Acidification described the behavior of plant species in a certain region when it is exposed to acidic conditions. The results of this work show that spatial occurrences in Brazil, represented by 33,167 species, indicating that this is a novel indicator. From the number of plant species occurring at each 0.1 pH unit ranking is consistent between locations with PM2.5 having the highest iFs, followed by NH3, SO2, and NOx. There is a considerable spatial variation of exposure per precursor between locations due to main factors of influence. NJ shows the highest iFs followed by WI and NY. For example, the NJ estimate for increased milk consumption. Emissions or underestimation in regions limited in NH3 from current estimates. However, current methodologies assessing impact along supply chains, such as LC-Impact and Impact Support the development of spatially differentiated characterization factors for biomass and forest land degradation. The exposure factor estimates the capacity of biodiversity to adapt to reduced water in a given water compartment by accessing alternative water sources. The effect factor accounts for the effects on taxa that are not able to counterbalance the lack of water. Further sub-factors, such as a severity factor, might be added to this framework. This framework clearly expands the way modelling water consumption has been considered in LCA.

Bioavailability of Substances with low water solubility in Ready Biodegradability Tests: S. Fleury, DR. U. NOACK-LABORATORIEN; H. Maischak; U. Noack, DR. U. NOACK-LABORATORIEN

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. 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 application technique and the agitation during testing need therefore special consideration. Careful investigation of the application method prior to the test start can enhance the degradation distinctly. 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, acceleration 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. These investigations involve the 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.
evaluate the biodegradability of the anionic surfactant sodium lauryl ether sulfate (SLES) contained in two commercial foaming agents. For this purpose, a set of microcosms was set up using two different soils treated separately with one of the two commercial products at the concentrations used for the mechanized drill. At selected times (0, 7, 14, 21, 28 d) soil samples were collected for assessing SLES concentrations by HPLC analysis. The implications of reliance upon populations of bacteria water soluble / water insoluble organic substances in seawater. Unlike similar properties. Assessment implemented will be conveyed of the suitability of the and ecosystem dynamics; J. Parsons, University of Amsterdam / IBED; W. Bending, University of Warwick / School of Life Sciences. Industry uses tests provided by the Organisation of Economic Cooperation and Development (OECD) to assess the potential for transformation of a chemical and its metabolites in the environment, before it can be approved for use. Various factors are important in determining chemical fate in the environment, but many are not considered in initial laboratory tests. In particular, light can be a major influence on microbial abundance, but these tests are carried out in the dark. Additionally, microbial community abundance and diversity are likely to change in time in relation to water quality and climate, but there is little consideration of time of year that sampling takes place. We investigated the impact of light on the transformation of C7-isopryrazam, a fungicide, in river microcosms, using water and water-sediment microcosms collected at defined seasonal time points across a 18-month period. Materials were collected from the River Dene (Wellesbourne, UK), and both water and water-sediment microcosms were pre-incubated in the dark or light (18 hour light/6 hour dark cycle) for 9 days prior to isopryrazam addition (0.1 mg/L). There was little degradation of isopryrazam in dark microcosms regardless of season. However, in microcosms incubated under a light-dark cycle, degradation was faster in all seasons, although the rate of degradation varied depending on time of year sampling took place. Microcosms sampled in autumn or summer gave the fastest degradation rates, whereas microcosms containing samples collected in winter gave the slowest degradation rate. This trend was seen in both the water and water-sediment microcosms, although there was a greater effect in water-sediment systems. There were also differing degrees of mineralization across time, but the extent of mineralization was not correlated with the overall isopryrazam degradation rate. To date, microbial community analysis has focused on phototrophic communities using chlorophyll a abundance as a measure. While phototrophs appear to be important determinants of isopryrazam degradation, overall abundance of phototrophic communities was not closely related to differences in degradation of isopryrazam across time. This suggests that phototroph community composition, or interactions between phototrophs and other taxa, could underlie temporal variations in isopryrazam degradation.

TH009
Multiple use of vegetation in dioxin contaminated soil
J. Wittebol, M.H.Wagelmans, E. Elferink, Bioclear
There is an ongoing global and socially relevant transition towards biobased production processes. Biobased production processes use renewable resources from living environment (biomass, green materials) as part of the sustainable economy. Due to long term and widespread use of POP’s for example PCB’s, PAH or dioxins soils can be polluted with these chemicals. This might result in pollution of renewable resources. The sugar beet for example is a versatile product. It is used to produce sugars, molasses and pulp. These sugars are sequentially used in foods, biofuel or PET + PEF bottles. PAH is detected in molasses and dried sugar beet pulp. Dioxins, PAH and PCB’s are lipophilic organic compounds and are more prone to absorb to the root or soil particles than being taken up by root into the inner root and for transport into the rest of the plant. There are however exceptions. The goal of this research is to investigate possibilities to connect the biobased production with the removal strategy of POPs from soil. The possibility of composting PCDD, PCB’s and PAH contaminated biomass was investigated in both aerobic as well as anaerobic digested systems. When organic waste streams are contaminated with POP’s the toxicity might be affected by additional processes for example biological degradation. This could either be the positive or the process negatively. And thus the application of contaminated waste should be considered in detail. With the application of the (possibly) contaminated renewable resources the value of the agricultural land as well as the “agricultural waste” can be increased. It is unknown if biorefinery production processes are affected by persistent contaminants like dioxins. The main question is which processes occur both during uptake and during biobased refinery. What does this mean for the final product and possible waste streams? And can “pollution” of the end product be prevented by additional processes in soil and during refinery?

TH010
Risks of Non Extractable Residues (NER) of chemicals in soil
J. Harmsen, Alterra, Wageningen-UR / CALM; D. Hennecke, Fraunhofer IME -
systems offer excellent opportunities to allow microorganisms to adapt to new chemical, while in the natural environment, microorganisms tend to adapt to exposure times do not allow for adaptation of microbial communities to a new biodegradability assessment. In OECD’s biodegradation testing, the short Because of its origin and history, the microbial inoculum is source of variability in the performance of enhanced biodegradation tests acknowledges that procedures are required. The work presented assesses the impact of different inoculum sources on the biodegradation of typical organic substances. Biodegradation was the performance of ready biodegradable are estimated in the range of 0.1 - 10 mgC/L. Our methodological concept is based on comparative evaluation of cell density measurement by flow cytometry and substrate disappearance measurement by CO2 evolution and gas chromatography under assimilable organic carbon restricted conditions. We conducted our experiments with lake water microbial communities at starting cell density of 10E4 cells/ml in a defined medium. Small molecules, benzoate, 1-octanol, antraniguanine and phenol are selected as primary positive controls for readily biodegradable compounds. We observed step-wise increase in the lake community size (at initial cell density of 10E4 cells/ml) at the expense of added positive control substrate at concentrations of between 0.1 – 2 mgC/L and 1–2 mgC/L, respectively. Yield approximations from the observed community growth allowed us to link population evolution to the target compound. For accurate mass balance between compound and community size, we will further simultaneously measure lake water community growth and compound disappearance by gas chromatography. Lake water communities that enable biodegradation of the test compounds will be analyzed for diversity changes, and we will also further isolate and characterize degrading bacteria. References: K. Sentichto V, Beggah S, Rey S, Seyfried T and Van Meer JR. (2013), Examining Chemical Compound Biodegradation at Low Concentrations through Bacterial Cell Proliferation. Environ Sci Technol. DOI: 10.1021/es303592c

TH013 Coupling traditional biodegradation assessments with spectroscopic tools for plastic wastes: case of Polyurethane wastes assessment M. Cregut, University of Nantes / GB; M. Durand, University of Nantes / UMR CNRS GEPEA CEBAC Laboratory; A. Assaf, University of Nantes / GEPEA UMR CBAC Laboratory; T. Gerard, University of Nantes / Microbiology

Polyurethane biodegradation: Polyurethane is a complex chemical material composed of organic compounds of different classes. The biodegradability of polyurethane has been studied extensively, but the mechanism of biodegradation is not fully understood. The aim of this study was to investigate the biodegradability of polyurethane and to identify the key factors that influence its biodegradation.

The biodegradation of polyurethane was assessed under different conditions, including temperature, pH, and the presence of specific enzymes. The results showed that polyurethane is biodegradable under aerobic conditions, but the rate of biodegradation is significantly affected by the presence of enzymes and the type of microorganisms used. The presence of specific enzymes, such as laccase and lignin peroxidase, enhanced the biodegradation of polyurethane, while the presence of inhibitors, such as heavy metals, inhibited the biodegradation process.

In addition to the study of polyurethane biodegradation, the project also investigated the potential of using spectroscopic tools, such as Raman spectroscopy and infrared spectroscopy, to monitor the biodegradation process. These tools provided valuable information about the structural changes occurring during the biodegradation of polyurethane.

The findings of this study suggest that polyurethane is biodegradable under aerobic conditions, but the rate of biodegradation is significantly affected by the presence of enzymes and the type of microorganisms used. The use of spectroscopic tools can provide valuable information about the structural changes occurring during the biodegradation process.
can, in addition to direct detrimental effects on aquatic organisms, indirectly alter the functioning of adjacent aquatic ecosystems.

**TH015**

Fullerol as a potential pathway for mineralisation of fullerene nanoparticles in biosolids-amended soils

D.G. Navarro, CSIRO / Environmental Contaminant Mitigation and Technologies; R.S. Kookana, CSIRO / Land and Water; M. McLaughlin, CSIRO/University of Adelaide / University of Adelaide and CSIRO Land and Water Flagship; J. Kerby, CSIRO / Advanced Materials Transformational Capability Platform Land and Water

Fullerene (e.g. C60, C70) nanomaterials are now used in a wide range of products and thus can enter the environment via waste streams. Although these contaminants could reach and potentially adversely affect the terrestrial environment via the re-use of biosolids, their fate in soil is poorly understood. This study examined the mineralisation of C60 and its transformation products. Fullerol (C60-OH with drastically different physico-chemical properties) in biosolids-amended soils. Using radiolabelled C60 and C60-OH, we established that mineralisation of C60 was less than the detection limit (< 0.025%) in three contrasting soils during a 55 day long experiment. In contrast, however, up to 3% of C60-OH was mineralised during this period. This difference in observed mineralisation highlights C60-OH's greater bioavailability compared to its parent compound, C60, in biosolids-amended soils. These results indicate that transformation of certain fullerenes, such as to fullerol, could provide a potential pathway for their mineralisation, but such transformation may be slow.

Glucose-induced respiration tests on C60 and C60-OH contaminated soils suggested that overall microbial activity was not compromised by the contamination. This study is also the first work to examine the fate of fullerol in soils.

**TH016**

Kinetics of rapid covalent bond formation of aniline with humic acid: ESR investigation with nitroxide spin probes

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The bioavailability of many soil and sediment contaminants depends on their interaction with the organic matrix. In biodegradation simulation studies, many of these xenobiotic chemicals exhibit a large fraction of residues, which cannot be further extracted even by harsh methods (non-extractable residues, NER). The paper presents a new approach of using stable paramagnetic spin probes for investigating the kinetics of covalent binding of xenobiotic functional groups with humic acids, the main organic matter fraction. Leonardite humic acid (LHA) was incubated with the nitroxide spin label Anilino-NO (2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-oxyl), which includes an aniline functionality susceptible to interaction with LHA. ESR spectra of LHA samples were recorded at X-band frequency (9.43 GHz) at room temperature. A broadened ESR signal was observed of Anilino-NO incubated with LHA, which indicates strong restriction of the reorientational motion of the spin probe, i.e. immobilisation due covalent binding of the aniline group. This signal increased immediately after incubation and was used to determine the kinetics of the covalent bond formation. A two-site model with two parallel first-order reactions was used for fitting the measured values and determining the rate constants. A fast reaction with a rate constant of 0.308 min⁻¹, i.e. a half-life of approximately 2.25 min, and a slower reaction with a rate constant of 0.012 min⁻¹, i.e. a half-life of 57.8 min, was observed. Laccase-treated LHA shows an increase in the amount of reactive sites, presumably quinones, by a factor of 7.2 for the slow reaction, which supports the conclusion of a nucleophilic addition reaction of the aniline moiety with LHA. Both reaction rate constants are only marginally enhanced for the laccase-treated LHA. Paramagnetic spin probing is an effective method to investigate the rapid formation of covalent binding of xenobiotic functional groups humic acids. For chemicals containing an aromatic amino group a covalent fraction of NER can be explained as covalently bound residues, which is important for the determination of persistence in soil and sediment required for PBT and risk assessment of pesticides, biocides and other chemicals.

**TH017**

Factors for biodegradation and mineralization of two labeled pesticides in soils under different environmental conditions

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**Objectives of the Study**
To study the turnover mass balance (CO₂ evolution, extractable contaminant residues and non-extractable residues) of ¹³C-labeled 2,4-D and ¹⁴C, ¹³N-labeled Glyphosate in soils under different environmental conditions. To investigate the incorporation of ¹³C-label into microbial fatty acids, and ¹⁴C-labeled N-label into proteins from the N-source UG 10 (unlabelled) and V (labelled).

**Results**
During the degradation of two pesticides under different environmental conditions.
To define how the environmental conditions (e.g. temperature, soil organic matter and soil pH) the biodegradation of pesticides and the extent of “biogenic residues” formation.

**Material and Methods**
Soil samples were collected from the 0 to 5 cm soil layer in the agricultural field located in Bad Lauchstädt, Germany. The soil organic matter (OM) of these soils was NRM, potential pH were between pH 6.0 and 4.0% OM and pH 5.5 and 6.0, accordingly. Thereafter, soil was spiked with labeled pesticides (¹³C-Glyphosate or ¹⁴C, ²⁴-D) and incubated according to the OECD 307 and at different temperatures (10°C, 20°C and 30°C). The ¹⁴C-OM evolution was quantified by Inorganic Carbon Analyzer and the isotopic composition was measured by GC-IRMS. After the transformation processes, the residual parental pesticide + its primary metabolites and microbial biomass components (amino acids) were extracted and quantified and identified using UHPLC (Glyophosphate) or GC-MS (2,4-D, amino acids) and the isotopic composition was measured by GC-IRMS. Results After 39 days of incubation, mineralization of ¹⁴C-Glyphosate in soil containing 3%OM and pH 6 at 20°C, 25°C and 30°C was higher than 20%, 45% and 50%, respectively. In the same experiment with soil at pH 6 and pH 5.5 at 10°C, 20°C and 30°C the mineralization of glyphosate was lower and reached about 16%, 35% and 40% of ¹⁴C-glyphosate equivalents, respectively. Mineralization of ¹³C, ²⁴-D in soil containing 3%OM after 32 days of incubation constituted more than 8%, 50% and 70% of ¹³C, ²⁴-D equivalents at 10°C, 20°C and 30°C, respectively. In the soil at lower pH (5.5 and 6), mineralization of this herbicide was lower and reached accordingly 2%, 20% and 20% at 10°C, 20°C and 30°C. Conclusion The first results showed a positive influence of the environmental conditions like temperature and OM content of soil on the extent of glyphosate and 2,4-D mineralization in soil.
completely degrade another β-triketone herbicide, mesotrione and producing already known metabolites (AMBA and MNBA). Microbial toxicity of sulcotrione and mesotrione and their related metabolites in bacterial cultures was estimated by monitoring 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme inhibition. Our results indicate that β-triketone herbicides toxicity linked to HPPD inhibition was due to parent molecules, and not to the formed metabolites. Attempts were done to identify the genetic localization of sulcotrione degradation in *Pseudomonas* sp. 1OP and *Bradyrhizobium* sp. SR1. Plasmid profiles of both strains revealed the presence of large plasmids (>12 kb and >50 kb, respectively). Curing experiments showed that *Pseudomonas* sp. 1OP lost its ability to degrade sulcotrione under non-selective conditions, therefore degradation capacity may be attributed to the presence of this plasmid. On the contrary, under the same conditions, *Bradyrhizobium* sp. SR1 plasmid was not cured and the sulcotrione-degrading ability of the strain was maintained. Furthermore, a 14,000 Tn5 mutant library was constructed using a Tn5 mat conjugation approach conducted on a *Bradyrhizobium* SR1 strain. Among this library, two mutants affected in their biodegradation capacity were identified. Full sequencing of SR1 and Tn5 mutants is ongoing to identify possible degrading gene candidates. Keywords: biodegradation, β-triketone, herbicides, metabolites.

**TH020**

**Fingerprinting Micropollutant Transformation in Hyporheic Zones**

M. Posselt, Stockholm University / Department of Environmental Science and Analytical Chemistry; A. Rikkenstäd, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; J. Benskin, Stockholm University / Environmental Science and Analytical Chemistry Hyporheic zones are key ecosystems for aquatic ecosystems. As dynamic and complex transition regions between rivers and aquifers, they are characterized by the simultaneous occurrence of multiple physical, biological and chemical processes. Assessing persistence of environmental contaminants in the hyporheic zone is non-trivial. In addition to advection processes, transformation can be influenced by sorption mechanisms and other characteristics of the sediments (e.g. porosity and bed morphology), chemical parameters (e.g. occurrence of electron donors or acceptors), and biological factors (number and type of microbial population). These variables can lead to difficulties in reproducing lab-based biodegradation experiments and in extrapolating estimates of persistence in the lab to field conditions. Benchmark chemicals (i.e. model substances which are co-incubated with a substance of interest in lab-based biodegradation experiments) have been proposed as a means of addressing inter-assy variability in sediment biodegradation experiments. These controls may aid in extrapolating among various lab-based experimental designs and conditions, and perhaps even to conditions encountered in the field. The present work takes an initial step towards characterizing a suite of benchmarking chemicals for use with sediment biodegradation experiments by measuring their degradation half-lives (DegT1/2) under a series of carefully controlled conditions (temperature, oxygen content, stirring) in closed-bottle experiments. Natural sediments from the river Erpe (Berlin, Germany) were collected and characterized prior to use. Marker chemicals (which included pharmaceuticals and industrial chemicals) were selected based on their environmental occurrence and to cover a range of physicochemical properties, transformation rates and pathways. We expect this approach to aid in the development of more appropriate assays specifically designed for probing microbial degradation processes in the hyporheic zones, for extrapolation of micropollutant half-lives between lab and field, as well as among diverse river sediments and within various locations within a river system.

**TH021**

**The role of solar radiation on the photodegradation of PAHs in soils**

M. Marques, Rivora i Virgili University / Chemical Engineering; N. Dominguez, URV/IISPV; M. Mari, Rivora i Virgili University / Chemical Engineering; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; M. Schuhmacher, Rivora i Virgili University / Chemical Engineering; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J. L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health. Polycyclic aromatic hydrocarbons (PAHs) are a family of widespread environmental pollutants, whose chemical structure is based on two or more fused benzene rings. PAHs are released from natural and anthropogenic combustion processes. Although these chemicals are more prone to be present in urban air, soil is considered as one of the major sinks of atmospheric PAHs, undergoing wet and dry deposition processes. Since the environmental fate and transport of PAHs is highly influenced by temperature and solar radiation, photodegradation is likely to be the main process related to the loss of PAHs on soil surfaces, leading to the possibility to use PAHs as potential indicators of possible biodegradation. This study was aimed at estimating the photodegradation rate of PAHs in soils under winter conditions in the Mediterranean region. Soil samples were taken from the A horizon of two common Mediterranean soils located in remote areas. Arenosol soil samples corresponded to Halpic Arenosol, an acidic and coarse-textured soil with granitic origin. In turn, Regosol soil samples belonged to Calcic Regosol, with a formed of sedimentary materials and fine-textured soil. Ten grams of cleaned soil were deployed in uncovered glass Petri dishes. Every sample was spiked with 250 µL of a stock solution containing 16 US EPA priority PAHs at a concentration of 100 µg/mL and exposed to sunlight radiation in a methacrylate box placed on the roof of the School of Chemical Engineering, Tarragona, Catalonia. Dark controls were also performed to assess slow sorption processes. Temperature and solar radiation were measured and used to model the experiment. The results showed that PAHs behaved differently according to their molecular weight, temperature, radiation and soil texture. A decreasing trend of PAH concentrations in both soils in samples exposed to daylight was observed. Low molecular weight PAHs are more influenced by volatilization and sorption, while photodegradation is more evident for medium and high molecular weight PAHs. Photodegradation was higher than that obtained previously in laboratory conditions, since the intensity of solar radiation is ten times higher than that emitted by fluorescents in a climate chamber.

**TH022**

**Bioavailability-related effects of dissolved organic matter on biodegradation of PAHs**

J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquimica y Conservacion del Suelo; C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol. de Sevilla / Agroquimica y Conservacion de Suelos; R. Posada-Baquero, J. García, M. Cantos, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC. In environmental sciences, dissolved organic matter (DOM) is usually differentiated from particulate organic matter as the size fraction of organic matter smaller than 0.45 mm. This fraction typically consists of a multitude of structurally different compounds, all typically present at low concentrations, although it can differ significantly in quantities of their bioavailability and persistence. The environmental fate of organic pollutants, such as PAHs, can be affected by DOM via increased apparent solubility, desorption, transport and biodegradation. In our group, we have specifically addressed the influences of DOM quality on bioavailability-related phenomena: chemotaxis, attachment and solubilisation. We used, for our studies, different model DOM sources like DOM from biodegradation, and these included Tenax extraction, dual 14C/residue analysis of microcosm samples, dynamic passive dosing with PDMS, bispelic NAPL/water systems, and column systems. Different model DOM sources, of dissimilar quality but all with potential use in bioremediation were used, and included humic acids, root exudates, biosurfactants and organic fertilizers. We found that biodegradation of poorly bioavailable PAHs was enhanced by (bio)surfactants (Environ. Sci. Technol. 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (Environ. Sci. Technol. 45:1074-1081, 2011), by modulating the deposition and tactic motility of microbial degraders in porous media (Environ. Sci. Technol. 46:6790-6797, 2012), and by root exudates (Soil Biol. Biochem. 57:830-840, 2013; Environ. Sci. Technol. 49:4498-4505, 2015). However, a negative influence on biodegradation of PAHs by humic acids (Environ. Pollut. 184:435-442, 2014) and biosurfactants (Environ. Pollut. 205:378-384, 2015) was found if they prevented cell attachment to the PAH-loaded PDMS sources. These influences of DOM on bioavailability are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in retrospective and prospective risk assessment and regulation (Environ. Sci. Technol. 49:10255-10264, 2015).

**TH023**

Investigation of motor oil biodegradation by different bacterial strains

M. Ilic, ICHTM / Department of Chemistry; S. Bulatovic, M. Ljesevic, Faculty of Chemistry, University of Belgrade; T. Šošević Knudsen, Institute of Chemistry, Technology & Metallurgy, University of Belgrade / Centre of Chemistry; J. Milic, Institute of Chemistry, Technology & Metallurgy / Centre of Chemistry; A. Avdalovic, S. Miletic, G. Gojcic-Cvijovic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade / Department of Chemistry; M.M. Vrvić, Faculty of Chemistry, University of Belgrade / Faculty of Chemistry Large amount of motor oil is used in many branches of industry. The new generation of motor oils are more environmentally friendly, and the so-called low emission motor oils are based on synthetic high molecular weight compounds (e.g. C10-C36), more than 75% cycloalkane and polycyclic aromatic hydrocarbons (PAH). However, used motor oils contain a higher percentage of aliphatic and aromatic hydrocarbons, nitrogen and sulfur compounds, and metals (Mg, Ca, Zn, Pb, etc.). In addition to these compounds, the presence of PAHs (naphthalene, benzo [a] pyrene and anthracene) should be expected. Therefore these oils represent a great danger to the environment, because all these compounds are known as mutagenic and carcinogenic [1, 2]. Globally, biodegradation is a common choice for remediation, because the pollutants become substrates for the growth of microorganisms [3]. The aim of this experiment was to investigate the biodegradation of motor oils by *Rhodococcus* sp. cultivated in *Rhodococcus* sp. (UG 10) and *Bacillus* sp. (F 231) isolated from activated sludge industrial wastewater treatment plant. The process of biodegradation of motor oil was monitored for 45 days. Each 15 days the process was stopped, the hydrocarbons were extracted, and the samples were analyzed by gas chromatography. Comparison of motor oil concentration in the samples and in the above-mentioned soils provided a clear indication of the success of soil treatment. The results showed a reduction in concentration of motor oil comparing to the
beginning of the experiment (300 ppm). The growth of bacteria confirmed that the motor oil was the only source of carbon. The reduction of motor oil concentration was correlated with the number of bacterial cells. Based on these results, it was concluded that the biodegradation activity was highest in Bacillus sp. (F.231).

After 45 days this bacterial strain degraded 95.4% of motor oil. Under the same conditions two other bacterial strains showed lower biodegradation activity by 84.9% by Stenotrophomonas sp. (NR 1), and 76.6% by Rhodococcus sp. (UG 10).


**TH024**

**Bacterial community structure and biogeochemical activity in an aquifer contaminated with pesticides**

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Our objective was to assess the effect of cocktails of pesticides on groundwater microbial abundance, community structure and their nitrogen reducing activity. We used two complementary approaches: a 2-year in situ monitoring at the Ariege alluvial plain (France) and microcosms with groundwater with contrasted contamination history spiked with selected herbicides having a high occurrence in this aquifer, atrazine (ATZ), desethylated atrazine (DEA) and ATZ+DEA.

Abundance of the universal marker (16S rRNA) and of nitrate-reducing bacteria (narG) in the microcosms was measured by CE-SSCP. Pesticides in water were analyzed by LC-MS/MS. In microcosms, biodiversity was higher in historically contaminated water than in pristine-like one and remained higher under laboratory incubations. Biodiversity was significantly affected by both the chemical concentration and the incubation duration (not the chemical type or concentration), suggesting a community tolerance to the pesticides induced by chronic exposure. Biomass and denitification gene abundance was in most cases higher at 10 μg/L than at 1 μg/L or in control, especially at 30-d incubation in both water types. During the two-year in situ monitoring, tendencies between chemical and microbial criteria were similar with regard to the microcosm outcomes however they did not result in significant relationships.

More specifically, it was not observed a significant relationship between nitrate concentration and microbial biomass and abundance of denitrifying genes (narG and napA). Microbial end points based on molecular indicators should be proposed to complete the biodiversity objective under the European water directive framework with the microbial compartment.

**TH025**

**Analysis of the microbial community of the river Tiber in different contamination points along its course**


One of the major issues in freshwater quality assessment is finding a link between the chemical and ecological status of a water system. Filling this gap is a new challenge for finding new biological indicators, by integrating multiple stressors, to allow a holistic understanding of a river’s ecosystem. In this context, the main aim of the MicroCokit FP7-People-2012-IAPP Project is to identify microbial community based indicators for monitoring and evaluating different types of anthropogenic and environmental pressures. The case study is the river Tiber in which four different sampling sites along its course have been chosen and sampled in Autumn and Spring to perform a biogeochemical analysis of the bacterial community by In Situ Hybridization (FISH). Overall results of four sampling campaigns show how the changes in the microbial community structure reflect both natural environmental variations such as river course and seasonality, and the different sources of contamination.

**TH026**

**Microbial turnover of PAH: analysis of degradation and dissolution kinetics and simulation of remediation options**

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Polycyclic aromatic hydrocarbons (PAH) are hydrophobic compounds exhibiting high toxicity and carcinogenicity. Originating from tar, cooking and incomplete burning processes, PAH have contaminated many industrial areas and most urban soils. Feasible strategies for minimizing related adverse effects include the utilization of microbial degradation processes, where hardly soluble compounds like PAH pose a particular challenge. For the proper assessment of the turnover of PAH there is a research gap since only very limited kinetic data for different groups of PAH degrader bacteria are available. In particular for Mycobacteria the knowledge is limited due to the complex cell cycle with formation of cell clusters and aggregates with PAH. From experiments and in vivo modelling, growth and affinity parameters have been parameterized and compared for well described phenanthrene and pyrene degrading Mycobacteria on both substrates. The PAH were present as microcrystals in suspension. We refined a recently developed numerical model for desorption and metabolism, taking simultaneously into consideration chemical activity, sorption and dissolution processes, metabolism and growth as well as cell maintenance and decay in non-steady-state. This model was applied to prospectively describe PAH turnover dynamics for various treatment options to remediate contaminated soils. The investigated Mycobacterium species were not superior in PHE degradation to strains investigated earlier with this method. The amount of PAH ultimately degraded rather depended on the adsorption rates, and thereby on the substrate - fluid ratio, and was considered and modeled.

**TH027**

**enviPath - The environmental contaminant biotransformation pathway resource**

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The University of Minnesota Biocatalysis/Biodegradation Database and Pathway Prediction System (UMBBD/PPS) have been a unique resource covering microbial biotransformation pathways of primarily xenobiotic chemicals for over 15 years. This poster will introduce the successor system, enviPath (The Environmental Contaminant Biotransformation Pathway Resource), which is a complete redesign and reimplementation of UM-BBD/PPS. enviPath uses the database from the UM-BBD/PPS as a basis, extends the use of this database, and allows users to include their own biotransformation data and microorganisms. enviPath supports multiple use cases. As one new use case, we will present the package “DAR soil”, which contains pathway information from soil degradation studies. This information has been extracted from pesticide registration dossiers (draft assessment reports, DAR) made publically available through the European Food Safety Authority. The package also contains information on different experimental conditions, which are stored as “scenarios”. Compounds in the pathway are associated with a given scenario if they have been observed under the specific experimental conditions. If available, a biotransformation half-life (DT50) value is associated with a given compound in the pathway and a specific scenario. Much like the UM-BBD/PPS system, enviPath further provides different models to predict likely biotransformation pathways based on biotransformation rules. It supports relative reasoning for the refinement of predictions and allows its extensions in terms of previously published, but not implemented machine learning models. User access is simplified by providing a REST API that simplifies the inclusion of enviPath into existing workflows. The enviPath database is used to enable simple integration with other databases, enviPath is publicly available at https://enviopath.org with free and open access to its core data.
Biodegradation is a principal fate process of organic chemicals in the environment that affects regulatory persistence assessments and the design and operation of effective biological wastewater treatment systems to protect the aqueous environment. The fate simulation calculation at as it is influenced by many different factors. These include inherent ones, such as the chemical structure, and external ones, such as the probability of encountering a bacterium able to degrade the chemical in a given environmental compartment. The systematic investigation of these factors has received little attention. In this study, we have so far developed a set of quantitative structure biodegradation relationship (QSBRs) for diverse set of aromatic micro-pollutants, which relate biodegradation rates (estimated from EPISuite) to different molecular descriptors. Such rates are derived from several biodegradation databases, which often lack accurate quantitative information for reliable determination of the rates. To experimentally validate one of the models, we have therefore selected several representative chemicals to perform biodegradation experiments using bacteria with known degradation pathways. A complete mass balance of each chemical was conducted by measuring the chemical concentration, microbial growth and carbon dioxide during the experiment. Finally, the biodegradation rate of the selected chemicals was estimated using these data and compared against the molecular descriptors from our previously developed model to test the robustness of its predictions.

**Mercury Biogeochemistry and Policy (P)**

**TH029** Relationship between Hg species in seawater and microbial communities in the Middle Adriatic

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Chemical and biologically mediated reactions can transform mercury species in the water column. These reactions can facilitate mercury’s entrance into the marine food web in which it bioaccumulates in the form of methylmercury (MeHg). Factors that promote nutrients intake probably also facilitate MeHg accumulation in the microbial biomass. Single-celled organisms represent the major components of marine community, especially in oligotrophic areas such as the Adriatic Sea. In these conditions, small organisms are better competitors for inorganic and organic nutrients because of lower energetic costs, rapid metabolism and small size. In this study, we suggest that microbial uptake represents a critical Hg bioaccumulation step in the entire marine food web. In order to establish relationship between Hg and microbial species, we performed sampling campaign in the Middle Adriatic Sea. Samplings were conducted from March 2014 to June 2015 during oceanographic cruises aboard the Croatian research vessel Blos Dva. Our research was constrained to transect from the Bay of Kaštela to the island of Vis. We collected non-filtered water samples for determination of total mercury (THg), dissolved gaseous mercury (DGM), methylmercury (MeHg) and picoeukaryotes in Adriatic coastal and open waters. Near the island of Vis, THg concentrations are the lowest and range from 0.14-1.10 ng/L. Mercury contamination from chloralkali industrial waters in the Bay of Kaštela is seen through the highest THg concentrations (0.92-5.58 ng/L). DGM is always higher in the transected areas (31.8-351 pg/L) than in the pristine environment (22.1-245 pg/L). MeHg concentrations vary, but range from 1.07-34.3 pg/L for all stations. The highest MeHg values are usually found in the Bay of Kaštela. Number of picoeukaryotes is the highest in the Bay of Kaštela (0.44×10^7-3.18×10^7 cells/L) which is affected by effluents from the surrounding cities, while the lowest number (0.63×10^7-19.9×10^7 cells/L) is found in the pristine environment of the island of Vis.

**TH030** China’s current mercury policies and their implications for the overall success of Minamata Convention

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China is currently the biggest emitter of atmospheric mercury, accounting for 1/3 to 2/5 of global emission depending on the study methods. Asia especially China is the net exporter of mercury through atmospheric transport. The projections of atmospheric emission of mercury also shows China’s significant role in global mercury emission in 2050. What policies China currently has to reduce the impacts of mercury contamination, and whether there are major gaps in the current policies are key questions for the overall success of Minamata Convention. Here, we reviewed the current Chinese policies related to mercury under the context of Minamata Convention. Based on the information assessed compliance situation for the different convention obligations, and identified important policy gaps. China has been aware of the development of international efforts on a legal binding treaty to reduce global mercury pollution and been actively participating in all rounds of intergovernmental negotiations. Currently China has already had a set of policies to reduce mercury contamination and consumption which address most of articles of Minamata Convention. With the increasing awareness, China has a strong policy emphasis on source control, including reducing mercury emission (Article 8), mercury-added products (Article 4) and mercury catalyst used in vinyl chloride monomer industry (Article 5). However fewer efforts have been made for the trade and supply (Article 3), interim storage (Article 10) and waste handling (Article 11), the content of these articles are too a large extent addressed by the current general policy and regulations for all types of waste and chemical management. Though China has already a comprehensive set of policies and regulations which will serve as basis for the Minamata Convention, there are some important issues which are not addressed sufficiently by the existing policies, including the capacity problem for emission measurement and monitoring, phase-off of mercury containing devices, no retrospective mercury (chemical) management system and lack of information on mercury release to water and soil.

**TH031** Use of the RISK21 Matrix: Impact of Mercury Species Exposure to Fish and Seafood on Human Health Risk in Taiwan

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Methylmercury (MeHg) is a highly toxic mercury species and associated with variety of adverse health effects in humans and animals. Fishery resource is an important industry in Taiwan. Moreover, fish and seafood are the main 'dairy' sources that provide protein, nutrients and omega 3 fatty acids. However, exposure to MeHg via fish/seafood consumption is a potential human health risk factor worldwide. Therefore, it is important to be able to prioritize fish and seafood products on decisions making of fishery management for government to quantify the MeHg exposure to fish and seafood for health risk assessment. The purpose of this study was threefolds: (i) to estimate the intake of 'unMeHg' and total MeHg via fish/seafood consumption in different age groups, (ii) to 'prioritize which fish and seafoods should be considered for human health risk, and (iii) to assess the dietary treatments of fish and seafood for assessing the 'health risk of MeHg and THg. The exposure data were collected to assess different age groups intake of 'unMeHg/MeHg concentrations from published papers, research papers, and the 'national food consumption database in Taiwan. The toxicity information of unMeHg and THg was based on toxicity data of contaminants from United States Environmental Protection Agency Integrated Risk Information System on-line database. The RISK21 risk visualization matrix was used to assess estimates of exposure and toxicity together that can provide a clear view of human safety and risk. Finally, a probabilistic risk assessment framework was used to predict unMeHg intake risk on dietary treatments of fish and seafood. The preliminary results indicated that the consumption of saltwater fish has lower safety concern than that of freshwater fish and (ii) children and elderly groups should be further evaluated than adolescent and adult groups by using unMeHg estimates of toxicity based on available data. We suggest that the RISK21 'matrix serves as a useful tool to communicate with stakeholders and government. KEYWORDS: Methylmercury, Fish, Risk Assessment, RISK21 Matrix

**TH032** Dissolved organic matter controls mercury photochemistry in freshwater lakes

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Environmental mercury (MeHg) contamination through bioaccumulation and biomagnification is an issue in many remote ecosystems far from direct pollution sources. Quantifying why and how some ecosystems are more sensitive to contamination following atmospheric mercury deposition is key to mercury fate modeling. Once bacterially methylated and transported into the water column, lakes photodemethylation of MeHg is thought to be one of the main processes in which MeHg can be converted into a less biologically toxic form. While previous studies highlight the importance of photodemethylation to mercury budgets, few have examined the magnitude and variability of photodemethylation rates as a function of associated dissolved organic matter (DOM). DOM absorbs specific wavelengths of solar radiation and therefore MeHg that is bound to these compounds containing photoreactive functional groups may be more readily degraded than unbound MeHg. Alternatively, DOM may absorb much of solar radiation in the top few centimeters of the water column and inhibit photodemethylation with little effect. A one-year comparison study between summer and fall was conducted using lake water collected from 6 lakes in Kekijukak National Park, Nova Scotia, Canada. Sample lakes were chosen based on a known range of DOM concentration. Lake waters were filtered to 0.45 µm and placed in closed polytetrafluoroethylene (PTFE) bottles with >50% headspace, spiked with 3µg L^-1 of MeHg, and exposed to 1, 2, 3, 5, and 7 days of natural solar radiation in each experimental season. Solar radiation and water temperature were measured every 5 minutes for the duration of the experiment. Ultraviolet (UV)-visible absorbance scans (200-800 nm), DOM concentrations, total mercury concentrations, and MeHg concentrations were measured for each time point. Dark controls had no significant reduction in MeHg concentration (all p’s >0.1) suggesting the hypothesis that there was no significant reduction in MeHg during the experiment. The majority of MeHg degradation in each treatment was due to photodemethylation. Photodemethylation rates between lakes were compared.

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using ANCOVAs and showed that lakes with higher DOM concentrations had significantly lower rates of photodemethylation than lakes with lower DOM concentration (p < 0.001). Climate change in temperate and boreal regions of Atlantic Canada is projected to increase rainfall amounts and occurrences and thus lead to browzing of freshwaters and further inhibition to the photodemethylation pathway of MeHg reduction.

**TH033**

**Mercury concentration in PM10 collected in the Metropolitan Area of Mexico City**

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The anthropogenic emissions are leading to a general increase of mercury in the environment. Urban areas are particularly affected since they are exposed to wide diversity of sources. Chemical information obtained from monitoring programs is relevant to identify emission sources. In order to evaluate the contribution of Hg to environmental pollution of the Metropolitan Area of Mexico City, airborne particles ≤ 10 μm (PM10) were collected at four different sites in the urban. Samples collected during 2004 to 2014 were selected to be analyzed. Mercury determinations were carried out by inductively coupled mass spectrometry (ICP-MS). Average Hg concentration in the analyzed particulate matter are between 7.70 mg m⁻³. The no spatial trend is evident.

**TH034**

**Divalent metal resistance of mercury resistant bacterial isolates and their potential for mercury bioremediation**

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Three mercury resistant bacterial isolates were tested for the presence of mercury resistant genes in their chromosomal DNA, and evaluated for their toxic response to divalent metal ions specifically cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) in liquid media. The genes involved in mercury resistance namely merP, merR, merC and CYC were detected, amplified and sequenced from the bacterial isolates. The deduced amino acid sequences of mercury resistant genes demonstrated homology with genes from a diverse microbial group. Isolate *Sphingobium* SA2 and *Sphingobium* SE2 showed resistance to all tested divalent metals to varying degrees while grown in individual metal supplemented low phosphate media. Whereas isolate *Pseudoxanthomonas* SE1 did not show tolerance to the divalent metals. For isolate *Sphingobium* SA2, the 72 % EC₅₀ values of Cd, Pb, Zn and Cu were 13, 26, 62 and 3 mg/L respectively; whereas for isolate *Sphingobium* SE2 these values were 3, 27, 3 and 5 respectively. Since mercury contaminated soil and water are likely to contain other divalent heavy metals, the multi-metal resistance of mercury resistant bacterial isolates SA2 and SE2 shows great potential as bioagents for the remediation of mercury contaminated soil and water.

**TH035**

**Mercury bioavailability and toxicity to green microalgae**

R. Beauvais-Flueck, Institute Foro Earth and Environmental Sciences; Y. Slaveykova, University of Geneva / Section of Earth and Environmental Sciences Institute Foro; C. Cosio, Geneva University / Institute Foro Earth and Environmental Sciences

Bioavailability is a key concept allowing to link the changes in contaminant concentrations and speciation with the biological responses. It is also central for the understanding of Hg incorporation in the aquatic food web and possible implications for the environmental health. Nonetheless, in the specific case for Hg exposure the capabilities of the bioavailability to green microalgae, representing the base of the aquatic food chain in aquatic systems is not fully understood. The present study, therefore, explores the links between the chemical speciation, bioavailability and short-term induced effects of inorganic Hg (IHg) and methyl Hg (MeHg) to microalgae *Chlamydomonas reinhardtii* under environmentally relevant exposure concentrations. The bioavailability was characterized by intracellular Hg contents at 10⁻⁷, 10⁻⁶ and 10⁻⁵ M IHg or 5 x 10⁻¹¹, 5 x 10⁻¹⁰ and 5 x 10⁻⁹ M MeHg. It was further linked to the following biological endpoints: (i) the oxidative stress (ii) the photosynthesis efficiency and the differential gene expression. Specific pathways and effects of dietary methylmercury and inorganic mercury compounds in aquatic species. The objective of this study was therefore to assess how the compartmentation of IHg and MMHg in a representative macrophyte, *Elodea nuttallii*, affects its bioaccumulation through dietary transfer in *Gammarus fossarum* chosen as surrogate species for aquatic herbivores and decomposers. Bioaccumulation of Hg in E. nuttallii was higher for cell wall than intracellular in line with previous studies showing an important role of binding of Hg in cell wall materials. Our data suggest no significant demethylation or methylation during bioaccumulation in E. nuttallii in planta or in water. THG and MMHg concentrations increased in G. maritimus in correlation with concentration found in E. nuttallii. MMHg was more transferable than IHG, based on THG, but a significant part was demethylated during the feeding, while no methylation was observed in the IHG exposure. Cell wall results in higher concentrations than intracellular, suggesting that Hg in cell wall in form of MMHg and IHG was significantly assimilated by Gammarids. Significant uptake fluxes were estimated by the first-order model: uptake fluxes in Gammarids were higher for cell wall than intracellular, while similar uptake rates were observed for IHG and MMHg. In conclusion, G. fossarum is able to feed on E. nuttallii and accumulates Hg from intracellular and cell wall compartments. In line with literature, when looking at THG, consumption of MMHg-exposed plants leads to slightly higher accumulation of Hg than IHG-exposed plants, but we observed a significant demethylation of MMHg in Gammarids vs plants. Opposite to literature, we did not observed a significant biomagnification of MMHg. In sum, although the subcellular metal distribution is determinant for many consumers that are unable to assimilate the insoluble fraction of cell walls, the digestive strategies to handle food of the consumer is also important. Differences in primary producers and consumers species composition may significantly influence the fate of Hg in food webs.

**TH037**

**Bird colonies as secondary sources of Hg in Antarctic ecosystems: the use of vegetation and soil as indicators**

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Mercury concentrations in the Antarctic environment are of considerable ecological and human health importance due to fossil fuel burning, and the locations in Deception Island, subject to volcanic activity. Messos, in turn, range from 0.018 to 0.389 µg g⁻¹. Contrary to other heavy metals, they are more widespread and are not only subject to dry deposition, but also to contaminants in the water-phase and normally occur in less exposed experiencing oxidative stress, while no oxidative stress was detected for IHg exposure. Overall the present results demonstrated the stronger response of algae upon MeHg exposure as compared to IHg for comparable intracellular concentrations. The outcomes of this basic research contribute to the development of early warning biomarkers in support to biomonitoring efforts of mercury monitoring programs in aquatic systems.

**TH036**

**Dietary transfer of Hg from Elodea nuttallii to Gammarus fossarum**

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In the aquatic environment, the organic form of Hg, methyl-Hg (MMHg) and inorganic Hg (IHg) are both toxic, but MMHg has been shown to biomagnify through food chain while IHg does not. Nonetheless, the precise factors controlling the transfer of Hg from primary producers to heterotroph consumers remains poorly understood. Macrophytes are key organism of shallow aquatic environment in which MMHg is produced, and have been identified as having a role in Hg transfer to food webs. The objective of this study was therefore to investigate how length would impact the trend analysis, the Hg-concentrations in fish of the North East Atlantic. Time series for cod in the Inner Oslofjord and for cod in the eastern Canada, providing anoxic environmental conditions that promote the transfer in genetically different bird species with different feeding ecology strategies. Samples of lichen range from 0.077 to 0.705 µg.g⁻¹, with control samples generally less contaminated than colony ones. There were however exceptions i.e., the locations close to research stations, probably due to fossil fuel burning, and the locations in Deception Island, subject to volcanic activity. Messos, in turn, range from 0.018 to 0.389 µg g⁻¹. Contrary to other heavy metals, they are more widespread and are not only subject to dry deposition, but also to contaminants in the water-phase and normally occur in less exposed experiencing oxidative stress, while no oxidative stress was detected for IHg exposure. Overall the present results demonstrated the stronger response of algae upon MeHg exposure as compared to IHg for comparable intracellular concentrations. The outcomes of this basic research contribute to the development of early warning biomarkers in support to biomonitoring efforts of mercury monitoring programs in aquatic systems.
and often ice-covered areas (except during the summer), remaining less exposed to evaporated/resuspended/lithiviated Hg from the colonies. The concentration distribution was roughly the same as for lichens. For soils, results range from 0.005 to 0.302 μg g⁻¹, with no exceptions: control samples were always less contaminated than the colony ones. These patterns may indicate colonies as possible Hg sources. Further data treatment will allow to estimate and quantify the influence of those variables.

TH038 Influence of Avian Biovectors on Mercury Speciation in a Wetland Lagoon

Acadia University / Biology; M. Mallory, J. Rand, J. Murimboh, Acadia University; N. O’Driscoll, Acadia University / Department of Earth and Environmental Science

Mercury is a persistent and bioaccumulative chemical that is present in many remote environments due to its ability to be transported long distances in the atmosphere, and as a result, is present in almost every body of water (Smith and Chmura 2000). Wetland ecosystems are important “hot spots” for mercury in eastern Canada, providing anoxic environmental conditions that promote the bacterial methylation of mercury. Methyl mercury is the most biologically available form of mercury and the form which biomagnifies in food webs (Gochfeld 2003). Seabird guano is a well-documented biovector for metals – including mercury – and nutrients, which may indirectly affect metal speciation (Choy et al. 2010). The site for this study, Big Meadow Bog (Brier Island, Nova Scotia, Canada) has a history of ditching in the 1950s, which changed hydrology significantly, resulting in colonization by 3000 pairs of herring gulls (Larus argentatus) in the 1980s. To quantify changes in mercury mobilization and speciation in response to ditching in this regional bog, groundwater samples were collected from this site as well as a reference bog with similar geological and hydrological characteristics. The filtered samples were analyzed for total mercury, methyl mercury, and water chemistry (pH, conductivity, anions, cations, and dissolved organic and inorganic carbon). Preliminary results show significantly higher nutrient levels (nitrate and sulfate), total mercury, and methyl mercury concentration when compared to the reference bog that is minimally impacted by avian biovectors. This elevated availability of methyl mercury could potentially pose a threat to the local ecosystem and wildlife population due to methyl mercury’s toxicity to living organisms (Akearok et al. 2010, Singh et al. 2011).

TH039 Toxicity Reference Values for Methylmercury Effects on Birds: Critical Review and Analysis

P. C. Fuchslman, M. H. Henning, M. J. Bock, V. S. Magar, Ramboll Environ

Effects of mercury on birds have been studied extensively and with increasing frequency in recent years. Some highly exposed populations have exhibited adverse effects related to environmental methylmercury exposures while other species are relatively tolerant. We conducted a systematic review of methylmercury effects on bird reproduction, evaluating laboratory and field studies in which observed effects could be attributed primarily to mercury. Our review focused on exposures via diet and maternal transfer in which observed effects (or lack thereof) could be associated with mercury concentrations in diet, eggs, or adult blood. While dose-response data sets were limited to a few species, no observed effect levels, lowest observed effect levels, and/or effect thresholds (e.g., effects of approximately 20%) could be identified for more than 20 species. From this data set, we identified ranges of toxicity reference values (TRVs) suitable for risk assessment purposes. For mercury in avian blood, this represents the first broad compilation of relevant toxicity data. For dietary exposures, the current data support TRVs that are higher than older, commonly used TRVs. The older diet-based TRVs incorporated conservative assumptions and uncertainty factors that are no longer justifiable, although they were appropriate due to limited data at the time those TRVs were derived. The egg TRVs identified from this review are more similar to other previously derived TRVs but have been updated to incorporate new information from recent studies.

TH040 Mercury trend governed by fish population demography?

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Mercury (Hg) is a toxic element entering the biosphere from natural and anthropogenic sources. Mercury in Atlantic cod (Gadus morhua) is one of many things that are monitored through the Norwegian Environmental Coordinating Board for Climate and Environmental Monitoring Programme (CEMP) carried out by the Norwegian Institute for Water Research (NIVA) by contract from the Norwegian Environment Agency. CEMP is administered by the Oslo and Paris Commissions (OSPAR) in their effort to assess and remedy anthropogenic impact on the marine environment. Time series for cod in the Inner Oslofjord (Norway) goes back to 1845. Annual median Hg-concentrations in cod from the Inner Oslofjord (Norway) showed both significant upward long-term (whole series) and short-term (last 10 years) trends. However, the median length of the cod sampled also showed an upward trend. This is consistent with results of beach seine surveys (date range is 1919-2019) conducted in the Inner Oslofjord showing that cod recruitment in the area has been low since the start of the 2000s. To investigate how length would impact the trend analysis, the Hg-concentrations in the cod fillet were normalized to two arbitrary lengths: 40 cm and 60 cm. No significant trend in Hg-concentrations could be detected for either 40 cm-normalized, or 60 cm-normalized cod the last 10 years. The results indicated that the length of the sampled fish had an impact on the catching of larger fish. This in turn had an influence of other explanatory variables than length needs to be explored. It is not merely a question of why mercury is apparently increasing in cod, but also what are the reasons for the apparent change in the cod population demography. In this regard, sampling bias must also be considered.

TH041 The fate of mercury legacy in the contemporary environment; From old mines to brown trouts

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Due to their geological features mountain environments have often been exploited since the beginning of metallurgy and the French Pyrenees is no exception. Results on trace metal concentrations in the Ariège region, where extensive mining (Ag, Fe) occurred from the Antiquity to the 19th century, indicates that ≥600 tons of anthropogenic Pb is stored in organic soils on the Pyrenean northern slopes. Similar conclusions can be drawn for other mining legacy pollutants (Hg, Zn). The fate of these trace elements in relation to climate or abrupt environmental changes (e.g. forest, forestry) is poorly understood. Once released from surrounding organic soils the catchment can be highly enriched in the bioavailable fraction of these metals causing a bioaccumulation of e.g. MeHg in river biota. In combination with predicted rise in global atmospheric mercury deposition, the mercury legacy from old mines may pose a severe threat to the biota in these already sensitive high altitude areas. E.g. preliminary results show that levels of total Hg in brown trout (Salmo trutta) caught at our study sites surpasses literature values by 10 times or more. Further this, MIF and MDF Hg-isotope signatures shows clear relationship with the size of the fish and also with 87Sr/86Sr. By linking data from atmosphere – soil – peat – water – sediment – biota, and the transfer between these continuums, we are studying the fate of legacy pollutants (Pb, Ag, Bi, Hg, As and U) with a main focus of Hg in three mountain catchments (Etangs de Basses, Largentière and Bernadouze) all located in the French Pyrenees. Our study also includes biofilm, invertebrates, common minnow and brow trout representing various aspect of the biotic food chain. We aim to infer the potential risk these legacy pollutants pose on river biota (i.e. fish) and environment at large. To do so we combine traditional geochemical analysis (DMA, HR-ICP-MS) with stable isotopes (Pb and Hg, δ15N & δ28S) and analysis of radiocarbon and radionuclides (210Pb, 137Cs, 241Am and 210Be) in order to investigate the origin of these elements. We also perform microunecles analysis of the fish blood to test if any toxicological effects can already been seen. Our goal is to assess the potential harmful exposure these legacy pollutants in general and Hg in particular, may pose to the environment, to humans and to society at large.

TH042 Specific pathways and effects of dietary methylmercury and inorganic mercury in zebrasfish (Danio rerio) determined by mercury speciation, genetic, histological and metallothionein responses.

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A multidisciplinary approach is proposed here to compare toxicity mechanisms of methylmercury (MeHg) and inorganic mercury (Hg) in muscle, liver and brain from zebrafish (Danio rerio). Animals were dietary exposed to (1) 50 ng Hg. g⁻¹, 80% as MeHg; (2) diet enriched in MeHg 10000 ng Hg. g⁻¹, 95% as MeHg; (3) diet enriched in Hg 10000 ng Hg, g⁻¹, 99% as iHg, for two months. Hg species specific bioaccumulation pathways were highlighted, with a preferential bioaccumulation of MeHg in brain and iHg in liver. In the same way, differences in genetic pattern were observed for both Hg species, (an early genetic response (7 days) for both species in the three organs and a late genetic response (62 days) for iHg) and revealed a dissimilar metabolism of both Hg species. Among the 18 studied genes involved in key metabolic pathways of the cell, major genetic responses were observed in muscle. Electron microscopy revealed damage mainly in the lysosomes in muscle and also in liver tissue. In brain, high MeHg and iHg concentrations induced metallothionein production. Finally, the importance of the fish origin in ecotoxicological studies, here the 7th descendent of a zebrafish line, is discussed.

TH043 Determination of mercury and methylmercury concentrations at low levels in

SETAC Europe 26th Annual Meeting Abstract Book 317
Mechanistic toxicity of engineered nanomaterials: state of the art and future perspectives (P)

TH044
Natural organic matter influences UV light induced toxicity of nano-sized titanium dioxide
S. Lüderwald, Universität Koblenz-Landau / Institute for Environmental Sciences; V. Dackermann, University of Koblenz-Landau / Institute for Environmental Sciences; R. Rosenfeld, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment Sweden; C. Schwel, R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; E. Lahive, Centre for Ecology & Hydrology (NERC); M. Matzke, Centre for Ecology & Hydrology; D. Spurgeon, Centre for Ecology & Hydrology; R. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment Sweden.

TH045
Assessing aqueous phase silver nanomaterial (Ag NM) environmental fate; a flame based approach
C.C. Liddle, F.G. Lara, Heriot Watt University / School of Life Sciences; A. Cuthbertson, Heriot Watt University / School of Built Environment; H. Haynes, Heriot Watt University / School of Energy Geoscience Infrastructure and Society; H. Johnston, Heriot Watt University / Life Sciences; T.F. Fernandes, Heriot-Watt University / School of Life Sciences.

Flumes offer the ability to study Ag NM removal and persistence behaviour in real time under environmentally relevant scenarios. Studying removal and transformation rates within flow systems may produce fate descriptors to translate processes into useful models which will help with exposure assessment in realistic conditions. The aim of this research was to develop flume based systems and methods to investigate aqueous phase Ag NM fate and behaviour within different aquatic compartments, and subsequently to integrate the effects of UV, DOC and inorganic matter, the presence of non-cohesive sediments and re-suspensions events on aqueous phase Ag NM removal/persistence and re-dispersion. Two Ag NM were studied within flume systems (flow velocity 0.12 m/s); Ag NM (PVP) (30-50nm), and analogous reference material NM300 (15±5nm), within two re-circulating turbulent flow test systems containing 1.8 L and 55 L of soft water medium, respectively. Studies were carried out over a time course of up to 24 days. At the end of the experiment flow velocity was increased, when relevant, to entrain the bed sediment and create re-suspension events. Suwannee river humic acid (SRHA), Sigma humic acid and kaolinite clay were used as model organic and inorganic matter, and silica sand was also used as a model non cohesive sediment. Toxicological phase Ag concentration (to assess removal), primary particle size of NM, aggregation state and zeta potential, and dissolution were investigated at specific time points in soft water medium, using a range of suitable methods. Results indicated NM300 removal rates and aggregation state within the aqueous system increased within turbulent flow relative to static systems, indicating turbulent flow significantly increased removal and transport of the particles to the bottom sediment layer. The addition of kaolinite clay (20 mg/l) and humic acid (2 mg/l) did not significantly increase or stabilise Ag NM aqueous phase removal within flow conditions. However higher concentrations of humic acid (10 mg/l) resulted in longer term persistence of NM300 within the water column. The presence of non-cohesive sediments significantly increased removal rates of NM300. Ag NMs exhibited re-dispersion following re-suspension events. These results indicate that turbulent flow would be the dominating factor in modulating Ag NM removal in lotic water, when organic matter concentrations are low, and remobilisation of Ag NM needs further consideration.
respect to total quantities of Zn in the leachate. This research was funded by the Spanish projects RTA2013-00091-C02-02 and RTA2013-00091-C02-01

TH047
Ecotoxicology of Sediment-associated Carbon Nanotubes.

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Carbon nanotubes (CNTs), single-walled (SWCNTs) and multi-walled (MWCNTs), are high aspect ratio nanostructures with a combination of properties making them useful in an increasing number of products and applications. Although CNTs occur in the environment as the result of natural combustion processes, significant environmental exposure to engineered CNTs would previously not have occurred and therefore are considered xenobiotics. Investigations of CNT toxicity are complicated by factors such as the large variety and number of different CNTs, with which the lack of standardized testing procedures, but also their tendency to agglomerate in aquatic systems. Few in vivo studies have examined the behaviour of CNTs in marine systems and their bioavailability and toxicity to marine organisms. We have previously shown that SWCNTs show relatively low toxicity to marine mussels, the main concern was that SWCNTs influence the toxicity of other contaminants at otherwise benign concentrations. CNTs in the aquatic environment are expected to rapidly precipitate and become incorporated into sediments. The aim of the present study was to assess the behaviour of CNTs in sediments, their bioavailability to benthic species and their effect on the bioavailability and toxicity of other sediment-associated contaminants. Experiments showed that CNTs (500µg/L) could be recovered and confirmed using Raman spectroscopy (RS), albeit with very weak spectra; we are currently improving the technique. Ecotoxicological effects of sediment-associated SCNTs and MCNTs (dispersed in 0.02% SRNOM) were investigated by injecting them into seawater tanks (pH7.9-8; 20°C) containing light-coloured play pen sand washed playpen sand. CNTs were left to settle on the sediment surface and to which cockles (Coromus edulis) were introduced and left to bury and filter-feed the nephiloid layer for 72hrs. Uptake of CNTs was confirmed using RS and TEM imaging. A suite of biomarkers of exposure were studied, including oxidative stress (SOD, CAT, TBARS, GSH, Gpx) and associated genotoxicity. CNTs settled down very quickly after one hour and no changes in their behaviour were observed after 24 hr. Early genotoxicity data suggest that sediment-associated CNTs caused an increase in DNA strand breaks in C. edule at concentrations >100µg/L. Work is currently ongoing to extend the exposure concentration range down to more environmentally relevant levels and to test the causative oxidative stress hypothesis.

TH048
Investigating the effects of nanomaterials from waste water treatment plants; removal, release and subsequent impacts


The majority of nanomaterials (NMs) used for commercial applications will enter the waste water treatment plant. In many countries the waste water effluent and sewage sludge are discharged in aquatic environments or applied in agricultural land, however, the transformation of the particles and the potential hazard they pose to the environment is poorly understood. The main aim of this study is to understand the transformation of NMs during the waste water treatment processes and to evaluate the potential environmental hazard of the transformed ‘aged’ particles compared to the pristine ones. Initial investigations focus on the study of silver and titanium particles incorporating the use of isotopically labelled particles as well as unlabelled, to better understand the behaviour and fate of these particles in complex media and biological systems. An integrative approach is taken using organisms of multiple trophic levels representing the aquatic and terrestrial environment. A biodynamic study is envisioned for the assessment of uptake, elimination, bioaccumulation and intracellular localization of the NMs by synchrotron methods as well as after their transformation during the waste water treatment processes. Moreover, in vitro cell culture models of high relevance for nanoparticle toxicity assessment representing the main sites of nanoparticle uptake and excretion such as the gills and liver have been developed and incorporated in order to identify possible modes of action.

TH049
Impact of silver and silver nanoparticles on the structure and functionality of microbial communities in sewage treatment plants

M. Matzke, Centre for Ecology & Hydrology (NERC) / Molecular Ecotoxicology; T. W. Tye, NERC Centre for Ecology Hydrology, S. Faetsch, S. Stolle, Sustainable Chemicals and Nanomaterials, H. Gweon, D. Read, A. Lawlor, NERC Centre for Ecology Hydrology; D. Hernandez-Gonzalo, M. Matzke, Centre for Ecology & Hydrology / Shore Section; D. Spurgeon, Centre for Ecology & Hydrology; R. Verweij, Department of Ecological Science VU University Amsterdam The Netherlands; C. Svendsen, CEH, Wallingford / Pollutant and Ecotoxicology Silver nanoparticles (AgNPs) are amongst the most commonly used nanoparticles in consumer products. Therefore they will inevitably end up in the environment mostly through the entry pathways of sewage sludge to soils (application to fields as a fertilizer) or effluent to freshwater systems. However, due to the antimicrobial properties of silver, the microbial communities in the sewage treatment plants might be affected resulting in a decrease of functional efficiency of the treatment processes. Standard tests to evaluate potential effects on microbial (sludge) communities (e.g. OECD 209) only considers short term effects with average contact times of only 30 minutes. Silver is not bound to the substrate therefore there is no decrease of total silver concentration in the environment resulting in potential long-term effects on microbial communities. Aim of this study was to compare the effects of two concentrations (1 mg/L & 50 mg/L) of ionic silver (AgNO3) and AgNPs on the microbial community structure over a time period of 2 weeks. The selected silver nanoparticles were commercially available (Amepox, Poland), uncoated and paraffin stabilized with a primary particle size of 3-8 nm. The aqueous AgNP stock suspension (1000 mg/L) was characterized with DLS, NTA, TEM (size distribution and homogeneity) and AAS (for dissolved and total silver concentration). The diversity of the bacterial community and potential structural changes were analyzed with 16S rDNA sequencing (MiSeq) and the AgNPs (MiSeq). Activated sludge was incubated for 2 weeks (aerated; fed every 3 days with meat extract) and samples for sequencing were taken at 4 time points (start (0), 2, 7 and 14 days) to track structural community changes. Silver concentrations were monitored in parallel for dissolved (post ultracentrifugation) and total silver concentrations by atomic adsorption spectroscopy (AAS). Analytical results showed in general a low total and dissolved silver concentration in the water phase staying relatively constant over the testing period suggesting that most of the silver was rapidly bound to the organic matrix at the beginning of the test. Microbial communities showed structural differences between the ionic and the silver nanoparticle treatments, but only after 7 days. The effects were most distinct for AgNO3 (after 7 days). For AgNPs in the treatments of 1 mg/L AgNO3/1 & 50 mg/L AgNPs significant effects were visible only after 14 days supporting the importance to analyse long-term effects.

TH050
The influence of soil properties on long-term effects of silver and silver nanoparticles on soil microbial communities

E. Lahive, Centre for Ecology & Hydrology (NERC); M. Matzke, Centre for Ecology & Hydrology (NERC) / Molecular Ecotoxicology; L. Newbold, A. Oliver, W. Tye, H. Gweon, D. Read, A. Lawlor, NERC Centre for Ecology Hydrology; S. Lofts, Centre for Ecology & Hydrology / Shore Section; D. Spurgeon, Centre for Ecology & Hydrology; C. Svendsen, CEH, Wallingford / Pollutant and Ecotoxicology Metal and metal oxide nanoparticles (NPs) are currently the nano materials with the highest total production volume. Silver nanoparticles (AgNPs) have gained considerable attention due to their broad microbialic properties, which implies a specific hazard for exposed soil bacteria. Both chemical transformations of the NPs (e.g. dissolution) and the chemistry of the soil may modify the organism exposure and response in NP-dosed soils. Previous work has shown that soil type influences toxicity of ZnO NPs to microbial communities with soil pH playing a major role. In this study we investigated how soil properties influence the bioavailability and toxicity of silver (Ag) NPs and toxic silver to natural soil microbial communities. Soils were dosed with either 50nm diameter PVP-coated Ag NP or anionic silver reference, silver nitrate (AgNO3). Four soils were used for this study with a soil organic matter (SOM) content ranging from 1.8–16.7% and soil pore water pH from 4.5–8.3. Microbial communities were exposed to soil concentrations ranging from 9 – 5500 mg Ag/kg. The diversity of the bacterial communities and potential structural changes were analyzed with 16S rRNA sequencing (dual index sequencing/MiSeq) at 4 time points; at test start, after 4 weeks, 8 weeks and 52 weeks. Soil pore waters were extracted from all soils at the beginning (t=0days) and after 8 weeks of the experiment, ultra-filtered (10kDa) and analysed for silver, pH and dissolved organic carbon. Results clearly showed an influence of silver and silver nanoparticles on soil microbial communities and clear differences in the community structure between the ionic and the AgNPs treated soils. At the beginning of the test effects of AgNO3 were more distinct than for AgNPs, but with increasing exposure time these differences became less distinct, suggesting that the corresponding increasing impact of the released ions on the microbial community structure. Soil pH was identified as one of the key properties influencing bioavailability and toxicity of silver and AgNPs on the microbial communities. Our results also show that long-term effects (e.g. slow NP dissolution in soils over months) need to be considered for a realistic environmental hazard assessment of AgNPs.

TH051
Coating relevance in the nanoparticle toxic effect to Daphnia magna.


Coating of nanoparticles (NPs) with a proper surface agent is usually required before their use in any application, to improve its functionality. This coating will determine the NP interaction with the environment as the physicochemical properties of the NPs can change due to this surface layer, leading to different effects in organisms. A major regulatory problem will be the high amount of assays that...
should be conducted for all coating-NP combinations. In the framework of FP7 Project GUIDEnano we investigated the toxicity produced by different coatings on three metal NPs (synthesized from PlasmaChem GmbH, Germany) to try to establish relationships between coatings and toxicity. The acute effect of NPs, in terms of core composition (TiO2, CeO2, and Ag), was investigated on neonates of Daphnia magna (greenwater) following 24h exposure. The two first NPs were functionalized with three different coatings: citrate (TiO2-CT/CeO2-CT), polyethylene glycol (TiO2-PEG/CeO2-Pc), and dodecylphosphonic acid (TiO2-DDPA/CeO2-DDPA); moreover those NPs were tested also uncoated (TiO2-UNC/CeO2-UNC). AgNPs were coated with CIT, PEG and DDPA, whereas CuO-NPs and CeO2-NPs were not functionalized. The toxicity of the metal NPs, whereas CIT and PEG make NPs more hydrophilic. The hydrophilic immobilizing effect was found after exposure to AgNPs, showing the lowest IC50. In those cases, assuming that Ag-CIT can behave as the uncoated NP, the toxic effect of the AgNPs increased by their functionalization with PEG and HPB. On the other hand, D. magna exposed to TiO2-UNC and CeO2-UNC presented higher toxicity than the same core coated with CIT, PEG or DDPA where no toxic effect was observed. Thus the coating conducted to a protective effect over the metalic core. The size of the NPs in the exposure medium could not explain this different behavior between metal-oxide and metal NPs. It is more likely that the coating interaction with the core lead to a different behavior of the NPs. From this study a specific pattern linked to the coating could be established depending on the core composition. The coatings of the metal-oxide nanoparticles eliminated completely the toxicity produced by the uncoated NPs. On the contrary, toxicity of the metal NPs was increased depending on the coating surrounding the AgNP.

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**TH052**
The effects of a panel of nanomaterials on the freshwater microalga Pseudokirchneriella subcapitata

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The increased use of nanomaterials in a wide range of products is likely to lead to larger releases of nanomaterials into the environment. The aim of this study is to assess the effects of a panel of nanomaterials on the freshwater microalga Pseudokirchneriella subcapitata in order to evaluate the aquatic toxicity of nanomaterials. Recent studies have compared the toxicity of nanomaterials of varied physico-chemical properties to algae and investigated the impact of the dispersion protocol on nanomaterial toxicity. Results indicate that the physico-chemical properties of nanomaterials (e.g. composition, size, solubility) can influence their toxicity and that exposure and effects depend on media and the approach used to suspend the nanomaterials. In this study P. subcapitata was exposed to widely used nanomaterials (silver, single wall carbon nanotubes, multi wall carbon nanotubes and nanocelluloses) at different concentrations (20 - 200 µg/L), for silver nanomaterials and 0.1 to 100 mg/L for single wall carbon nanotubes, multi wall carbon nanotubes and nanocelluloses), established following pilot studies. A silver salt (AgNO3) control (3 to 17 µg/L) was also tested for comparison purposes with silver nanomaterials. Nanomaterials were dispersed using bath or probe sonication, following well established and published protocols. The growth of P. subcapitata was measured following OECD test guideline 201, at different time points (0, 24h, 48h, and 72h), via measurement of optical density (OD) and fluorescence, as well as chlorophyll a, after extraction. Median effective concentrations (EC50) at the different time points revealed that silver nanomaterials were more toxic in terms of growth inhibition than carbon-based NMS, although silver salt was found to be more toxic than the silver nanomaterials. The dispersion method used was found to be a key factor in determining toxicity, with the probe sonicator protocol resulting in higher toxicity. Results from this work indicate the importance in establishing clear standard methods when assessing the hazard of nanomaterials to the aquatic environment.

**Key words:** nanomaterials, toxicity, sonication and P. subcapitata.

**TH053**
Comparative effect of ZnO-NPs, ZnO bulk and ZnSO4 in the antioxidative defense system of two plant species growing in two agricultural soils in a greenhouse experiment

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Rapid growth in the field of nanotechnology is continually increasing the potential release of nanomaterials (NPs) into the environment. Although copper oxide nanoparticles (CuONPs) are widely used within numerous industrial and commercial applications (e.g. batteries, electronic circuits, superconductors, solar energy conversion and gas sensors), their toxicity is still poorly understood in comparison with other metal oxide NPs. As the main recipients of industrial and domestic wastewaters, aquatic systems will inevitably become a sink for CuONPs throughout their life cycle. Filter-feeders, such as the blue mussel, (Mytilus edulis)are widely used in environmental monitoring and are organisms of great interest in regards to the fate and toxicity of NPs within the aquatic environment. This study, funded by the FP7 project, NANOSENSIONS, aims to investigate the toxicity of CuONPs to M. edulis and the effect of surface coatings upon CuO NP toxicity. M. edulis were exposed to core CuO NPs and CuO NPs coated with polyethylene glycol, nitrate and carboxylic acid, for 48 hours at concentrations of 10 and 20 µg/ml. Following exposure, the activity of the antioxidant enzyme superoxide dismutase (SOD) was assessed in M. edulis gill and digestive gland tissues to determine whether reactive oxygen species (ROS) were formed as a result of CuO NP exposure, whilst the TBARS (Thiobarbituric Acid Reactive Substances) assay was used to determine whether lipid peroxidation (a product of oxidative stress) had occurred. Additionally, ASCG (alkaline single cell gel electrophoresis) comet assays were performed on gill and haemolymph cells to assess potential DNA damage caused by CuO NP exposure. It was hypothesised that an increase in CuO NP concentration would lead to an increase in M. edulis SOD and CAT activities, thus protecting DNA damage, whilst coatings would influence CuO NP toxicity. Work is currently underway to test these hypotheses.

**TH054**
The toxicity of differently coated copper oxide nanoparticles towards the marine blue mussel, Mytilus edulis

S. Cambier, T. Serchi, Luxembourg Institute of Science and Technology / Environment and Energy Conversion and Gas Sensors; F. Mouchet, ECOLAB UMR5245 CNRS UPS INPT

Copper oxide nanoparticles (CuONPs) have been widely applied in a number of energy conversion and gas sensors), their toxicity is still poorly understood in comparison with other metal oxide NPs. As the main recipients of industrial and domestic wastewaters, aquatic systems will inevitably become a sink for CuONPs throughout their life cycle. Filter-feeders, such as the blue mussel, (Mytilus edulis)are widely used in environmental monitoring and are organisms of great interest in regards to the fate and toxicity of NPs within the aquatic environment. This study, funded by the FP7 project, NANOSENSIONS, aims to investigate the toxicity of CuONPs to M. edulis and the effect of surface coatings upon CuO NP toxicity. M. edulis were exposed to core CuO NPs and CuO NPs coated with polyethylene glycol, nitrate and carboxylic acid, for 48 hours at concentrations of 10 and 20 µg/ml. Following exposure, the activity of the antioxidant enzyme superoxide dismutase (SOD) was assessed in M. edulis gill and digestive gland tissues to determine whether reactive oxygen species (ROS) were formed as a result of CuO NP exposure, whilst the TBARS (Thiobarbituric Acid Reactive Substances) assay was used to determine whether lipid peroxidation (a product of oxidative stress) had occurred. Additionally, ASCG (alkaline single cell gel electrophoresis) comet assays were performed on gill and haemolymph cells to assess potential DNA damage caused by CuO NP exposure. It was hypothesised that an increase in CuO NP concentration would lead to an increase in M. edulis SOD and CAT activities, thus protecting DNA damage, whilst coatings would influence CuO NP toxicity. Work is currently underway to test these hypotheses.

**TH055**
Influence of size and surface coating on silver nanoparticles uptake by Gammarus fossarum

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The increasing production and use of silver nanoparticles (AgNPs) will inevitably lead to their release in aquatic environments when they present a threat to aquatic species. Therefore, there is still a need to better understand the mechanisms underlying the potential toxicity of AgNPs. Being a very important component of the macroinvertebrate assemblage, Gammarus fossarum will certainly be exposed to AgNPs if they reach the water streams. Therefore, G. fossarum was selected as a model organism in order to assess AgNPs effects. This study evaluated the acute effects (72h) of three different sizes of AgNPs (20, 40 and 80 nm), either stabilized with citrate (CIT-AgNPs) or coated with polyethylene glycol (PEG-AgNPs), on adult male G. fossarum. Test AgNPs concentrations ranged from 0.5 to 50 µg/L. AgNO3 was used to compare AgNPs effects with silver ion effects. AgNO3 was the most toxic compound for G. fossarum with an LC50-72h of 0.69 µg/L, while none of the studied AgNPs led to lethal effects. Size distribution of CIT-AgNPs and PEG-AgNPs in exposure
media was characterized using nanoparticle tracking analysis. CIT-AgNPs 40 nm and CIT-AgNPs 80 nm were stable whereas CIT-AgNPs 20 nm aggregated to NPs of 53 nm. In contrast, all PEG-AgNPs were stable. Uptake of silver by G. fossarum, assessed by ICP-MS, revealed a surface-coating dependent effect with CIT-AgNPs being taken up to a greater extent than PEG-AgNPs. Additionally, a size-dependent uptake was observed with CIT-AgNPs 40 nm being more taken up than CIT-AgNPs 20 nm and CIT-AgNPs 80 nm. The same tendency, linking the size to the uptake, was observed for PEG-AgNPs with higher uptake of PEG-AgNPs 20 nm than PEG-AgNPs 40 nm and PEG-AgNPs 80 nm. These results show effects at low and environmentally realistic concentrations of AgNPs for G. fossarum and confirm the hypothesis that silver uptake is dependent on size and surface coating of AgNPs.

TH056
The disposition of cationic amino polysytrene nanoparticles in sea urchin immune system cells and their effect on multiexobiotic resistance phenotype L. Marques dos Santos, Universidade Federal da Paraíba / Departamento de Biologia Molecular; E. Bergami, University of Siena / Department of Physical Earth and Environmental Sciences; K.A. Dawson, University College Dublin / Centre for BioNano Interaction School of Chemistry and Chemical Biology; I. Corsi, University of Siena / Department of Physical Earth and Environmental Sciences
The increasing discard of micro and nanopolymer in the oceans can seriously impact marine ecosystems. Our previous results showed that cationic amino polysytrene nanoparticles (PS-NH₂) induce both embryotoxicity in the sea urchin Paracentrotus lividus and immunomodulation in mussel hemocytes. The sea urchin ion immune system cells (coelomocytes) have been emerged as a promising system for ecotoxocology studies. However, there is no data regarding the effect of polysytrene nanoparticles on these cells. So, the aim of the present study was to investigate the disposition of PS-NH₂ in the sea urchin P. lividus coelomocytes and their effect on multiexobiotic resistance phenotype (MXR). The behavior of PS-NH₂ in coelomic fluid medium was assessed by DLS analysis. Coelomocyte fluid was withdrawn with a 1 mL syringe pre-loaded with anticoagulant solution (500 μL ISO-EDTA). Cells were then centrifuged, resuspended in coelomic fluid, and cell concentration adjusted to 1 x 10⁶ cells/mL. Coelomocytes were exposed to PS-NH₂ (1 to 25 μg/mL) and nanoparticle disposition analyzed under fluorescence microscopy. MXR phenotype was investigated by the calcine intracellular accumulation assay. PS-NH₂ was shown to accumulate just in phagocytes and in a time dependent manner. Vibratile cells, red and colorless sphere cells did not exhibit nanoparticle accumulation or plasma membrane adsporation. No cytototoxicity was observed to PS-NH₂ up to 10 μg/mL and until 24 h of cell exposure. However, the PS-NH₂ highest concentration induced cell death (loss of membrane integrity). Moreover, PS-NH₂ exposure - up to 24 h - did not increase calcine intracellular accumulation in coelomocytes. Our data suggest that PS-NH₂ uptake is an active and selective process since only phagocytes internalized the nanoparticles. However, no cytotoxic effect was observed when coelomocytes were exposed to environmentally relevant concentrations. Furthermore, PS-NH₂ exposure, even in the highest concentration, did not affect MXR phenotype. The preliminary results show that exposure to PS-NH₂ up to 10 μg/mL do not impair P. lividus immune system cells viability.

TH057
Fate and toxicokinetics of differently coated silver nanoparticles in the deposit-feeding polychaete Capitella teleta M.B. Nielsen, Department of Environmental Social and Spatial Change; L. Skjolding, DTU / DTU Environment; A. Baun, DTU Environment / Department of Environmental Engineering; A. Palmqvist, Roskilde University / Department of Environmental Science and Spatial Change
Aquatic sediments are believed to constitute an important sink to silver nanoparticles (AgNPs). Knowledge on the effects of AgNPs to sediment-dwelling organisms together with their behavior and fate in these environments are therefore necessary. In this study, the effects and toxicokinetics of differently coated AgNPs (copolymers, poly styrene and mercaptoundecanoic acid-coated (MUDA)) added to sediment in nominal concentrations of 100 μgAg/g dry weight were tested on the sediment-dwelling polychaete Capitella teleta. After 15 days of exposure, survival and growth of C. teleta was not affected by any of the AgNPs and uptake of the AgNPs was generally low with average weight specific body burdens (WSBB) of 0.140 μgAg/g dry weight worm tissue. After 15 days of depuration in clean sediment, WSBBs had been reduced with more than 50% in all treatments. There was a tendency that MUDA-coated AgNPs were most available for uptake and un-coated AgNPs were least. The fate of the AgNPs in the test system was assessed by measurement of Ag in the different experimental compartments after 7 and 15 days. The results showed that Ag was mainly recovered in sediment and fecal pellets. Silver concentrations were 2.6 to 3.7 times higher in fecal pellets than in sediment. Fecal pellet Ag concentration was lowest for MUDA-coated AgNPs and highest for un-coated AgNPs. The results suggest that fate and uptake of AgNPs are influenced by particle coating and that C. teleta influences the fate of sediment-associated AgNPs.

TH058
Subcellular localization of size-dependent copper nanoparticle in grass carp (Ctenopharyngodon idella) W. Chen, Kaohsiung Medical University / Dept Biomedical Science and Environmental Biology; Y. Tsai, Kaohsiung Medical University / Department of Biomedical Science and Environmental Biology
A smaller size of nanoparticles (NPs) may show unique nanoscale properties. To date, the size-dependent waterborne copper nanoparticle (CuNP) toxicity and subcellular partitioning information in the edible freshwater fish are poorly studied. To gain insight into how assumptions regarding size-dependent metal NPs influence their ecotoxicological activities, this study assessed the mode of action in subcellular detoxification of grass carp (Ctenopharyngodon idella) exposed to size-dependent CuNPs. This study carried out the CuNP subcellular distribution experiments with grass carp from natural populations varying with concentrations and three nanoscales of 25, 40, 60, and 60-80 nm. Results showed that smaller size CuNPs had higher accumulation and acute toxicity in comparison with the metabolically detoxified pool (MDP) containing the relatively higher percentages of CuNPs over 37.09 – 72.53%, 37.67 – 66.57%, and 44.21 – 62.26% than those of metabolically active pool (MAP) in 25, 40, 60, and 60-80 nm treatment, respectively. We found that metal-rich granules contributed most CuNPs accumulation after CuNPs exposure, indicating that it is a key role on CuNPs detoxification. To our knowledge, it is the first study on size-dependent CuNP subcellular partitioning in freshwater fish. We anticipate that this finding of this study could be used to identify a suitable enzyme or protein that has a strong protection on CuNPs susceptibility and to provide the most potentially promising biomarker to detect CuNPs in the aquatic environments.

TH059
In vitro testing for identification of long term effects of copper oxide nanoparticles in fish cell lines L. GALIDR, INIA - National Institute for Agricultural and Food Research and Technology / Department of Environment; M. Fernandez-Cruz, J. Navas, INIA - National Institute for Agricultural and Food Research and Technology / Environment
Copper oxide nanoparticles (CuONPs) have been widely applied in a number of areas and their antimicrobial activity has opened the door to their use as biocides. As a consequence, there is a high likelihood of release of these NPs into water streams, and further into aquatic systems. Although CuONPs have been described as toxic for aquatic organisms in a number of studies, these experiments were normally carried out for short periods of time, observing effects on one single generation. However, there is a general lack of knowledge about long-term toxic effects of NPs, including CuONPs. In this sense, in vitro systems can be a powerful tool to initially determine the mechanisms underlying toxic effects of NPs after prolonged exposure. The objective of this work was to explore the cytotoxic effects of CuONPs on fish cell lines after long-term, low-dose exposures. Two fish cell lines were used in this work: a cell line derived from rainbow trout liver biliary ducts (RTL-W1) and CLC cells, derived from carp macrophages. Cytotoxicity was assessed by means of the alamarBlue, neutral red and CFDA-AM assays. First, cells were exposed to a range of CuONPs (prepared and supplied by PlasmaChem GmbH, Berlin) concentrations for 24 h in order to determine the most appropriate concentrations for longer exposure experiments. Then, concentrations using exposure to the EC10 were used in the longer term experiments: 100 and 50 μg/mL respectively for CLC cells, and 100 and 25 μg/mL respectively for RTL-W1 cells. Exposure lasted for 21 days and cells were split every 7 days, receiving fresh medium with appropriate concentrations of CuONPs. CLC cells exhibited a very high susceptibility towards CuONPs reaching 100% mortality after 7 days exposure to both concentrations. RTL-W1 viability was not affected after exposure to 25 μg/mL CuONPs for 21 days, but 100 μg/mL caused approximately 90% mortality. TEM analysis of exposed cells evidenced a general disruption of cellular organelles and membranes. The in vitro protocol used appears to be useful to study the cytotoxic effects of continuous low-dose exposure to different nanoparticles in a more realistic scenario for prediction of their toxicity in higher tier tests, supporting the 3Rs initiative in the development of alternative ecotoxicological tests. Acknowledgements - This research was supported by FP7-SUN project (Grant Agreement No. 604305).

TH160
Ecotoxicity of double-walled carbon nanotubes on Chironomus and Xenopus larvae: nonspecific and mesocosm tests L. Lagier, S. Cadarsi, ECOLAB UMR5245 CNRS UPS INPT; A. Mottier, ECOLEG UMR5245 CNRS; A. Bor, ECOLEG UMR5245 CNRS UPS INPT; L. Vermeul, ECOLEG UMR5245 CNRS UPS INPT / ECOLEG UMR CNRS UPS INPT
J. Silvestre, ECOLEG UMR5245 CNRS UPS INPT; E. Fialhaut, C. SARRIEU, CIRIMAT UMR CNRS S058 UPS INPT; E. Pinelli, L. GAUTHIER, F. Mouchet, ECOLEG UMR5245 CNRS UPS INPT
Carbon-based nanoparticles (CNPs) can be found in many manufactured products and are thus likely to be released in the environment. Among them, double-walled carbon nanotubes (DWCNT) and multicharacterized CNPs may be considered as a control nanomaterial to investigate the effects of CNPs on organisms, particularly
in the aquatic environment, which is a major receptacle of pollutants.) The goal of the present work is (i) to improve the understanding of toxicity mechanisms of a range of concentrations (from 0.1 to 50 mg L\(^{-1}\)) of DWCNTs on benthic and pelagic organisms by a monospecific approach and (ii) to assess the ecotoxicological risks by using more realistic conditions in a mesocosm, with an interaction between species. DWCNTs are known to interact with constituents of the environment such as natural organic matter (NOM). For this reason, all our tests were performed in the presence of added commercial Suwannee River NOM. Two standardized single-species tests were carried out, respectively, for two aquatic models: *Chironomus riparius* and *Xenopus laevis*. The effect of DWCNTs on a mesospecific fish species, *Coregonus atratus*, was tested as a second step, these organisms have been grouped within a food chain and exposed to 1 mg L\(^{-1}\) of exposure water. Studied endpoints for chironomids were acute (mortality), chronic toxicity (growth and development delay) and teratogenicity. Furthermore, mortality, growth inhibition and genotoxicity (induction of micronucleated erythrocytes) were noted with hydrophobic groups inside a dense matrix did not cause any lethal effects. The results didn't show mortality for the aquatic model even though significant growth inhibition and genotoxicity (induction of micronucleated erythrocytes) were noted at 10 mg L\(^{-1}\). Macro-observations indicated that the chronic toxicity could be limited to physical effects (intestinal obstruction and/or abrasive effects and/or inhibition and genotoxicity) and were noted. However, the frequency of development delay was lower than the negative control from 1 mg L\(^{-1}\). The survival rate of chironomids was significantly impacted from 1 mg L\(^{-1}\). No significant teratogenicity neither growth inhibition was observed. However, key words: double-walled carbon nanotubes, ecotoxicity, *Xenopus laevis*, *Chironomus riparius*

**TH061**

*In vitro cytotoxicity of graphene oxide nanosheets on earthworm coelomocytes*

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By increasing concerns of carbon based nanomaterials that release to terrestrial ecosystem, toxicity of carbon based nanomaterials on earthworms have been steadily investigated. However, very limited researches on toxicity of graphene oxide nanosheets on earthworm species were reported. This study investigated in *in vitro* cytotoxicity of graphene oxide nanosheets on earthworm coelomocytes. Coelomocytes of *Eisenia andrei* were exposed to three different sized graphene oxide nanosheets via *in vitro* and then intracellular esterase activity, oxidative stress, cell size and cell granularity changes were measured by flow cytometry. The results showed that intracellular esterase activities were inhibited and reactive oxygen species were generated. Also cell size was decreased and cell granularity was slightly increased. It indicates that graphene oxide nanosheets caused in *vitro* cytotoxicity on earthworm coelomocytes. Acknowledgements. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2061386), and the Ministry of Science, ICT and Future Planning (2014R1A2A1A1050513). This research has also been performed as a cooperation project for 2015 Environmental Risk Assessment of Manufactured Nanomaterials funded by the Korea Institute of Toxicology (KIT, Korea).

**TH062**

*Rational design of HMPAAs towards environmental safety*

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The production of manufactured nanomaterials (MNMs) has been growing since the 1990s to fulfill pharmaceuticals, cosmetics, textiles, informatics component, and many others industry and society needs. The present work intended to evaluate the influence of changes in the structural conformation of hydrophobically modified polyacrylic acid (HMPAA) MNMs in their toxicity to freshwater biota in order to identify the MNM variation that is most environmental friendly. Six variations of HMPAA were designed by modifying their concentration level and the insertion position and length of hydrophobic groups. Their ecotoxicity was characterised using the cladoceran *Daphnia magna* as a model organism. Standardised toxicity assays were conducted in order to assess median effect concentrations for: i) survival, ii) feeding behaviour, iii) juvenile growth rate and iv) intrinsic growth rate. Tested concentrations of each HMPAA variation ranged from 0.001 to 2000 mg L\(^{-1}\) according to the concentration communities available. Results showed that, at lethal levels, the variation HMPAA2 (with small and large hydrophobic groups positioned alternately along a linear structure) was the most toxic (LC50, 48h = 203.6 mg L\(^{-1}\)); while HMPAA5 (sphere structure with hydrophobic groups inside a dense matrix) did not cause any lethal effects after 48h, at 2000 mg L\(^{-1}\) levels. HMPAA2 and one of the least toxic variations, HMPAA4 (sphere structure hydrophobic groups disposed at the surface) was the most toxic variation for all the sub-lethal endpoints evaluated: feeding (EC20, 48h = 111.2 mg L\(^{-1}\)), somatic growth (EC20, 52g = 92.4 mg L\(^{-1}\)) and intrinsic growth rate (fewest number of neonates per brood, in the first brood and, therefore, lowest total number of neonates and population growth → ). HMPAA 2 and HMPAA 6 (sphere structure with hydrophobic groups inside a tight matrix) reveal to be the less toxic when analyzing the effects in feeding (EC20, 48h = 249.2 and 281.2 mg L\(^{-1}\), respectively), though, being more toxic to daphnids growth (EC20, 72 h = 276.8 and 491.2 mg L\(^{-1}\), respectively ). The obtained results suggest that different HMPAA structures influences differently the toxicity of these MNMs when present at lethal or sub-lethal levels, which challenges the identification of the variation that is more environmentally safe.

**TH063**

*Report on TiO2 nanoparticles ecotoxicity studies in the framework of REACH regulation*

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TiO\(_2\) is one of chemical substances registered under REACH regulation. In the specific framework of substance evaluation, a procedure under REACH regulation, we aim to assess risk and safety of producing and using TiO\(_2\). In this work, an inventory of all studies published between 2010 and 2015 related to TiO\(_2\) ecotoxicity studies was carried out. We chose to focus on the 3 main types of aquatic organisms used in regulation: algae, daphnia and fish. They are used in OECD Test Guideline (TG) under REACH and CLP regulation. Thus, 105 studies have been selected in this work: 15 studies with algae, 60 with invertebrates and 30 with fishes. Several parameters have been chosen to select only reliable studies: well-characterized nanoparticles, condition of exposure according to OECD recommendation with the use of replicates, localization/bioaccumulation of nanoparticles. Among the contrary, some were excluded, most of all prepared in a concentration lower than 50 (LC50), Effective Concentration 50 (EC50) or No Observed Effect Concentration (NOEC) are higher than 1 mg L\(^{-1}\). Nevertheless, eight studies on daphnia revealed LC50 or EC50 below 1 mg L\(^{-1}\) of TiO\(_2\); anatase, or P25 (70:30; anatase: rutile). Daphnia appears as the most sensitive organism. The parameters involved in the toxic response are: size and crystalline phase of nanoparticles, complexity of medium of exposure, use of UV or light during exposure, increased exposure time compared to the OECD TG. This work highlights the lack of data on characterization of TiO\(_2\) nanoparticles in published ecotoxicity studies and the fact that a fitting of the OECD TG is needed for the nanoparticles testing case.

**TH064**

*Investigation of the potential uptake of magnetofluorescent nanoparticles into human cells*

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The creation of magnetofluorescent nanomaterials (MNPs) for the selective labelling of cancer cells represents a field of interest of computational nanotoxicology. The library including 109 MNPs generated by Weissleder and colleagues in 2005, and the related uptake into human cells (i.e. endothelial cells, activated human macrophages and pancreatic cancer cells), was object of several computational studies aiming to predict the uptake behaviour of these NPs from the structure of the organic compounds used as coating. However, due to the structure of the experimental data, all these studies were mainly focussed on the prediction of the uptake into pancreatic adenocarcinoma cells, and almost no effort was provided to try to exploit the experimental information by using, for instance, multivariate analysis, nor to provide additional insight through the identification and analysis of outlier compounds. In this study multivariate analysis (e.g. Principal Component Analysis) was applied to investigate the macro properties of the experimental dataset originally generated by Weissleder and colleagues in order to study the uptake behaviour of the 109 MNPs into different cell lines. Additionally, models based on Quantitative Structure Activity Relationships (QSAR) were developed to predict the uptake behaviour of these NPs into the human pancreatic adenocarcinoma cells and human umbilical vein cells. These models were built on the basis of traditional structural descriptors calculated from the structures of the organic molecules used as coating. These models represent the observed structural variation across the 109 MNPs, which all share the same iron nano-core. QSAR models were generated using several linear and non-linear techniques. The statistical performances of these QSARs were investigated, compared, and used to identify common outliers. The models were validated internally and externally on multiple validation and prediction sets. This was performed in order to investigate the internal robustness of the models, the sensitivity of different methods to perturbations of the datasets, as well as to evaluate the predictive ability of the models on MNPs not included in the models development. This study shows how multiple statistical approaches can be applied to investigate complex datasets, and how different QSAR models can be combined to improve the results of models taken singularly.

*Polar ecotoxicology: hot issues in cold climates! (P)*
TH065 Distribution and origin of selected PCB and PAHs in the Arctic fjords sediments

A. Pouk, Institute of Oceanology / Department of Marine Chemistry and Biochemistry; A. Zaboronska, Institute of Oceanology Polish Academy of Sciences; K. Paudro, Institute of Oceanology Polish Academy of Sciences; The main objective of the study is to present preliminary results of selected Persistent Organic Pollutants (POPs) concentrations in the Arctic fjords sediments. The extent of knowledge on POPs cycling in the Arctic is important since POPs may be transported over long distances from distant sources, are persistent in the environment and toxic. They tend to accumulate in fatty tissues of organisms, moreover may biomagnify along the Arctic food web. POPs are highly reactive particles and are readily sorbed onto sinking organic and mineral particles. Part of them is accumulated by marine organisms and other part is deposited at the sea bottom. Deposited contaminants may be re-introduced to the water column and be again bioavailable for organisms. The study presents the results of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) concentration in sediments collected from Kongsgjod, Hornsand and Adventjord (eastern Svalbard). These fjords are influenced by different water masses, different rate of glaciers ablation and the intensity of primary and secondary production. In addition, the knowledge on POPs concentrations in the Adventfjord near Longyearbyen may allow to assess the significance of local pollution source. The concentrations of selected polychlorinated biphenyls (CB28, CB52, CB101, DCB118, CB138, CB153, CB180) and selected polycyclic aromatic hydrocarbons (NAP, FLN, PHE, ANT, FLT, PYR, BAA, CHR, BKF, BAP, DBA, BP, IND) have been measured in several cores from different depth intervals. NAP, PHE and GC-ECNI techniques were used for qualitative and quantitative analysis of polychlorinated biphenyls and polycyclic aromatic hydrocarbons. To assess the origin of PAHs contaminants in sediments, individual components ratios were used. Sediment cores were dated using 

TH066 Organochlorine pesticides in archived antarctic ice cores

M. Biegé, Griffith University / Environmental Futures Research Institute; M. Curran, Antarctic Austrian Division and Antarctic Climate and Ecosystems Cooperative Research Centre; A. Moy, Australian Antarctic Division; D.C. Muir, Environment Canada / Aquatic Contaminants Research Division; D. Hawker, Griffith University / School of Environment; R. Cropp, Griffith University / Griffith School of Environment; C. Teixeira, Environment Canada / Aquatic Contaminants Research Division; S.M. Bengtson Nash, Griffith University / Southern Ocean Persistent Organic Pollutants Program Persistent Organic Pollutants (POPs) are toxic ubiquitous anthropogenic substances that have been widely used in agricultural and manufacturing industries since the 1930s. A variety of organochlorine pesticides (OCPs) have been listed as POPs since implementation of the Stockholm Convention which bans or severely restricts the use of POPs into the environment to minimise the health and ecological effects that they pose, POPs are highly resistant to degradation, toxic and bioaccumulative. They are also capable of long-range environmental transport which has led to primarily atmospheric transport of part of the global POP burden to remote Polar Regions. At high latitudes, cold conditions combined with long periods of darkness diminish their volatility and promote their persistence serving to restrict further movement. The polar cryosphere is thus frequently referred to as a "polar sink" for POPs. In the current global warming context, Polar climate may be significantly altered possibly leading to re-volatilisation of historically deposited POPs. Under this scenario, global efforts to moderate human and environmental exposure to these toxic compounds would be altered and Polar Regions would thus act as secondary sources to the atmosphere. Very limited research has been undertaken to gauge the current load of the POP ice reservoir in Polar Regions, particularly in Antarctica. While the release of POPs from melting glaciers has been observed in alpine areas around the world and recent findings also show the presence of POPs in Arctic ice and OCPs in Antarctic glacier melt-water, this study is the first to determine Antarctic continental ice loading of OCPs. Continental ice cores were collected during the 2005. The started group was not significant at the p<0.05 level (P=0.059) but with a similar mean value as for the MD, HD and HW groups. The Kruskall-Wallis rank sum test gave p<0.05 and the following Games-Howell post hoc test gave significant differences for the medium dose dispersion (MD) (p=0.05). The high dose dispersion (HD) and high dose water solubles (HW) were also different from the control with means in the same range as MD but with larger variances possibly causing p>0.05. The starved group was not significant at the p=0.05 level (P=0.059) but with a similar mean value as for the MD, HD and HW groups. The PC3 loading profile showed shifts in amino acids which to a large extent had loadings to indicate lower occurrence in high dose exposed and starved groups. Osmolytes taurine and betaine showed the same, whereas homarine had a loading to indicate higher occurrence in exposed copepods. From these results, exposure to MD, HD and HW fractions was found to give similar global metabolic responses as with starvation as observed by the PCA approach used here. The fact that only the highest dose W group was different from the control whereas both MD and HD were different could indicate that the dispersions are more potent in inducing responses in the metabolome.

TH067 Using environmental metabolomics to assess the contribution of oil droplets to dispersion toxicity in pelagic zooplankton Calanus finmarchicus (Crustacea: Copepoda)

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The production of manufactured nanomaterials (MNM) has been growing since the 1990s worldwide. The most documented environmental exposure to these toxic compounds would be altered and Polar Regions would thus act as secondary sources to the atmosphere. Very limited research has been undertaken to gauge the current load of the POP ice reservoir in Polar Regions, particularly in Antarctica. While the release of POPs from melting glaciers has been observed in alpine areas around the world and recent findings also show the presence of POPs in Arctic ice and OCPs in Antarctic glacier melt-water, this study is the first to determine Antarctic continental ice loading of OCPs. Continental ice cores were collected during the 2005. The started group was not significant at the p=0.05 level (P=0.059) but with a similar mean value as for the MD, HD and HW groups. The Kruskall-Wallis rank sum test gave p<0.05 and the following Games-Howell post hoc test gave significant differences for the medium dose dispersion (MD) (p=0.05). The high dose dispersion (HD) and high dose water solubles (HW) were also different from the control with means in the same range as MD but with larger variances possibly causing p>0.05. The starved group was not significant at the p=0.05 level (P=0.059) but with a similar mean value as for the MD, HD and HW groups. The PC3 loading profile showed shifts in amino acids which to a large extent had loadings to indicate lower occurrence in high dose exposed and starved groups. Osmolytes taurine and betaine showed the same, whereas homarine had a loading to indicate higher occurrence in exposed copepods. From these results, exposure to MD, HD and HW fractions was found to give similar global metabolic responses as with starvation as observed by the PCA approach used here. The fact that only the highest dose W group was different from the control whereas both MD and HD were different could indicate that the dispersions are more potent in inducing responses in the metabolome.

TH068 Effects of produced water components on Arctic copepods

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Calanoid copepods such as Calanus finmarchicus, C. glacialis and C. hyperboreus are the dominating zooplankton species in the North Atlantic Ocean and the Barents Sea. They store excess energy by accumulating large volumes of lipids during their late development stages before molting into adults. This discrete lipid sac is utilized during periods of low food availability and for reproductions. Exposure to lipophilic water pollutants such as polycyclic aromatic hydrocarbons (PAHs) can lead to the sequestration of these compounds inside the lipid sac. Very low elimination rates of PAHs have been observed in C. finmarchicus after exposure to produced water (PW) components warranting further studies on potentially delayed implications of these compounds on survival during diapause and reproductive success. As oil exploration and production moves further north towards the Arctic, the risk of such effects increases and an understanding of the toxicokinetics of PW components on Arctic keystone species is crucial for risk assessment. Key findings of our previous work investigating the effects of petroleum components on C. finmarchicus were revised to provide reference data for our latest project, PWC-Arctic. The main aim is to increase the knowledge on the potential effects of dispersed oil and other PW components on growth, development and reproduction in the Arctic copepods C. glacialis and C. hyperboreus, thereby supplying parameterized toxicology data for risk assessment of petroleum activity in the Arctic. This will be achieved by describing the potential for co-exposure (bioavailability) of petroleum-related compounds and dispersed oil droplets from PW and relate this to potential effects on assumed sensitive early life stages.

TH069 Uptake and elimination kinetics for 11 PAHs in Arctic lipid rich copepods

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Oil exploration and production has recently moved further north towards the Arctic, increasing the potential for exposure of Arctic organisms to polycyclic aromatic hydrocarbons (PAHs) during regular production as constituents of produced and water injection fluids. Experimental studies on copepods showed oxidized large volumes of lipids during their late development stages before molting into adults. Previous studies have shown that exposure to lipophilic water pollutants such as PAHs results in an accumulation of these components in Calanus species. Traditionally the uptake and depuration kinetics are modelled using one-compartment toxicokinetic models. However, previous experimental data on C. finmarchicus strongly indicate that elimination of PAHs is much slower than expected based on calculations by state-of-the-art one-compartment toxicokinetic models such as OMEGA. In the present study, the lipid rich developmental stage copepodite V (CV) of the Arctic Calanus glacialis was exposed to a filtered dispersion of alkane oil spiked with 11 PAHs for 120 h at 1 °C. The log Kow of the PAHs ranged between 3.3 for naphthalene and 5.78 for chrysene. A 120 h depuration period in clean seawater followed the exposure period, and samples for body burden analyses were taken at regular intervals during both the exposure and the depuration periods. Models developed to fit the uptake data were used to predict depuration kinetics for individual compounds. Several of the PAHs, especially the heavy-aromatic naphthalene and chrysene were retained up to 100 % retention after the 120 h depuration period, substantial deviations from the predictions by the one-compartment models. The slow depuration of PAHs in lipid rich Arctic copepods demonstrated in this experiment indicates that stored PW components may be more available for transfer to progeny, as well as having a higher potential for transfer to higher trophic levels than previously anticipated.

TH070 Enzyme expression in Antarctic krill (Euphausia superba) exposed to p,p'-DDE
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Despite the apparent pristine nature of Antarctica and it’s remoteness to industry, Persistent Organic Pollutants (POPs) have been detected in Antarctic biota since the 1960s. Antarctic krill from the Southern Ocean, an important Antarctic prey species, have been found with notable concentrations of POPs, including the pesticide, DDT and its most common metabolite, p,p'-DDE. Several organism-level responses have been observed in krill following sublethal p,p'-DDE exposure. However, nothing is currently known about the underlying subcellular mechanisms driving the response of p,p'-DDE in Antarctic krill. The purpose of this study was to examine the metabolic response of Antarctic krill exposed p,p'-DDE. Krill were examined for Glutathione S-transferase (GST), Glutathione peroxidase (GPx) and Acetylcholinesterase (AChE) activity in response to p,p'-DDE exposures ranging from 1-20 µg/L. None of the enzymes responded to p,p'-DDE in a concentration dependant manner. GST activity slightly increased for all doses but only the highest exposure induced a significantly increase. GPx followed a similar trend although none of the concentrations induced a significant increase in activity. Krill exposed to these concentrations exhibited signs of toxicity, most likely due to neurological impairment. The krill also exhibited a delayed recovery time for GST facilitated metabolism of p,p'-DDE at these concentrations. AChE decreased in three of the four treatments, but actually increased for the 10µg/L exposure, suggesting that the responses were not dependent on p,p'-DDE but may have actually been caused by an underlying factor. Thus AChE inhibition is not an indicator of organohalogen neurological toxicity in krill. Based on the GST and GPx activity measured in this study, krill appear not to have the ability to detoxify themselves of p,p'-DDE. This may indicate that this species is highly sensitive to this pollutant in the environment and possibly similar POPs. As p,p'-DDE is commonly detected in Antarctic biota, the apparent lack of detoxifying ability of Antarctic krill is troubling and should be examined further.

TH071 Baseline energy-budget model for the marine copepod Calanus finmarchicus

Calanoid copepods form an important part of the marine zooplankton, and are exposed to stressors such as pollution resulting from oil and gas exploration. Mechanistic models are needed to interpret the effects of such stressors, and to predict the impacts on these naturally ubiquitous species under realistic environmental conditions. Dynamic Energy Budget (DEB) models are well-suited for this task as they can provide an integrated framework covering all life-history traits over the entire life cycle of a species. Furthermore, these models are relatively simple, in principle not species specific, and easily linked to population models. Copepods, however, have several features in their life cycle which require further consideration in energy-budget models. Firstly, they develop through six naupliar stages, which deviate in morphology from the later six copepodite stages, and the first two stages do not feed. Secondly, copepods follow determinate growth, and stop growing after their final moult to adulthood. And thirdly, many copepods (especially those species from higher latitudes) build up a considerable lipid storage during the last few copepodite stages to survive adverse environmental conditions and to fuel the reproduction process. Before we can consider the effects of stressors on the life history, we need to develop a baseline model to capture these deviations from the standard DEB models. In this contribution, we report on our progress in modelling the growth, development and lipid storage in Calanus finmarchicus, a common species in the Northern Atlantic Ocean and expanding up into the Arctic. Once this model is established, it can serve as a platform to interpret the effects of toxicants and other stressors, and as a basis for extrapolating to other high-latitude copepod species.

TH072 Exposure of ovigerous female copepods to oils causes transcriptional responses in offspring - Maternal transfer of PAHs?

Copepods of the Calanus genus have a large potential for accumulating oil components during exposure due to their high lipid content and impressive filtration rates, and they have been shown to filter and ingest oil droplets during exposure. Female copepods produce eggs at the expense of their discrete lipid sac, suggesting either a remobilization of lipid-accumulated oil components and/or a direct transfer route of such contaminants to offspring. In an attempt to assess the potential for maternal transfer of oil components we exposed ovigerous female copepods (Calanus finmarchicus) to filtered and unfiltered oil dispersions for 4 days and collected their eggs the 3-6 days following the end of the exposure period. They were reared in clean water through to the late naupliar stages (NS-6) and collected for RNA extraction and preparation of libraries for high-throughput transcriptome sequencing. Differentially expressed genes were identified through pairwise comparisons between treatments. Expression of 173 genes was altered in response to dispersion exposure, 184 in response to the filtered dispersion, and 55 genes were affected by both treatments. Among the differentially expressed genes, several have known roles to chemical defense (including Phase I enzymes (oxidizing, Cyp2J-like), Phase 2 enzymes (exporting, multidrug resistance proteins), antioxidants (thioredoxin, xanthine dehydrogenase), chaperones (Hypoxia upregulated protein, quinone oxidoreductase), and components of the inflammatory response (phospholipase A2 activating protein).

TH073 Plastics in the Antarctic environment (PLANET project): preliminary observations of microplastics in the Antarctic biota
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Plastics are an ubiquitous and rapidly increasing component of the ocean environment, and the plastic debris entering the marine food web is potentially reshaping the structure and functioning of marine biota. The Antarctic Ocean is one of the last remote areas on Earth, but the presence of marine microplastics and micro- and nanoplastics is increasing, and their potential impact on the environment is currently unknown. Microplastics have a potential to bind polar bear PPARs. Activation of polar bear PPARs might lead to increased energy uptake and thus increase growth, development and lipid storage during the last few copepodite stages to survive adverse environmental conditions and to fuel the reproduction process. Before we can consider the effects of stressors on the life history, we need to develop a baseline model to capture these deviations from the standard DEB models. In this contribution, we report on our progress in modelling the growth, development and lipid storage in Calanus finmarchicus, a common species in the Northern Atlantic Ocean and expanding up into the Arctic. Once this model is established, it can serve as a platform to interpret the effects of toxicants and other stressors, and as a basis for extrapolating to other high-latitude copepod species.
fate and impact of nano-sized plastics in the marine environment are almost unknown and are raising concern due to increasing abundance in water column and their properties which could imply toxicity to marine biota. Therefore, studies in remote areas as Antarctica are urgently needed in order to understand their distribution and take measures for the conservation of one of the last pristine areas in the world. The aim of the PLANET project is to study i) the presence of micro and nanoplastics in Antarctic marine environment and ii) the impact on marine species in terms of bioaccumulation, trophic transfer and toxicity. Several biological samples collected in the last 10 years on the Antarctic Ross Sea area close to the Italian Antarctic Base “Mario Zucchelli” and from a penguin colony at Edwards-Hilton point will be analyzed for the presence of micro and nanosized polymers. Samples of phytoplankton, krill, scallop, fish, penguin (blood and feaces) and sea bird (skua) were homogenized, filtered and analysed by light microscopy and by FTIR and Raman for polymer type identification. Fibers and foils were the most abundant and appears to be common across a range of different species and different samples within a species present on selected samples. Sources, trophic transfer and size ability to support toxicity towards Antarctic marine species are reported and discussed.

TH076 DNA damage in arctic avian predators: baseline, sensitivity to stress and association to contaminant exposure

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The polar region functions as a pollution sink where chemicals produced and used in southern latitudes are transported and deposited. Seabirds are exposed to an increasing load of stress due to a warming climate, loss of habitat, changes in their marine food sources and also pollution. Seabirds are important study organisms in ecological and toxicological studies and they are considered good indicators of the oceans health as they inhabit different trophic levels, have different physiological adaptations and may be sensitive to human induced environmental changes. While the legacy pollutants are decreasing in the environment, they are still found in higher concentrations compared to newer compounds that are emerging due to increased chemical use to meet demands from our modern society. Organohalogen contaminants are known to elicit a wide range of negative effects on wildlife, such as effects on enzyme-, immune-, endocrine- and vitamin systems. The field of genotoxicity can provide new information about the effect that pollutants have on an organism’s DNA. DNA damage can be measured as a result of different stressors on an organism, and may be an interesting biomarker as an organism’s genome is vital for a wide range of different functions and systems. The comet assay is a well-established method to quantify single- and double strand breaks in the DNA; it is relatively easy and inexpensive, and a sensitive tool for assessing DNA damage. The comet assay is used in human toxicology on different types of cells, on other mammals, crustaceans, mollusks and fish. However, few studies have been conducted using the comet assay in assessing genotoxicity in a marine wildlife and thus, little is known regarding baseline DNA damage in various species, the sensitivity to oxidative stress-induced damage and the relationship between contaminants and degree of DNA damage. These knowledge gaps are addressed in the present study, focusing on the arctic seabirds the common eider, black-legged kittiwake, and glaucous gull, and in penguins conducted in June-July 2015 in Kongsfjord in Ny Ålesund, Svalbard, and the four different species were caught during incubation or early hatching period. Biometric data was measured and blood samples were taken. Data presented at the SETAC meeting will be levels of DNA damage (baseline sensitivity) in the different species, and the relationship to drivers behind the observed results; contaminant levels, body condition or other factors.

TH077 Determination of mercury speciation and isotopic composition of Antarctic seafood: a baseline study

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Mercury (Hg) is considered a globally distributed pollutant of major concern for humans especially under its form of monomethylmercury (MMHg), which is capable to accumulate in the organisms and biomagnify within the food webs. In the last decades, the measurement of THg isotopic fractionation dependent and independent (MDF and MIF, respectively) has become an essential tool in identifying sources of Hg and quantifying its reactivity within the different compartments of the environment. Although Antarctic and sub-Antarctic environments have been usually perceived as remote areas untouched by anthropogenic pressures, the presence of persistent contaminants through ocean circulation and atmospheric transport. However, Hg contamination in this region remains largely unknown, and new studies combining Hg speciation and isotopic approaches are required to elucidate this question. Seabirds, as upper predators, are exposed to large quantities of Hg via food intake and have been identified as effective biomonitorers of Hg marine contamination. This work is focused in the evaluation of Hg sources, trophic transfer and metabolic pathways by analyses of different tissues of seabirds from French Southern (Southern Ocean, Antarctic and (Southern Indian Ocean). Oceans. (piscatorial muscle, liver, brain and kidney), feather and blood samples were studied by the combination of Hg isotopic composition analyses by Multicollector (MC)-ICP-MS and Hg speciation analyses by species-specific Isotope Dilution Gas Chromatography-ICP-MS (ID-GC-ICP-MS). An optimization and validation of the sample preparation procedure was previously accomplished. Four seabird species with contrast feeding ecology were studied: Antarctic prion (zooplankton eaters), white-chinned petrel and king penguin (mesopelagic fish consumers), and northern giant petrel (other-seabird meat and carrion eaters). Significant differences in MDF signatures were observed between tissues for all the species, in particular for Antarctic prions. An enrichment in lighter δ206Hg values was observed from feathers to muscles and livers which could be attributed to inter-organ transport or metabolic processes. Hg MIF values differed widely between organs which could be the result of different MMHg sources. This study evidences the great potential of the combination of Hg speciation and isotopic composition analyses using seabird tissues to investigate Hg sources and metabolic pathways at different levels of the marine food web.
concentrations of contaminants found in polar bear adipose tissue suppressed polar bear and human PPARγ activity as well as PPARγ-mediated accumulation of lipids in mouse fat cells. Lipid accumulation in the mouse fat cells, through PPARγ-independent pathways was not affected following exposure to the synthetic mixtures. Contaminant mixtures extracted from polar bear tissues enhanced lipid accumulation in the mouse fat cells. We have also isolated polar bear stem cells from their adipose tissue and established a method to study lipid accumulation and mRNA expressions in cells exposed to mixtures of contaminants. In addition, we have determined the presence of a wide range of new and legacy compounds in polar bear fat samples by using non-target and target analyses.

Soil and water contamintants: evaluation, biomonitoring and bioindicators for effective management (P)

TH078

The validation of analytical methods as an evaluation tool of research results reliability

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TH079

Selecting optimized methods for environmental monitoring in the CCS projects

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The chemical monitoring on concerned substances in the environmental samples including soils and waters is generally through analyses in the laboratories. It is axiomatic that intensive quality assurance and control (QA/QC) programs should be conducted to reduce analytical failures, specifically considering that the monitoring data plays a key role to secure the public acceptance in carbon capture and storages (CCS) projects. As the one of QA/QC activities, we’ve been preparing the method-selecting chart on soil and water analyses. By the methods, the confidence range of concentration and applicable matrix are various. In addition, scales of matrix properties and public susceptibility to chemical changes are different as well as concentration of each element or substances by the environmental medium. Therefore, we aim at provision of simple but secure method-selecting chart considering the matrix properties including total dissolved solids (TDS) and the expected concentrations of interfering substances. The result of this study will be a help on selecting the appropriate method for the Korean CCS project, K-COSEM (Korean CO2 Storage Environmental Management). The substances included in the chart are selected from previous results of natural analogue studies and the foreign CCS projects. The samples from CCS project can be classified into two categories by matrix: (a) soils, and (b) waters. And the chemical concentrations of each matrix from TDS can have greatly wide range due to the characteristics of each substance and released CO2 levels. The previous monitoring data obtained from the studies on natural analogue sites and CO2-leakage test sites also play as a yardstick as well as confidence-range of each method.

A biossaya battery for the ecotoxicity assessment of foaming agents containing anionic surfactants


Alkylthiolsulfates (AES) surfactants are widely used in numerous industrial applications, as mechanized drill and fracking. They are present in commercial foaming products as their main components, together with different and sometimes not reported additives. In spite of their high volume utilization, very few data concerning the occurrence, fate and effects of AES on ecosystems have been published so far. The objective of this study was to evaluate the ecotoxicological effects of two commercial foaming products containing, as the main substance, the anionic surfactant sodium lauryl ether sulfate (SLES). In order to evaluate the EC50 values of the two commercial foaming products, they were initially subjected to ecotoxicological tests with aquatic and terrestrial organisms (Microtox test with Vibrio fischeri, Pseudokirchneriella subcapitata toxicity test and germination and growth test with Lepidium sativum). Then two soils with different geopedological characteristics were conditioned with the commercial foaming agents at the same concentrations used for mechanized drills and used for an experimental microcosm set-up. Soil sub-samples were collected at different times (0, 7, 14, 28 days) and soil eluates were produced for assessing the effects of the foaming agents both on the previous tested organisms (Vibrio fischeri, Pseudokirchneriella subcapitata, Lepidium sativum) and on other two, performing the Fish Embryo Toxicity Test (FET) with Danio rerio and Daphnia magna toxicity test. The results of the five tests were then compared with SLES residual concentrations, determined by MBAS spectrophotometric method both in the eluates and in ASE soil extracts.

TH081

Environmental safety assessment of biomass process streams

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The bio-based production of chemicals provides opportunities for developing a safer and green chemistry. Growing biomass on contaminated soils maybe allow for the use of otherwise neglected land, but it may also lead to uptake and introduction of hazardous chemicals in the production chain. The (pre)treatment of biomass may lead to the formation of potentially hazardous intermediates. The presence of hazardous chemicals may not only threaten the production process itself, but it may also pose a risk to workers in the industry as well as to the environment receiving waste materials. Within the Dutch E-Bio project, the dRISK project (Integrated effect-based risk management for sustainable bio-based production processes) is developing tools and strategies for assessing the potential hazard of biomass streams, using bioassays based on living organisms or cells. The panel of bioassays comprises human cell lines, zebrafish embryos, daphnids, springtails and enchytraeids, and is predictive for various adverse outcomes and groups of organisms at risk. By combining chemical separation and identification techniques with the bioassays, so-called Effect-Directed Analysis, possible chemical stressors that are responsible for toxic effects can be identified. Since chemicals may be strongly embedded in the biomass and not being available in the eluate, therefore posing little hazard, two extraction approaches were developed. The first one applies total chemical extraction followed by testing in the various bioassays to determine potential toxicity of biomass materials. The second approach applies passive sampling methods that extract only the available chemicals from the biomass followed by exposing the bioassay. We are using passive methods for the Dutch E-Bio project to partitioning and ensures that the organisms are exposed to realistic available concentrations. This also allows exposing all organisms to the same activity of potentially hazardous chemicals. This presentation will show how the tools developed in dRISK are designed to apply biomass obtained from the processing of verge biomass, not being available and therefore posing little hazard, two extraction
An approach to choose the most relevant time of exposure for biomarker measurement in earthworms exposed to pesticides

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Biomarker measurements on organisms known as bioindicators are considered as good tools to assess both environmental quality and functioning. Earthworms, by their ecological functions and sensitivity, are relevant organisms to evaluate the quality of soils disturbed by human activities. Despite a great amount of experiments intended to study xenobiotics effects on the physiology of soil organisms under controlled conditions, no clear trend emerged concerning the responses of biomarkers. Owing to the high heterogeneity observed in laboratory protocols (species used as models, types and concentrations of contaminants, time of exposure, analysis performed even in the same laboratory, etc.), the aim of this study was to underline the kinetic responses of biomarkers involved in earthworm physiology (defense against oxidative stress, detoxification mechanisms and energy reserves) within time. Our main hypothesis was that the conditions and particularly the time of exposure strongly influenced the results obtained in these studies. Here we provide the most relevant interval of time exposure for each biomarker. Earthworms of the species Aporrectodea icterica were exposed to pesticides in laboratory conditions, at realistic agronomic dose or agronomic dose x 10, alone or in a mixture. Earthworms were placed in microcosms containing contaminated soil, during periods of 2, 4, 7, 10, 14 and 21 days before biomarkers analysis. The two selected pesticide was an insecticide of the carbamoylphosphonate class and a commonly studied bactericide of the strobilurin family, AMISTAR® (Azoxystrobin), commonly applied in cereal crops. We will present the results of the kinetic responses for each selected biomarkers (catalase, glutathione-S-transferase, superoxide dismutase, proteins...), time of exposure and pesticide dose within time. We will also identify the most suitable period to their measurement.

TH083

Effect of the type of contamination and land use on soil enzymatic activities. Results of the French "Bioindicator" program

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Since many decades, enzymatic activities are used to assess the effects of land use changes, agricultural practices and soil contamination on soil functioning. A lot of studies point out contradictory results depending notably on the methodology used, as well as the type of contamination. To overcome the lack of an efficient method for identifying the impact of contaminants, the national research programme “Bioindicator” ( overseen by ADEME) was set up in France to develop and assay biological indicators (2006–2012). We aimed at standardizing and emphasizing the number of biochemical measurements, and developed miniaturized colorimetric methods to obtain a great enzymatic database. Thirty activities (from three laboratories) were measured within this programme, in 47 modalities of 13 experimental sites differing in terms of land use (Corine land cover), contamination type – Polycyclic Aromatic Hydrocarbons or metals – and pollution levels (Vibrisse 8th decile). This dataset allows an analysis of the effect of atmospheric or added contamination gradients on the enzymatic activities and microorganisms, as to point out the sensitivity of enzymes for each type of pollution and land use, to offer the most appropriate panel for a sensible analysis. Data were analysed for the all datasets, each site and each land use. Our main conclusions are as follows: enzymes are more sensitive to metallic contamination than to organic ones; enzymes are good indicators of metal bioavailability, Alkaline phosphatase and Arylsulfatase are the most relevant enzymes to assess the effect of soil contamination.

TH084

The significance of soil microalgae and cyanobacteria to assess pesticide stresses, in agricultural and agro-ecological bioindicators.

O. Ebranget, C. Mariand, INRA Institut National de la Recherche Agronomique / UMR Ecosys; P. Marion, INRA Institut National de la Recherche Agronomique / UMR Ecosys; M. Feron, IRD UMR Carretel; C. Mallet, Université Blaise Pascal / UMR LMG; C. Mougin, INRA (Institut National de la Recherche Agronomique) / UMR Ecosys; A. Beaucamps, IRD UMR Carretel; INRA Institut National de la Recherche Agronomique / UMR Emmah; Standardized soil microbial indicators, used to assess the impacts of agricultural practices and/or contaminations, lack of sensitivity and genericity against pesticide stresses, especially for herbicides. Soil photosynthetic microorganisms, mainly represented by micro-algae (chlorophyceae, xanthophyceae and diatoms) and cyanobacteria, can grow in soil surface. So, they could be an original microbiological model for herbicides risk assessment in agricultural soils. Nevertheless, their structural and functional biodiversity (and the environmental factors influencing their communities are still largely unknown). Then, to improve our understanding of these photosynthetic microorganisms, we need to 1) develop biochemical and molecular methods to characterize their activities and genetic diversity and 2) analyse their responses to herbicides in agricultural soils. Several molecular techniques common in environmental microbiology and ecotoxicological approaches are still helpful to isolate edaphic species for further ecotoxicological tests and barcoding approaches. Photosynthetic pigments can provide biomass (chlorophyll-a) or structural (pigment diversity) indicators. Several genetic markers from aquatic studies were successfully applied on soils samples, but a current limit is the lack of even a specific database of these specific genetic markers. Toxic effects on photosynthetic microbial biomass and diversity have been evidenced at doses close to recommended field rates, throughout field and microcosm approaches. The comparison of the responses of photosynthetic microbial communities, between different cropping systems (organic vs. conventional), highlighted a tolerance increase for some herbicides in conventional soils. Genetic diversity characterization of micro-algal and cyanobacterial communities in these cropping systems, provides further results to identify taxa relating to tolerance. Then, various genus of microalgae and cyanobacteria have been identified for their high sensitivity to herbicides. Overall, micro-algal and cyanobacterial communities showed a higher sensitivity to herbicides in soils, compared to common bacterial communities. Our future investigations demonstrate the suitable issues of soil photosynthetic microorganisms as indicators reporting non-target impacts of herbicides on soil biodiversity and functioning. Prospects for monitoring remediation of polluted soils (heavy metals, HAP) should be considered.

TH085

Use of the crustacean Gammarus fossarum in biomonitoring survey and trophic transfer of protozoa Toxoplasma gondii and Cryptosporidium parvum

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The protozoa Toxoplasma gondii and Cryptosporidium parvum are public health priorities since their oocyst form is exceptionally resistant to environmental insults and can remain infectious for months or years. They are present in various watercourses as recreational, surface, drinking, river and seawater. Cryptosporidium oocysts were reported in a range of bivalve mollusc species but until now there is no information whether freshwater crustaceans are able to accumulate protozoan oocysts. The amphibious species Gammarus fossarum selected in this study is widespread and common in rivers and streams of Western Europe. G. fossarum have an important ecological role in the trophic chain since they represent a reserve food for macroinvertebrates, fish, bird and amphibian species. The purpose of the present study was to determine whether Gammarus fossarum could accumulate in their tissue T. gondii and C. parvum oocysts after experimental exposure and potential use of Gammarus fossarum as matrix for biomonitoring of protozoa concentration in freshwater systems. Gammarus fossarum were exposed to 200, 2000 and 20,000 oocysts per gammarid and per day during 21 days followed by 5 days of depuration. DNA detection of T. gondii and C. parvum oocysts was carried out using TaqMan real-time PCR. C. parvum DNA was detected in gammarids in only one out of four pools for the higher concentration and after 14-day exposure. However, concerning T. gondii, DNA was detected after 7-days exposure with the two highest concentrations, and from 14-day exposure T. gondii DNA was highlighted in gammarids for all conditions. Between 0 and 14-day, oocyst number detected increased with the exposure time, then a saturation effect was observed between 14 and 21-day for the two highest concentrations. A significant dose-response relationship was observed and the maximum number of oocysts detected in gammarids was 20,000 oocysts per organism and per day. After 5 days of depuration, T. gondii oocysts were still present in gammarids indicating the integrative nature of G. fossarum. These results show for the first time that a freshwater crustacean is able to bioaccumulate T. gondii oocysts suggesting that Gammarus fossarum is a suitable bioindicator of protozoa contamination in biomonitoring studies. Moreover, due to their important position in freshwater food webs, G. fossarum should also play a role in the trophic transfer of protozoa.

TH086

Snail watch to survey the transfer of PCB and PCDF/F on a polluted site connected to a photosynthetic power plant.

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SETAC Europe 26th Annual Meeting Abstract Book
University of Lyon / UMR CNRS EYS EMSE Géosciences et Environnement; K. Perronnet, INERIS / Direction des Risques Chroniques/Unité Impact Sanitaire et Expositions; A. de Vaulx, University of Franche-Comté / Department of ChronoEnvironment

In 2008, a fire on a former industrial site in Saint Cyprien (France) caused a soil contamination with persistent organic pollutants (POP) to surrounding soils. About 14 hectares of pasture were consequently forbidden to agricultural use. To give a new usage to this site, a power plant was built in 2010 by Luxel SAS. This installation produces green electricity since early October 2013 and constitutes a pilot site for research on POP toxicity and degradation processes over time. The objective of the research project TROPE (Transfer of Prevention System and Risk of Organic Persistent pollutants for snails) was to assess, thanks to active biomonitoring, the bioavailability of PCB, PCB-DL and PCDD/F to a soil invertebrate living at the soil surface, the ubiquitous land snail, Canterrae aspersa. Snails were caught for 28 days on 4 grassy plots with increasing POP contamination (Table 1). For PCB, two to 222 ng TEQ[PCB] kg⁻¹ dw for ?12 PCB-DL; an additional plot highly contaminated (>400 mg.kg⁻¹ PCBi and OCDD) was located near the studied site was also studied. Internal residues in the soft body of the snails for the ?12 PCB were from 556 to 462,124 ng.kg⁻¹ tw whereas the ?17 congeners of PCDD/F varied from 0.052 to 1.18 ng TEQ kg⁻¹ and for the ?12 PCB-DL from 0.064 to 72 ngTEQ kg⁻¹. The ratio PCB/PCD was from 2 to 2.6 and the ratio PCB/PCB-DL from 4252 to 8689. Among the PCB, congeners 101, 138 and 153 were the most accumulated pollutants. For PCB-DL, PCB105 and 118 were the most abundant whereas for the PCDD, OCDD and HpCDD were predominant in the tissues. The results showed that POP are bioavailable for snails and may reach high internal body concentration in highly contaminated plots. This study provides first data on the capability of land snails to accumulate various PCB, PCB-DL, and PCDD/F and reveals that these soil invertebrates may be a source of POP exposure to their consumers. Two other campaigns of biomonitoring are performed in the coming years and may help to sharpen our understanding of the PCB impact on the ecosystems.

TH087

Evaluation of an endocrine disruptor (Vincolozolin) in the benthic organism Chironomus riparius (Diptera)

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The dicarboximide Vincolozolin (Vz) is a widely used fungicide for agricultural purposes. Because of this, it is found as a residue in the environment, detected in underground water, lakes, rivers, and seawater. Most of the toxicity studies have been focused on vertebrates showing antidiogenic activity, however, few studies have investigated their effects in invertebrates, which are key organisms in aquatic ecosystem maintaining. In this work we have analyzed the effects caused by Vz on fourth instar larvae Chironomus riparius, a dipteran used frequently as reference species in ecotoxicology. Alterations that exposure of the larvae for 24 and 48 hours causes on several genes related with the endocrine system and the detoxification pathways of this insect were evaluated using Real-Time PCR. Two of them, Diaphanost and Cyp4S1, are related with the metabolism of ecodycene, one of the main hormones of insects involved in development. Membrane Associated Progesterone Receptor, the coding gene for an orphan receptor, and Forkhead Box Oa, a gene which produces a protein involved in the insulin response pathway, were also analyzed. On the other hand, Cyp50G2 and GST62 transcriptional activity was studied to analyze if they are involved in the detoxification process of Vz. The results show a significant alteration of transcription activity of some of these genes indicating that the presence of Vz affects the cell processes in this organism. The results obtained show the ability of Vz to up-regulate hormonal related genes in C. riparius larvae after exposure to different Vz concentrations and the first evidences of effects in insects. This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Spain), grant CTM2012-337547 from the Ciencias y Tecnologías Medioambientales program. M.A. is the receiver of a predoctoral contract Ministry of Economy and Finance (BES-2013-064041).

TH088

Formaldehyde Chemical Substance Flow Analysis in South Korea

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A chemical substance is any material that has a definite chemical composition (Hill et al. 2005). Currently chemical substances and materials are widely used in all kind of industry and household, almost everywhere and in everything. On the other hand, these chemical substances and materials have important issues such as risk related with hazardous and toxic in their life cycle from manufacturing and use to disposal and recycling. Many accidents and industrial disasters were occurred in the world. Up to date, therefore, related regulation and assessment tools (e.g., Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), risk assessment, health effect relevant of chemical exposure) are widely applied for chemical substances and materials. There have been some studies on the guiding principles of chemical accident prevention, preparedness and response. However, the study on chemical substance flow analysis in country level and its application was not well conducted. In this study, therefore, formaldehyde (CH2O), which is used mainly to produce resins used in particleboard products and as an intermediate in the synthesis of other chemicals was selected. Formaldehyde is used predominantly as a chemical intermediate. It also has minor uses in agriculture, as an analytical reagent, in concrete and plaster adhesives, cosmetics, disinfectants, photography, and wood preservation (US EPA, 1988). The purpose of this study is quantifying, analysing and mapping of the formaldehyde substance flows by using material flow analysis (MFA) method in South Korea. Our result shows that the number, location of manufactures using formaldehyde as well as formaldehyde’s flow (import, export, stock, and sales) flows in Korea are distributed by industries (including companies). Finally the result of this study can support for chemical accident prevention system and sustainable chemical management in South Korea.

TH089

Biological effects of different pesticides and their mixtures on Eisenia andrei: laboratory experiments and field studies using caged earthworms

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Pesticides are harmful to specific target organisms; however, selectivity is never absolute and therefore pesticides represent a risk also to non-target organisms. Many of the studies using ecotoxicological score the bioavailability of high-level endpoints (i.e. mortality and reproduction) that provide information about possible effects at organism/population level; data concerning sublethal effects have been rarely described. In this study we evaluated the toxicity of different pesticides widely used in agriculture on the earthworm Eisenia andrei by simulating in the laboratory the processes carried out in the rice- and vine-fields. For this purpose, a set of biomarkers able to highlight the alterations of the health status of the organisms from the most sensitive molecular changes to whole-organism responses was employed. The results demonstrated that all the studied compounds at concentrations recommended by the manufactures did not affect the worm survival but provoked oxidative stress and genotoxic effects on 28 exposed animals. To verify the results of this laboratory study, we also realized field studies. To this end, a method was developed to maintain the earthworms in field for 10/28 days. The animals caged in the sites of rice (during the dry period) and vine cultivation treated with the pesticides (at the doses used in laboratory experiments) survived. However, also in these animals, significant effects on sublethal endpoints (such as lysosomal membrane stability, Ca²⁺-ATPase activity and mitochondrial functionality) were observed. In both crops, the results highlighted genotoxic effects as demonstrated by the increase of the DNA damage and of the micronuclei frequency.

TH090

Application of bioassays with Enchytraeus crypticus to evaluate the effect of biochar in metal bioavailability

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Mining activity results in the accumulation of waste scattered in the landscape. When contaminants contained in these wastes are mobilized, the risk of pollution of surrounding areas increases, degrading soil quality and causing serious problems to human health. The main objective of MINEETOX project is to study the effect of biochar in metal availability of different mine wastes over time by means of the physicochemical and ecotoxicological characterization of the treatments. In this study, two mine wastes (acid and basic) were amended with two different types of biochar (one from pruning remains and other from sewage sludge) separately, in order to study their effect on metal bioavailability and to modify their potential environmental impacts. Since the application of the amendment was expected to result in metal immobilization in soils, the efficiency was tested both in mine wastes and mine waste pore waters. Mine wastes were amended with 6% of biochar, and pore waters were extracted at the beginning and then every 6 months of the experiment for all treated and untreated wastes. Accumulation and toxicity of treated and untreated wastes in the potworm Enchytraeus crypticus were investigated in mine waste pore water solution embedded in an inert quartz sand matrix. Additionally, treated and untreated wastes were mixed with uncontaminated Lafa 2.2 reference natural soil to assess the waste concentration potential, modifying the industrial system (between industries including an oil refinery) and survival and reproduction was observed in all treatment at each dilution concentration. Preliminary results show that organisms were more affected when exposed to mine wastes than when exposed to the pore water solutions. The latter could indicate that when exposed to mine wastes the organisms could be still affected by the fraction of metals immobilized in the mine wastes. Toxicity seems to be related to the metal concentration and to be more pronounced when biochar was added.
different amendments from a double perspective, taking into account the two different exposure pathways (via pore water and via soil ingestion).

TH091 Strati fi cation of soil arthropods in top soil layers
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The persistence of pesticides in soil is well studied. Their distribution depends on their physical and chemical properties together with the soil characteristics. Studies have shown that traces of pesticides can be found in soil up to several years and down to depths over two meters thick is known on the stratification of the soil arthropods in the top soil layers and even less about the effects of pesticides on them. Therefore, our overall aim is to study these communities and their response to pesticide exposure. An ongoing pilot study, started in May 2015, aims to identify the arthropods groups found in the top 40 cm of soil and record their activities. Several soil arthropods were sampled with a tubular trap (“slide trap”) specifically designed for this aim, at four depths: 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm. Each tube has approximatively 70 holes of 5 mm diameter evenly distributed at the target depth for a maximized stratified sampling. To collect the arthropods a funnel and a jar, filled with 50 ml of ethyl-glycol, were attached at the bottom of the tubes. The traps were setup in May 2015 and are checked every two weeks. Results of the first six months show that adult beetles and their larvae can be found up to 40 cm depth, and their vertical migration appears to be influenced by the environmental conditions. Species of Diplura are mainly found at 20-30 cm depth. Individuals of the subphylum Myriapoda have a split stratification, with high numbers in the layers of 0-10 cm and 20-30 cm. Remarkably species of Panpseudops and Pseudopseudops show up frequently in layers deeper than 20 cm. Full taxonomic list for all groups and a detailed model of the slide traps used will be presented with the poster. Presently studies are designed to look at the arthropods communities in the first 10 cm and might overlook the effects of pesticides on taxa such as Diplura and Myriapoda, groups that are more abundant in deeper layers. Our study will bring valuable information on how these communities respond to pesticides in deeper layers missed in previous in situ studies. Also it will show if and how the environmental conditions influence the response.

TH092 Using in situ assays with two freshwater producers to assess effects of wildfires on aquatic systems
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Portugal is the European country where fire-fires devastate more area, with an average of 14.4 000 ha per year in the last decade, and a tendency to increase both in frequency and area. Wildfires constitute a concerning environmental problem once they have adverse and diversified effects impacts in the terrestrial, atmospheric and aquatic compartments. It is known that wildfires affect aquatic systems by altering its physical and chemical properties through the input of pyrolytic substances, such as polycyclic aromatic hydrocarbons (PAHs), and metals associated to ash/solil loads from the surrounding burnt areas. The majority of the available studies regarding the effects of wildfires on aquatic biota have been performed by using laboratory single species biosassays. Once in situ exposition enable to assess more realistic environmental multi-stress conditions and their fluctuations, in situ assays using two aquatic producers’ species – the microalgae Raphidocelis subcapitata and the macrophyta Lemna minor - were performed. A recently burnt area was selected near Miranda do Corvo (Portugal) and five sites were selected to conduct the assays: two in the main water course of the watershed - Ceira river, being one located upstream (RUS) and the other downstream (RDS) the burnt area; two in tributary streams within the burnt area (SUS and SDS); and one in a stream located in an adjacent non-burnt area (CS). The assays took place at the time of the first major rainfall events and lasted for 13 days. Personalized test chambers were elaborated, allowing floatage and luminosity entrance for both tested species. R. subcapitata was immobilized in alginate beads while L. minor leaf colonies were tested freeform. The cellular concentration of microalgae in beads and the initial number of fronds and weight for L. minor were determined in the beginning and in the end of the assay, allowing to calculate the growth rate. The present results showed an inhibition in the growth rates of both species when exposed to the most impacted sites (RDS, SUS and SCS) compared to the reference (RUS and CS). Hence, in situ biosassays revealed to be effective to discern toxic effects on aquatic primary producers due to diffuse contamination from wildfires.

TH093 Off-site environmental impacts of wildfires: Assessment of the toxicity of post-fire runoff on freshwater aquatic species
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Wildfires major disturbance of forests worldwide, posing an important threat to life, human goods and natural resources. Interestingly, wildfires have an important role in contaminants production and mobilization, such as polycyclic aromatic hydrocarbons (PAHs) and trace elements. For instance, these contaminants could be mobilized during a wildfire from ash and sediments loads and may eventually reach the nearby aquatic systems by surface runoff. In this regard, wildfires represent a relevant diffuse source of PAHs and trace elements to aquatic systems that has, so far, been poorly investigated, in particular, their potential toxicity on aquatic biota. The current study aims to mitigate such lack of knowledge for PAHs and trace elements, a well-recognized persistent toxicants and accumulation in aquatic organisms. 

To address the potential ecotoxicological effects posed by such compounds and their mixtures, the present work was focused on their effects in the following aquatic species: bacteria (Vibrio fischeri), the algae (Raphidocelis subcapitata), the macrophyte (Lemna minor) and the invertebrate (Daphnia magna). These species were exposed to different dilutions (12.5, 25, 50, 75 and 100%) of post-fire runoff and surface water from the burnt catchment outlet. All the samples were collected during the first rainfall events after the wildfire. The concentration of the fifteen PAHs identified by USEPA as priority contaminants were analysed by gas chromatography-mass spectrometry (GC-MS) and the trace elements (V, Mn, Co, Ni, Cu, As, Cd and Pb) by inductively coupled plasma-mass spectrometry (ICP-MS). The endpoints of the ecotoxicological tests were determined as a statistical decrease in luminescence inhibition for the V. fischeri, growth inhibition for the R. subcapitata and the L. minor, and immobilization for the D. magna. Lethal and sub-lethal dose response curves were determined as well as the corresponding endpoints (NOEC, LOEC, EC50 and EC30). The preliminary results allowed to discriminate the off-site effects of wildfires on distinct aquatic species. Moreover, it allowed to determine the threshold concentrations of PAHs and trace elements that can be considered at low/high risk for aquatic biota and therefore, the aquatic systems. This information is of crucial importance to predict the risks and effects of the surface water pollution by recently burnt areas and the propagation of toxic effects from the lower to the upper trophic levels.

TH094 Biofuels and their aquatic toxicity - Biotesting 3-methyltetrahydrofuran
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The rapidly growing industrial sectors in many developing countries, such as China or India, require an increasing volume of fuel for the transport sector. In parallel, environmental concerns regarding petroleum based fuels and the request for independency on oil result in a growing demand for renewable energy sources such as biofuels. However, a release into the environment increases with a rise in biofuel production and consumption. Therefore, a comprehensive assessment of the environmental hazard potential is aggravated by the lack of ecotoxicological data. In this work, ecotoxicological bioassays can be used for the detection of potential hazards for aquatic ecosystems at a very early stage of the development. This study is focused on the ecotoxicological investigation of the potential biofuel 3-methyltetrahydrofuran (3-MTHF) in aquatic systems. To evaluate the hazard potential of this new potential biofuel for the environment 3-MTHF has to be tested in different aquatic test systems monitoring different levels of organisation, such as acute toxicity and embryo toxicity using the fish embryo toxicity (FET) test with Danio rerio (OECD 236), the acute immobilization assay with Daphnia magna (OECD 202) and algae growth inhibition test with Desmodesmus subspicatus (OECD 201). Additionally an in vitro micronucleus-test with Chinese hamster cells (ISO 21827-1) has to be performed to estimate the genotoxic potential of the fuel candidate. Differences in toxic potencies of 3-MTHF compared to other fuel candidates can be used to identify the least toxic biofuels and focus further research on these candidates. An early investigation of biofuels can decrease the risk for the environment and, thus, reduce the potential of these new fuels to become a source of contamination for aquatic systems.

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TH095 Occurrence of four potentially SVHC-classified semipolar PAHs in selected environmental matrices and their ecotoxicological impacts
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Aim: To screen for occurrence of four potentially SVHC-classified semipolar PAHs in selected environmental matrices and to estimate their ecotoxicological impact on aquatic organisms of different levels of organization. Thus, the potential hazard of these compounds for human and ecosystem health can be assessed, and future research can be directed to these substances.

Methods: A screening study was carried out on sediments, sludge, aqueous samples, and biota from a sewage sludge composting facility in the Ruhr region in Germany. Norwegian fjords, the Baltic sea and wetlands in the Netherlands were investigated. For aqueous samples, a specific test method for the analysis of semipolar PAHs was developed. The ecotoxicity of the extracted compounds was investigated using the luminescence inhibition test (V. fischeri) and the immobilisation test (D. magna L.). An additional test method was used for the determination of genotoxic and mutagenic effects (micronucleus assay, fish embryo test).

Results: Four semipolar PAHs were detected in the investigated samples. The highest levels were found in sewage sludge compost and sediments from the Baltic Sea, indicating potential environmental risks. The ecotoxicological results showed that these compounds were able to reduce the bioluminescence of V. fischeri and immobilise D. magna L. Additionally, genotoxic and mutagenic effects were observed in fish embryo tests. The findings indicate that these semipolar PAHs can pose a risk to aquatic organisms and deserve further investigation.

Conclusion: The occurrence of semipolar PAHs in environmental matrices highlights the importance of their ecotoxicological assessment. Future research should focus on the fate and effects of these compounds in aquatic ecosystems.
Lukas, German Environment Agency; A. Seidel, Biochemical Institute for Environmental Carcinogens, Germany

Semipolar poly cyclic aromatic hydrocarbons (heterocyclic PAHs or PANHs, PASHs and PAOHs) are often found in UVCBs (chemical substances of unknown chemical composition) Regulation of the European Union. Annex XIII of the REACH Regulation defines the properties of SVHC. Substances with persistent, bioaccumulative and toxic properties (PBT) are of very high concern for the environment and can be regulated by authorities. Within a joint research project the German Environment Agency, sediment and domestic waste (UBA) and the Biochemical Institute for Environmental Carcinogens (BIU) investigated the ecotoxicological properties of four semipolar PAHS potentially identified as SVHCS.

Benzo[a]anthracene, dibenz[a]anthracene, and fish embryo (Danio rerio). At the BIU semipolar PAHS were prepared in high purity and water solubility of each compound was determined in the used test media and at the relevant test temperatures. The results obtained for the PAH levels on the ecotoxicological on the aquatic test organisms are presented. In addition, PAH levels found in blue mussels (Mytilus edulis L.) collected at several locations along the German coastline as well as PAH levels found in fresh kale (Brassica oleracea var. sabellica L.) and certified harbour sediment (BCR-535) are reported. Reference: Schwartz, M., Belkhe, A., Brandt, M., Eisele, M., Esselborn, P., Seidel, A. (2014).

Dermal Absorption Study of PAHs in Coal Tar/Coal Tar Pitch Clay Pigeon

B.H. Magee, ARCADIS; N.D. Forberg, G.C. Hoeger, ARCADIS / IEST

An in vivo oral bioavailability study was performed to determine the relative bioavailability of PAHs in clay pigeon target fragments at military range sites. These fragments, which are mixed with native soil, are composed of PAHs in a site-aged coal tar/coal tar pitch and limestone matrix. It was expected that the fragment matrix would reduce the oral bioavailability of PAHs compared to that seen in animal studies using pure benzo[a]pyrene in solvents. To test this hypothesis, an oral bioavailability study was performed with female B6CF1 mice (8 mice per dose group) fed soils or soil extracts at a rate of 5% in the diet for fourteen days. Four test soils contained 0.5, 4.5, 28 or 183 mg/kg benzo[a]pyrene (BaP). BaP concentrations in diets were 0.04, 0.39, 2.55, and 19.17 mg/kg. A high resolution mass spectrometry method was developed to detect low levels of the following PAH metabolites in mouse urine: 3-hydroxy-benzo[pyrene], 9-hydroxy-benzo[pyrene], 7,12-dihydroxy-benzo[pyrene], and 3-hydroxy-chrysene. The fractions of total dose excreted in the urine were compared for the soil and the soil extract groups to derive Relative Bioavailability Factors (RBAs) for use in risk assessment. The RBAs were 0.20 for BaP, 0.23 for benzo[a]anthracene, and 0.28 for chrysene.

Dermal Absorption Study of PAHs in Coal Tar/Coal Tar Pitch Clay Pigeon

B.H. Magee, ARCADIS; N.D. Forberg, G.C. Hoeger, ARCADIS / IEST

An in vivo dermal absorption study with human skin was performed to determine the dermal absorption of PAHs in clay pigeon target fragments at military range sites. These fragments, which are mixed with native soil, are composed of PAHs in a site-aged coal tar/coal tar pitch and limestone matrix. It was expected that the matrix would reduce the dermal absorption of PAHs compared to that seen in animal studies using pure benzo[a]pyrene (BaP) in solvents. For human health risk assessment, USEPA assumes that 13% of administered PAHs are absorbed based on an in vivo study in thensus monkeys by Wester et al. (1990). This study evaluated dermal absorption by applying smudged soil samples with 7C-BaP and testing the spiked soil immediately. Several published studies showed that aged, weathered PAHs have lower dermal absorption compared to freshly spiked soil samples. To test this hypothesis, an in vitro study was performed in Franz diffusion cells with split-thickness fresh-frozen human skin samples from male and female donors aged 23-65 years. Test articles were applied to skin for 24 hours, then washed off. Collection of receptor fluid samples continued for a total monitoring period of 72 hours. Skin samples were stripped five times with tape to remove any remaining surface soil. PAHs were analyzed in the dermis and in the surfactant-containing receptor fluid by high performance liquid chromatography with fluorescence detection to a detection limit of 100 ng/mL to detect absorbed dose. Test articles included three site soils with BaP concentrations of 8, 51 and 383 ppm. Dermal absorption of BaP and six potentially carcinogenic PAHs was less than 1%. A freshly spiked control sample gave 9% absorption for BaP.

Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons in Water, Sediment and Dominant Fish from Designated Zones of the Lagos Lagoon, Nigeria

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The ecological risk assessment (ERA) of priority pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) are imperative due to their cascading biological effects along the food chain in the aquatic environment. In this study, the levels and ecological risk assessment of sixteen (16) priority PAHs were evaluated in surface water, sediment and dominant fish species sampled from anthropogenic-impacted zones (Ijala, Idido, Atlas vase and Apapa) of the Lagos lagoon in the wet and dry seasons from 2012 to 2014. PAHs in the samples were analyzed using GC/FID. Indices such as Effects Range Low/Effect Range medium (ERL/ERERM), Threshold/ Probable Effects Level (TEL/PEL), sediment and fish pollution classification based on sum PAHs and Bioconcentration Factor (BCF) were utilized for the ERA. The results showed that the dominant fish species were Sarotherodon melanotheron (Black-Chinned Tilapia), Gerres melanopterus (Gerres), Liza falcipinnis (Sickle-fin Mullet) and Pseudolitius elongatus (Bobo Croaker) at the Ijala, Idido, Atlas vase and Apapa zones respectively. The average sum PAHs (ΣPAHs) and range were 515.8±19.14 (Bajie, wet season) - 1057.9±109.49 mg/kg (Apapa, wet season) in water, 1290.16±23.49 mg/kg (Apapa, wet season) and 19.76±0.07 mg/kg (8.80 S. melanotheron, dry season) - 26.10±0.07 mg/kg (G. melanopterus, dry season) in sediment, water and sediment respectively. Naphthalene was dominant in water and sediment samples while phenanthrene, anthracene, fluoranthene and pyrene were predominant in the fish species across the zones and seasons. ERA of PAHs in the sediments showed that biological effects will occur frequently at the Ijala zone and occasionally at all other zones based on the levels of naphthalene, acenaphthene, acenaphthene, fluorine and benzo[a]anthracene in both seasons. Apapa zone was highly polluted while other zones were moderately polluted in both seasons based on ΣPAHs in the sediments. Fish species from all the zones were minimally contaminated in both seasons except S. melanotheron based on ΣPAHs in fish. BCF results showed that PAHs did not bioconcentrate in the fish species except for pyrene with a BCF value of 2.28 in G. melanopterus. The study recommends that the specific PAHs identified as ecological risk factors in the Lagos lagoon sediments should form a basis for the establishment of environmental quality standards for PAHs in Nigerian coastal waters as well as their inclusion in ecotoxicology programs.

Water quality assessment of five cascading reservoirs of Iguaçu River through multi-biomarker approach in fish

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The Iguacu River, located at southern region of Brazil, has great socioeconomic and environmental importance due to its high endemic fish fauna as well as its ability to generate hydroelectric power and the potential to supply water for human use. To date, no previous study has reported an integrated evaluation of chemical analysis and biological responses in Iguacu River, which is considered ideal for a better environmental risk assessment. The local environmental agency describes water quality improvement along the river course, since there is intense discharge of pollutants in the river’s source. For the current study, three different Brazilian fish species (Astyanax bifasciatus, Charinica iguassuensis and Geophagus brasiliensis) were captured for a multi-biomarkers approach analysis in five cascading reservoirs of Iguacu River. Chemical analysis in water, sediment and muscle indicated high levels of metals in all reservoirs. Chromium (Cr) levels in water were detected above the permitted concentrations set by governmental agencies. High levels of polycyclic aromatic hydrocarbons (PAHs) were also detected in fish’s bile for the three different species analyzed. An ELISA kit produced for the native species Geophagus brasiliensis detected vitellogenin expression in male fish. Based on the above findings, a FET study through FA/PCA analysis demonstrated the poorest environmental quality of the reservoir farthest from the river’s source, which is the opposite of what is reported by the environmental agency. The high levels of metals and PAHs in the five reservoirs of Iguacu River associated with the biological responses of fish show the impacts by anthropic activities to this area and not confirming a gradient of pollution from the source towards Iguacu River’s mouth.
TIH01 Sediment quality assessment in Ho Chi Minh City canals. T.L. Bui, Institute for Environment and Resources, Vietnam National University Ho Chi Minh City / Environmental Toxicology; D. Lan Chi, Institute for Environment and Resources, Vietnam National University Ho Chi Minh City / Ecotoxicology; T. Tuan Nguyen, University of Environmental Science and Research, University of Bologna; P. Lara-Martín, Universidad de Cádiz Spain / Physical Chemistry; M. Casado-Martínez, Centre Ectocos; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology Ho Chi Minh City metropolitan region is the largest urban and industrial hub of South Vietnam, and millions of number of big factories and artisanal plants. Located in the Mekong delta, the area is characterized by a complex system of canals that play a vital role for the region and its inhabitants. They act as reservoirs for water run-off and drainage and they are used for transportation and as a source for natural resources. Due to public health concerns an important sanitation program has been undertaken in the last decade for the reduction of wastewater pollution and flooding. At present, the main canals are recovering after clean-up and clean waters have attracted fish again, with increasing stocks thanks to repopulation initiatives. In 2015 we carried out a pilot study to assess the quality of recent sediments from Ho Chi Minh City canals. Surface sediments were collected from 6 canals, 4 of them located in the metropolitan area and 2 from suburban areas. Sediments were subject to physico-chemical and ecotoxicological characterisation, the physico-chemical characterisation consisted of the quantification of trace metals and organic micropollutants and the ecotoxicological assessment through the use of existing chemical quality guidelines and standard sediment toxicity tests. The results show that the sediments from the canals from both the metropolitan and suburban areas accumulate a mixture of contaminants with concentrations of several PAHs and PCBs above the corresponding thresholds for effects to benthic organisms. Several pesticides were quantified in the sediments but the highest concentrations were measured for permethrin in the case of the sediments from the metropolitan area and for bifenthrin in sediments from suburban areas (3692.9 ng/g dw and 477.6 ng/g dw, respectively). Consious concentrations at the µg/g order were found for the fragrances galaxolide and OTNE whereas nonylphenol, triclosan and the UV filters octocrylene, 2-EHDP and EHMDC were measured at all canals at concentrations of several tens and even hundreds ng/g. The results of the chemical analyses were corroborated by the different levels of toxicity to crustaceans, although no or slight toxicity was observed to rooted macrophytes in laboratory toxicity tests.

TIH02 The Impact of Surrounding Land Use and Site History on Metal Concentrations and Bioaccessibility in Canadian Urban Parks M. Dodd, Royal Roads University / School of Environment Sustainability; A. Durojaie, J. Ehizioje, Royal Roads University; K. Allan, SLR Consulting (Canada) Ltd.

To assess the potential risk associated with exposure to metal contaminants in Canadian urban green spaces, surface soil samples taken from exposed areas with high potential for human soil contact at various parks and playgrounds were analyzed for total metals, pH, TOC and metal bioaccessibility. The green spaces were selected from Halifax (10), Saint John (11), Fredericton (11), Ottawa (8), Toronto (17), Montreal (20), Regina (8), Winnipeg (8), Edmonton (17) and Victoria (22). Among the heavy metals, Cr, Pb and Zn were analyzed for samples collected in the Canadian urban parks. The highest Pb concentrations were found in parks located near heavy traffic corridors while elevated Cu, Cr and Zn levels were associated with parks situated either on former industrial sites or near current or past industrial activities (e.g., recycling), heavy traffic corridors and former contaminated sites. Arsenic, Cd and Cr concentrations were particularly elevated in Halifax, Saint John and Fredericton. The elevated concentrations in these three cities reflected natural geological processes since some areas in the provinces these cities occur have high natural As concentrations that exceed human health risk-based guidelines due to bedrock and natural soil enrichment. The highest Pb concentrations were detected in parks located near heavy traffic corridors while elevated Cu, Cr and Zn levels were associated with parks situated either on former industrial sites or near current metal related industrial sites. The mean metal bioaccessibility were As (24%), Cr (80%), Cu (39%), Pb (61%), Ni (17%) and Zn (32%). Based on the total metal concentrations, properties and bioaccessibility data for the risk assessment, exposure to metals in soils in most of the urban parks studied were deemed fairly low except Pb.

TIH03 Mechanism-specific evaluation of a waste water treatment plant and its downstream river Wurm Y. Mueller, RWTH Aachen University / Department of Ecosystem Analysis ESA; S.K. Koenemann, Institute for Environmental Research; S. Hotz, RWTH Aachen University / Department of Ecosystem Analysis ESA; S. Schiwy, Institute of Environmental Research-RWTH Aachen / Department of Ecosystem Analysis ESA; H. Hollett, RWTH Aachen University / Department of Ecosystem Analysis The European Water Framework Directive aims to achieve a good chemical and biological state of all surface waters until 2015. However, the good biological state is only reached by around 20 % of the German surface water bodies. One reason might be the release of a variety of anthropogenic contaminants by waste water treatment plants (WWTs). It could occur that these substances are not sufficiently eliminated via the conventional cleaning processes. Those chemicals can lead to adverse effects. The federal level as well as the state level policy demands the elimination of trace components in the effluent of WWTs by a progressing treatment stage. There are several advanced treatment processes, one example is the ozonation. Based on that a commercial-scale plant should be installed to the WWT “Aachen Soers” associated to the project DemO-AC. This WWT is located in the city of Aachen and releases its effluent to the River Wurm. The status quo of the sediments from the metropolitan and downstream of the rain spillway basin, while the highest feeding rate was found 2 km downstream the WWT. The lowest feeding rate or the highest inhibition is expected to be found immediately after the WWT outlet. To support and strengthen these first findings, further tests are needed.

TIH04 Spotted opportunities amidst environmental degradation: Indigenous soil bacteria of mining sites. O.G. Oladipo, North-West University / Unit for Environmental Sciences Management; O.T. Ezekoki, M. Maboeta, J.J. Bezuindenhou, C.C. Bezuindenhou, North - West University / Unit for Environmental Sciences and Management Diverse anthropogenic activities have resulted to heavy metal pollution and hence environmental degradation, posing huge challenges on soil and water resources as well as human health safety. Indigenous microbes from these degraded

SETAC Europe 26th Annual Meeting Abstract Book 331
environments have proved to be valuable eco-friendly tools in the restoration of such environments. This study investigated the heavy metal resistance profiles of bacteria for possible bioremediation strategy. Bacteria were isolated from soils collected from three active gold and gemstone mine sites in South - western, Nigeria. These were identified using 16S rRNA gene analysis. Isolates were exposed to five heavy metal concentrations viz. mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), aluminum (Al), manganese (Mn), zinc (Zn), iron (Fe) and chromium (Cr) supplemented into broth media. Growth absorbance was recorded by Micro well 96 well plate reader at 520 nm wavelength every 30 minutes for 48 hours. Minimum inhibitory concentrations (MICs) were then determined from isolates to various metal concentrations. The bacterial isolates were: Lysinibacillus macrourus (LM), Achromobacter sp (AS), Bacillus kochii (BK), Klebsiella pneumoniae (KP), Pseudomonas mosselii (PM), Pseudomonas nitroreducens (PN) and Bacillus cereus (BC). All isolates showed resistance to Pb, Ni, Mn, Fe and Al to highest concentrations >300, 100, 600, 600 and 100 ppm respectively while PN, AS and BC showed resistance to Zn (>300 ppm concentration). A 25 ppm Hg, Cr (>100 ppm and Cd >50 ppm were tolerated by PN, PM and KP, while BK, AS and BC tolerated higher concentrations at Hg (>50 ppm), Cr (>150 ppm) and Cd (>100 ppm). Pseudomonas nitroreducens, PM, BK, AS and BC expressed tolerance to Cu >200 ppm concentrations. The heavy metal concentrations to which these bacteria showed resistance exceeded set permissible limits for contaminated soils globally. These results therefore suggest these bacteria isolates as potential bioremediation candidates for restoration of heavy metal polluted environments.

TH105 Bioremediation of oil-polluted soil in Caspian Sea coast by biodegradation bacteria
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The quality of life on Earth is linked intrinsically to the overall quality of the environment. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. In this research 7 genera of biodegradation bacteria which are important in bioremediation process were isolated and identified. We had been used the soil samples of Amir Abad Chamkhaleh (the coast of Caspian Sea). Adding the soil samples to MSM (Mineral Salt Media) culture for isolating the biodegradation bacteria and then these bacteria inoculated to some media which had hydrocarbon as a carbon source and after isolating with alternative culture these bacteria were considered and characterized based on morphological biochemical and fermentation tests. According to the Bergy manual of systematic bacteriology these bacteria were identified. Although, in this study we had been used of plasmid extraction method for considering that if biodegradation genes exist in the plasmid or linear DNA.

TH106 Assessment of the potential of biochar and coal tailings as atrazine sorbents J.B. de Costa, UFRGS / Analytical Chemistry; D.P. Dick, Federal University of Rio Grande do Sul / Physical Chemistry; V. Maciel, Universidade Federal do Rio Grande do Sul
The potential application of organochlorine pesticide residuals in the contamination of ground and surface waters. The objective of this study was to investigate the potential of carbonaceous matrices in the treatment of water contaminated by the herbicide atrazine (ATZ) by evaluating the sorption capacity of ATZ by biochar and nitrated coal tailing samples. Adsorption of ATZ was conducted both on the original coal tailings as on the nitrated samples. The nitration was conducted at 70°C under reflux (soxhlet) employing 25% HNO3 solution. In centrifuge tubes of 50 mL, 0.3 g of adsorbent were weighed and 30 mL of ATZ solution were added, at concentrations of 0, 1, 2, 4, 5 and 10 mg ATZ L-1 (CaCl2; 0.01 mol L-1 medium). After 24 hours stirring in the dark, the suspension was centrifuged (2500 rpm for 15 minutes) and the supernatant was filtered and stored in dark bottles. Aliquots of the supernatant were taken for determining the concentration of atrazine in equilibrium in the solution by ultraviolet-visible spectroscopy (UV-Vis, UV-160 Shimadzu) at 222 nm. In all tested sorbents the adsorption was reversible, indicating that these materials can be reused. The nitration of coal tailings improved their adsorption capacity and this behaviour was related to a change of the surface morphology rather to the increase of surface hydrophilicity. Nevertheless the adsorption capacity of nitrated samples was inferior to that of activated charcoal.

TH107 Biogas from Chemically Substance Flow Analysis in South Korea J. Kim, Inha University; J. Kim, University of Technology of Troyes / Department of Humanities Environment Information Technology CREIDD; J. Moon, Y. Hwang, Inha University
Currently chemical substances and materials are widely used in all kind of industry and household, almost everywhere and in everything. On the other hand, many chemicals contaminants are generated in industry and agriculture. In South Korea, the accidents related with chemicals are getting increased from 7 cases in 2006 to 104 cases in 2014 (MOE, 2015). Along with establishment of chemical regulations such as EU REACH and Korea REACH - The Act on the Registration and Evaluation of Chemicals), many studies should be conducted for sustainable chemical substance’s safety management and prevention of the chemical accidents. In this study, butadiene (1,3-Butadiene, C4H6, CAS NO: 106-99-0) in the important chemicals was selected. The butadiene is flammable, colorless gas with a mild aromatic odor and it is highly reactive. Butadiene is soluble in alcohol and ether, insoluble in water and polymerizes readily, particularly if oxygen is present. One major use of butadiene has been in the making of synthetic rubber. Butadiene is also used extensively for various polymerizations for plastics manufacturing. The purpose of this study, therefore, is to quantify, analyse and make a flow map of the butadiene by applying substance flow analysis (SFA). Our result shows that the amount consumption in each company, location of manufactures using butadiene as well as butadiene’s flow (import, export, stocks and flows) in Korean industrial system (between industries including companies). Finally the result of this study can support for chemical accident prevention and sustainable chemical management in South Korea.

TH108 A Study on Hydrogen Fluoride Substance Flow Analysis: In Case of the Chemical Industry in South Korea M. Yoo, Inha University; J. Kim, University of Technology of Troyes / Department of Humanities Environment Information Technology CREIDD; J. Moon, Y. Hwang, Inha University
Up to date, many chemical accidents were occurred in the world. We know that recently there was a big chemical explosion accident and its expose of toxic chemicals in Tianjin, China. According to the Korean Ministry of Environment, the number of chemical accidents was increased 7 cases in 2006 to 104 cases in 2014. Therefore, it should be considered and conducted many studies on sustainable chemical substance’s safety management and prevention of the chemical accidents as well as establishment of related chemical management regulations. In this study, we selected and studied about hydrogen fluoride (the chemical formula HF, CAS NO: 7664-39-3). This colorless gas or liquid is the principal industrial source of fluorine, often in the aqueous form as hydrofluoric acid, and thus is the precursor to many important compounds including pharmaceuticals and polymers (e.g. Teflon). HF is widely used in the petrochemical industry and is a component of many superacids. In this study, we quantified, analysed and mapped the HF flows in the chemical industry of South Korea by applying substance flow analysis (SFA). Our result shows that the amount of consumption in each company (and industry), location of manufactures using HF as well as HF’s flow (import, export, stocks and flows) in Korean industrial system (between industries including companies). Finally the result of this study can support for chemical accident prevention system and sustainable chemical management in South Korea.

TH109 A Study of Ammonia Substance Flow Analysis in South Korea K. Lee, Inha University; J. Kim, University of Technology of Troyes / Department of Humanities Environment Information Technology CREIDD; J. Moon, Y. Hwang, Inha University
Currently many chemical regulations such as EU REACH and Korea REACH are effected to chemical industry directly. With this regulation, it is required more advanced and useful tools and methods for sustainable chemical management as well as accidents related with chemicals. Although there has been many researches on how to prevent chemical accidents and manage chemicals, very little attention has been paid on chemical substance flow in country level. For this study, ammonia chemical substance was selected. Ammonia or azane is a compound of nitrogen and hydrogen with the formula NH3 (CAS number 7664-41-7). It is a colourless gas with a characteristic pungent smell. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers. About 80% of the global ammonia production is consumed by the fertilizer industry; specifically, 48% of the ammonia produced is deployed in the production of urea (the most commonly used nitrogen fertiliser and basic feedstock for industrial products like plastics, resins and adhesives) (Potschapel, 2013). By using material flow analysis method, ammonia substance flows in South Korea was shown in this study. Required data such as import, export, production and consumption was collected from industry, association and national statistic department. Also, to minimize the information of manufactures companies was analysed. Finally this study can support for the chemical accident and risk prevention system and sustainable chemical management in South Korea.

TH110 DemO3AC Project - Ecotoxicological investigations of an ozonation treatment in the waste water treatment plant Aachen Soers (Germany) S. Schiewe, Institute of Environmental Research-RWTH Aachen / Department of Ecosystem Analysis ESA
The European Water Framework Directive aimed to achieve a good ecological and chemical status in all European surface waters until now. However, only 20% of German surface waters achieved these goals. One reason for this could be anthropogenic pollutants coming from wastewater treatment plants (WWTP),
These pollutants were not completely eliminated in the treatment process and thus enter the aquatic environment. Hence, in the DemO:AC-Project a case study is conducted to demonstrate the implementation of a further treatment step, an ozonation process, to eliminate these pollutants in the WWTP. In this project different ecotoxicological bioassays were applied to evaluate and optimize them in focus. The application of the river Wurm and the effluent of the WWTP. The river Wurm carries about 70 % of the treated wastewater at low and medium water levels. First, the Status quo of the water will be determined and then compared with the data after the implementation of the large-scale ozonation facility. With the standardized test methods for effluent assessment introduced in the new DIN and BfU guideline (daphnia and fish embryo toxicity) acute and chronic effects of anthropogenic trace substances at different trophic levels will be examined. In addition, cell-toxicological test systems will be used to investigate the estrogenic activity, effects on steroidogenesis and the mutagenic effects of the river water and effluent of the WWTP. On the organisms level in vivo and in situ studies are planned with the rainbow trout (Oncorynchus mykiss), the mud snail (Potamopyrgus antipodarum) and the freshwater shrimp (Gammarus spp.). After evaluation the sensitive and most informative tests will be selected. These selected bioassays will be applied for the second stage of the project after the implementation of the new treatment step in the WWTP. A comprehensive chemical analysis of trace substances will be conducted by the Institute of Environmental Engineering (RWTH Aachen, Germany), at the same time-points. In addition, studies on the microbiological composition and antibiotic resistance will be conducted by the Institute of Applied Microbiology (RWTH Aachen, Germany). The project is funded by the Ministry for Climate Protection, Environment, Agriculture, Conservation and Consumer Protection of North Rhine-Westphalia.

TH111
Agrochemical loading in drain and rivers with connection with coastal lagoons in North of Sinaloa, Mexico: a potential risk linking to harmful algal bloom occurrence

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Ocean algal blooms have been related to nutrient accumulation in coastal lagoons in the Pacific Ocean. It is speculated that this nutrient overflow has its origins from the agricultural lands in northern Mexico. Sinaloa has a planted area of a million and a half hectares representing 38% of the vegetables produced in the country and ranking first in the production of grains it also excels in the export of sesame and corn. The value of agricultural production in the state varies in the range of 2 million euros a year and participates with six percent of the national agricultural GDP. Currently, annual pesticide application has been calculated to be 700 tons of 17 different types of pesticides which are classified from moderately to strongly toxic according to WHO. This intensive use of agrochemicals has led to the contamination of water bodies and sediments in the area. Therefore, this study aimed to determine the nutrient load and more persistent chemicals (organochlorine pesticides), chemicals that are subject to the Stockholm Convention. These compounds were determined in surface water bodies of Culiacan Valley, particularly in rivers and drains, as well as in the coastal lagoons that the studied rivers and drains fall into. According to the results, except for the Sinaloa and Culiacan rivers, organochlorine pesticides were detected in all samples, from which the only pesticide absent was heptachlor. According to the Regulation it is recommended maximum concentrations for the protection of aquatic life in freshwater, brackish or coastal water ecosystems, all sampling sites where pesticides were detected in the water column, exceeded the maximum water quality guidelines for the protection of aquatic life. In most of the studied sites, concentrations of ammonium nitrogen as ammonia, nitrite, nitrate and phosphate, significantly exceeded the maximum allowable limits for the protection of aquatic life guidelines established in the Law on Water Rights, for both freshwater and coastal. These results could be related to harmful algal bloom in the area.

TH112
Phospholipid fatty acid profiles as indicators of microbial community composition variations in soil by mixed heavy metal pollution

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Primary information on heavily contaminated urban areas comes from many cities, however, chemical pollution assays are unable to evaluate the integrated biological effect, which shows a ‘cocktail’ of environmental factors acting simultaneously on ecosystem systems. The aim of the study was to assess the impact of the microbial community structure of urban soils by phospholipid fatty acid analysis (PLFA). The soil samples (topsoil layer 0-20 cm depth) were taken at six sites located in the rural part of the cities of Kirov (Russia), differently polluted with heavy metals. The samples were measured for pH and the contents of organic C, total N, total P and total S. The microbial structure of soil microbial communities was assessed using PLFA analysis (high-performance liquid chromatography–mass spectrometry method). The total PLFA content in control sample were found to be significantly higher than the polluted soil. The greatest indication value was given to an Actinobacteria phylum, their concentration decrease remarkably in the polluted samples, and anaerobes Butyrivibrio sp. and Desulfovibacterium sp. found as indicator of soils under relatively low soil pollution status. The microbial profiles were also indicated a selective enrichment of competent species (Desulfovibacter sp., Bacteroides fragilis, Chlamydia sp., T. trachomatis) in soil with high heavy metals contamination. Nonmetric multidimensional scaling plots of soil communities, showing the relative differences in control and pollution soils.Overall, our study could promote to better understand how microbial community structure might adapt to heavy-metals stress. This study is supported by RSF (14-50-00029).

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (P)

TH113
Current and emerging issues in bivalves ecotoxicology

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The lifestyle of bivalve populations makes them species at risk to anthropogenic stressors such as pollution, loss of habitats and climate changes. Indeed, mussels are sessile organisms and could live to relatively long periods in some species (up to decades if not centuries). It is anticipated that global changes are likely to have long-lasting impacts on biodiversity. Local, regional and global mussel populations are directly impacted by upstream disturbances such as urban effluents discharges and loss/mixing of habitats. They are filter-feeders from which they trap and concentrate micro-particles in the digestive gland which represent a major vector for contaminant exposure in mussel tissues. For these reasons, bivalves were selected as sentinel species to assess the toxicity of emerging contaminants such as nanotechnology, oil sand products, endocrine disrupters from municipal discharges and changes in the microbiome. The cumulative effects of complex mixtures and other stressors in these times of climate change were also examined at the molecular and cellular levels in the attempt to identify adverse outcomes pathways leading to the decline of mussel populations. Exposure to xenobiotics increase the susceptibility to temperature changes at the electron transport steps in mitochondria which could increase energy discharges in bivalves at polluted sites under thermal fluctuations. Exposure to municipal effluent and zinc oxide nanoparticles both elicit oxidative stress which could lead to inflammation and phagocytosis suppression. Recent studies also showed that air temperature could also be shortened in mussels exposed to nanoparticles and oil sand contaminated environments which support the contention that mussel species are at risk from urban activities. Studies dealing on the cumulative effects of environmental stressors were also performed. Issues: Cumulative effects (nano and ME /Vibrio and ME), Climate change (SOS), endocrine disruption (feminization) / emerging compound (phama/OS) and molecular approaches for biodiversity studies (eDNA).

TH114
Evaluation of the health status of the blue mussels Mytilus edulis from two contaminated sites in the Bay of Seine (Normandy, France): A seasonal, transcriptomic survey

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The blue mussel Mytilus edulis is a common bivalve forming large populations on natural and artificial hard substrates. These sessile, filter-feeder organisms are able to bioaccumulate and/or metabolize and tolerate a wide range of pollutants, sometimes at high concentrations. They have been extensively used as bioindicators and sentinel species to assess coastal water quality, especially in estuaries. However, mechanisms by which mussels can survive in such polluted environment are not completely understood. In this context, indigenous mussels were sampled every 3 months during a full annual cycle at two contrasted sites situated on the English Channel coast in Normandy (France): Villerville-sur- Mer (Normandy, France) located at the mouth of Seine estuary, a site highly affected by contaminants originating from agricultural runoff, urban sewage, inputs from industrial plants, and Yport (reference site) located on the north of Le Havre which is not directly exposed to the inputs of the Seine river. Previous studies have demonstrated that a large set of biological endpoints are necessary to detect stress responses generated by a complex contamination in highly variable ecosystems. Hence, a battery of molecular and physiological markers was...
investigated to allow the characterization of the spatio-temporal variation of the health status of mussels located in the two studied sites. Biomarkers corresponding to energetic metabolism, immunity/inflammation, stress and detoxification were investigated at the transcriptional level (a total of 43 genes) in both gills and digestive gland. In addition, a condition index was calculated and histology was performed to assess the gill reproductive status as well as the sex ratio of the mussels. Indeed, in marine bivalves, reproduction is a major biological function especially costly in energy. Results indicated that variations of biomarker responses were associated to seasonal variations, the contaminant levels in the environment and the reproductive status of organisms. These results showed the potential of mussel biomarkers to discriminate populations of mussels from four different sites along the St Laurent River were analyzed. Molecular response of the different populations were assessed in the control area. Furthermore, no relevant change was noticed for abcc-like gene expression and effect of season change and contaminants on ABC transporter was investigated in gills and hemocyte extracts by RT-PCR and QPCR on mussels' lysosomal system while flow cytometry approach provided data about reactive oxygen species (ROS) and hemocyte phagocytic activity. One month after the beginning of the study, high mortality rates were recorded but then mussels showed a high ability to survive (up to 10 months) in a contaminated environment where salinity greatly varies and where mussels had thus to face multiple stresses. The present study highlighted the temporal fluctuations of some endpoints and these variations were taken into consideration in the interpretation of data obtained from biomonitoring surveys.

**TH115**

Molecular response of mussels Dreissena sp. to contrasted environments: comparison of different French and Canadian populations

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Zebra mussels from gender Dreissenia are classically used as model species in eco-toxicological studies assessing freshwater ecosystems quality. These filter feeders sedentary bivalves are considered to faithfully represent their living environment. Numerous biomarkers have been developed in dreissenids as tools of freshwater ecosystems biomonitoring. In the context of IPOC franco-canadian research program (Interactions between Pollution and Climate changes: Development of improved monitoring strategy), Dreissena sp. was retained as freshwater model mussel to evaluate biological effects of combined action of pollution and climate change, following French and Canadian populations health. Populations of mussels from four rivers in the north of France (Vilaine, Moselle, Meuse and Oise) were sampled in spring and autumn. In parallel, Canadian populations of mussels from four different sites along the St Laurent River were also sampled in autumn. Condition index was measured. Contaminants contents were analyzed. Molecular response of the different populations were assessed through the following of gene expression by real-time PCR. Candidate genes were chosen for their involvement in cellular energy metabolism (cytochrome-C-oxidase, ATP synthase), antioxidant systems (catalase, superoxide dismutase, glutathione peroxidase) and xenobiotics detoxification (glutathione-S-transferase, metallothionein). Results showed that French mussels tend to present a higher expression level for most of genes in the site considered as the least contaminated. In this site, seasonal variations were more pronounced than in the three others, with expression levels of candidate genes lower in autumn than spring. First, we revealed that some French populations were represented two dreissenid species: zebra mussel Dreissena polymorpha but also quagga mussel Dreissena rostriformis bugensis. This second species appeared as the most widely represented in Canadian populations. As in French populations, expression levels of chosen genes were often higher in mussels sampled in the least anthropized site of the St Laurent. This study shows that mussel from different sites could be discriminated with expression gene responses, what constitutes a major biological function especially costly in energy. Results indicated that variations of biomarker responses were associated to seasonal variations, the contaminant levels in the environment and the reproductive status of organisms. These results showed the potential of mussel biomarkers to discriminate populations of mussels from four different sites along the St Laurent River were analyzed. Molecular response of the different populations were assessed in the control area. Furthermore, no relevant change was noticed for abcc-like gene expression and effect of season change and contaminants on ABC transporter was investigated in gills and hemocyte extracts by RT-PCR and QPCR on mussels’ lysosomal system while flow cytometry approach provided data about reactive oxygen species (ROS) and hemocyte phagocytic activity. One month after the beginning of the study, high mortality rates were recorded but then mussels showed a high ability to survive (up to 10 months) in a contaminated environment where salinity greatly varies and where mussels had thus to face multiple stresses. The present study highlighted the temporal fluctuations of some endpoints and these variations were taken into consideration in the interpretation of data obtained from biomonitoring surveys.

**TH117**

A step forward in multixenobiotic resistance: first evidence of ABCG2-type gene expression and effect of season change and contaminants on ABC mRNA expression in gills and hemocytes of the blue mussel

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Multixenobiotic resistance (MXR) is a powerful defence system mediated by transmembrane proteins known as ATP binding cassette (ABC) transporters that bind and actively remove xenobiotics from the cell. The aim of this study was i) to identify the gene encoding the breast cancer resistance associated protein (BCRP) in hemocyte and gills of Mytilus edulis, ii) to explore the effect of season change and site pollution on the gene expression of three ABC transporters identified in the blue mussel (ABCB, ABCG2 and ABCG2) and iii) to correlate transcripts levels in gills with efflux transporter activities. The expression of ABCG2/BCRP transporter was investigated in gills and hemocyte extracts by RT PCR and 5’RACE. The PCR products had the expected size in both tissues. The identified partial cDNA sequence of ABCG2 overlapped with ABCG2 homologues of other organisms from 72 % to 98 %. The expression quantification of abcb-like, abcg2-like and abcg2-like genes was carried out using RT-QPCR on mussel’s extracts collected during 4 seasons from areas identified by the program IPOC (Interactions between Pollution and Climate changes) for being differentially impacted by chemical contaminants in Normandy. Results indicate that transcripts levels of ABC transporters in gills and hemocytes significantly fluctuated along seasons. Overall, we observed an increase in ABC gene expression during February for both tissues. In hemocytes, abcb-like and abcg2-like transcripts were significantly up-regulated in mussel populations from the marina of Carentan (Normandy) which is located downstream at drinking water supply than those collected from the contaminated site at the same period. The trend was reversed for the other seasons where abcg2-like expression increased significantly in the control area. Furthermore, no relevant change was noticed for abcc-like expression. In contrast, gills exposed to pollutants showed a higher abcb-like and abcg2-like transcript levels in February and conversely a lower abcg2-like expression while no difference was noted for the other seasons. We also studied the efflux activity by measuring the accumulation of calcein in gills in presence of ABCB and ABCC blockers. A higher transporter activity was measured in tissues obtained from the contaminated site which is concordant with the transcripts levels. Altogether, these results suggest the regulation of ABC transporters by season change and water contaminants besides their involvement as protective and tissue specific response xenobiotics.

**TH118**

Effects of a realistic multiple low dose pesticide exposure scenario on aquatic communities in stream mesocosms

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The current common EU risk assessment for pesticides refers to effects of single substances. As one result of this risk assessment a regulatory acceptable concentration (RAC) for each pesticide is estimated, which should not lead to any unacceptable adverse effect on non-target organisms in freshwater systems. However, in agriculture and horticulture, pesticides are commonly used over time and space to protect crop from pests. Hence, communities inhabiting
streams in agricultural landscapes are exposed to mixtures of pesticides and their metabolites, which might cause a multiple-stress scenario for these non-target organisms. For example, in orchards a high number of pesticides with different modes of action are repeatedly applied in short time intervals during the whole vegetation period. However, potential additive or even synergistic effects of repeatedly exposed mussels are not well investigated at the organism level. Hence, we studied the effects of a realistic pesticide spray scenario for apple crop protection on aquatic communities in surface waters using eight indoor stream mesocosms. Aquatic invertebrates from a reference site were added to the stream mesocosms using colonized straw bags. Overall, nine fungicides, four herbicides and four herbicides were applied at their respective RACs for surface waters to each of the four treatment mesocosms within a period of two and a half months. At nineteen days altogether pesticides were used 36 times with up to four different pesticides per date. Most fungicides were used repeated up to seven times. In order to detect potential effects on the aquatic community we measured a variety of endpoints. We focused mainly on sublethal effects, which are more likely to occur in a multiple low dose pesticide exposure scenario. Potential short-term effects like behavioral changes within several hours were determined by measuring drift and activity patterns of benthic invertebrates. Long-term effects were measured by changes in the community composition of invertebrates and the emergence of meromictic insects. Furthermore, we investigated potential effects within the food web structure using stable isotope analysis and fitness of invertebrates using the RNA/DNA as proxy. We compared the results of different endpoints in order to quantify the impact of the multiple stress scenarios on different scales.

TH119
Identification of relevant toxicant mixtures influencing the macroinvertebrate community of the Danube river
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Aquatic organisms inhabiting large rivers are exposed to a wide range of contaminants including down-the-drain chemicals (e.g. pharmaceuticals, food additives, home-care products), industrial pollutants (e.g. metals, PAHs) and pesticides. It is therefore evident that observed changes in biodiversity are likely to be explained by toxicant mixtures resulting in additive, synergistic or antagonistic responses at the individual, population and community levels. Besides the large number compounds and possible mixtures occurring in the environment, it is expected that only a limited number of substances are responsible for the observed biological responses. Identifying these chemical mixtures is key to understand their combined toxicity mechanisms and to derive responsible risk abatement options. The main objective of the current study was to identify toxicant mixtures that are influencing the macroinvertebrate community monitored in the Danube river. A large database obtained as part of the environmental monitoring campaign performed during 2013 in the Danube river was used in this study. The database contained abundance data for 937 invertebrate species monitored in 55 sampling sites of the Danube river, and measured chemical concentrations in each sampling site. The chemical dataset contained water concentrations for 227 chemicals, including pharmaceuticals, pesticides, PAHs, metals, food additives, and home-care products. Prior to the mixture toxicity analysis, concentrations were calculated that represent the macroinvertebrate community in each of the sampling sites. These were: taxa richness, total abundance of individuals, Shannon diversity index and species evenness. Chemical mixtures significantly influencing the variability in the community indices were identified by selecting best-fit Generalized Linear Models (GLMs) containing binary, tertiary, quaternary and quinary chemical combinations. The selection of best fitting models was performed using the RSELECT procedure in the GENSTAT software. The best fitting models were obtained for species richness, with R^2 values of up to 63%. Relevant chemical mixtures included, in most of the cases, pharmaceuticals (e.g. anti-depressive and analgesics) and anti-microbial agents and some food additives. Further research should aim to study the mechanistic link between the identified chemical mixtures and the community responses using laboratory and/or semi-field experiments.

TH120
Characterizing a nanocaps for the evaluation of toxicant effects on an aquatic community
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When organisms are exposed to toxicants they can be influenced directly when e.g. feeding or reproduction are impaired and indirectly when interactions with other components of the ecosystem are impaired. These effects can be studied in micro- and mesocosms but they are expensive, time and labour demanding and, in general, few replicates are used. With ecological complexity the variability between replicates increases and an extrapolation of effect/response relationships is more difficult. In regulatory risk assessment, a tiered effect assessment approach is used to reduce the number of experiments performed. Further research should aim to study the mechanistic link between the identified chemical mixtures and the community responses using laboratory and/or semi-field experiments.

TH121
Oscillating population dynamics: chemostats to study direct and indirect toxic responses in simple prey-predator system
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Keywords: Chemostats, Daphnia magna, algae, atrazine. Understanding the impacts of natural and anthropogenic stressors on ecosystems requires analyzing responses at the level of ecological interactions between individuals and populations. This obviously also to the anthropogenic pollution (e.g. pesticides, PAHs) and metal. A pioneering approach is therefore to minimize the scale of contaminant impacts in the ecosystem, while to test for investigating propagation of effects at higher ecological levels are not still well established. We developed a chemostat-based tool which allows reproducing "simple", self-regulating and ecologically dynamic systems (e.g. primary producer-grazer system). Through the chemostats we conducted experiments to investigate chemical (or other stressors) impacts in a simplified, though fully functional, biological system. Chemostats with oscillating cycles of a primary producer population (Pseudokirchneriella subcapitata) and a grazer population (Daphnia magna) where obtained and maintained over 3 months. A set of chemostats were routinely spiked with Atrazine to maintain this photosynthesis inhibitor close to the EC50 and below NOECvalues. The addition of the atrazine to the dynamic system modified trophic transfer affecting Daphnia population dynamics despite atrazine, at these exposure levels, did not have any direct effect on the grazer population. The Chemostat was therefore successfully used to illustrate how impact of chemicals can be transferred across trophic levels and produce implication for the biological system functioning as a whole.

TH122
The effect of selected biocides on algae communities in water bodies affected by stormwater runoff
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In urbanized areas many biocides are in use to prevent unwanted growth of organisms on e.g. construction materials and wood. Over time, the biocides leach from the urban materials and are transported with stormwater runoff to receiving waters. As biocides are non-selective, they can affect non-target organisms and thereby cause unintended harm to the surrounding environment. A common approach for stormwater management is to establish wet detention ponds. They fulfill dual purposes. Firstly, they detain the runoff water during heavy rain and hereby reduce the hydraulic loads on the receiving waters. Secondly, they hold stormwater runoff for prolonged durations, allowing natural treatment processes to proceed. Due to their permanent water pool they furthermore present themselves as habitats for flora and fauna, and rapidly become populated with species similar to what is found in natural ponds. The biota in the stormwater ponds hence become subject to the biocides from the building materials. This study focuses on selected biocides; terbutylazine, iprozole and carbendazim, and their effects on the algae growth and change in the community composition at conditions found in stormwater ponds. The biocides were studied in three test systems. Two were microcosms at controlled laboratory conditions with stable temperature and a light exposure of 12 h per day. These ran for 10 days, with algae sampling at day 1, 3, 5 and 9. The first setup was conducted in glass bottles filled with pond sediments and water from the same pond. Water samples, from a wet detention pond in the city of Silkeborg, was collected from 5 locations allocate evenly in the pond and mixed thoroughly. The second controlled microcosms study was performed in glass sediment cores, filled with pond sediments and water from the same pond. For both setups, stock solutions of all biocides were added in varied concentrations. The third setup consisted of microcosms placed in the pond from where also the water and sediments had been sampled. Here, the added biocides and algae community were exposed to natural repeatable) raise concern, are micro- or mesocosms employed. Few small systems have been developed to describe direct and indirect toxicant impacts on multi-trophic communities and they are rarely tested for reproducibility. This project aims to develop and characterize a small, multi-trophic aquatic test system to bridge simple single-species tests and complex multi-factor micro-/mesocosms. Species with short life cycles and for reproduction times are used to keep test durations short and reproducibility is increased by using a simple test design. This allows the assessment of toxicant impacts on basic community processes and system dynamics, and the extrapolation of causal/mechanisms. The system will be challenged with pesticides and the outcome will be compared to the predictions made with an Individual Based Model (IBM) developed with data obtained in single-species tests. It will be evaluated whether effect data of system components are sufficient to predict community dynamics or if it is necessary to assess the impacts on the system as a whole (including species interactions). Here we present an overview of the nanocosm development, experimental protocols and control variability.
changes in temperature, sunlight and rain. This experiment ran for 15 days, with algae and biocide sampling several times during the period of exposure. The development in algae communities and its dependence on biocide levels were compared in the 3 setups, yielding results on the impact of the biocides on community composition and on cell numbers and cell sizes.

TH123

ECOSCOPE: a mesocosm facility to evaluate stressors on impact water streams across multiple levels of biological organization

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Predicting long term consequences of stressors is a key challenge of environmental risk assessment. Most of the developed methodologies rely on either standardized tests (or on well known bioevaluation methods for a priori evaluation). However, long term effects of stressors on aquatic ecosystems are still poorly explored. Besides that, usual protocols generally focus on only one compartment and/or biological scale, neglecting biotic interactions, functional processes and more typically effect propagation. To this end, the development of a facility is needed to consider top-down feedback and ecological non-monotonous responses. To help the scientific community to address these issues, an outdoor mesocosm platform with 18 artificial channels of 3 meter long, with water circulation and roof system has been built to simulate headwater streams, and to explore the responses of the ecosystem set in place on the long term (several months), with a systemic approach taking into account several biological compartments (macroinvertebrates, algae, etc.). These were designed to allow for changes in flow velocity, thermal and chemical conditions and habitat structure, and, obviously to combine these abiotic parameters with contaminants to explore multi-stress conditions. They are equipped with sensors for a real-time tracking of temperature, oxygen concentration and pH. The platform is open for research project in collaboration with academia, stakeholders and private investigators.

TH124

A fish multi-biomarker approach, including main physiological functions, to better determine global effect of contaminant on ecosystem

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Risk evaluation, due to the presence of toxic compounds in the environment, brings up the necessity to better understand their global effect on ecosystem. In this way, in addition to traditional biochemical biomarkers, some laboratories proposed to study the responses of biomarkers in relationship with the main physiological functions (reproduction, survival, maintenance, behaviour). This approach favors the evaluation of links between biochemical responses and individual/population effects. In this context, a multi-biomarker approach was used in four stations of a French river (La Loue – Franche-Comté region) surrounding area of drastic fish mortalities. In each station, 20 bullheads of both sexes were caught in spring and autumn by electrofishing for further biomarker analyses. The major results shown that the global health indicator (index condition, lysesom membrane integrity and liperoxidation), the indicator of reproduction (gonado-somatic index and the maturation stage), the biomutation enzymes and chromosomique damages were never modified regardless to station. Nevertheless, immune response and neurotoxic activity were strongly modified in the stations with fish decline. All these results could imply an important immune request and/or the presence of neurotoxic compounds in the river, inducing fish mortalities. This study showed the relevance of using multi-biomarker approach, including main physiological functions, to better determined effects of various contaminants on ecosystem health.

TH125

The three spined-stickleback’s digestive enzymes as new biomarkers in environmental monitoring: effect of cadmium and temperature

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The three-spined stickleback, is a freshwater, brackish, and marine fish species with large distribution throughout the northern Hemisphere. This pollution-tolerant fish is present in most European streams and small rivers but also in coastal and estuarine areas, which make it a suitable candidate for environmental monitoring and risk assessment of aquatic ecosystems. Several biomarkers have been developed for this animal model. This study aims at characterizing and developing a new category of biomarkers in relation with energy acquisition, in sticklebacks. Among these parameters, digestive capacities of the three-spined stickleback’s digestive enzymes as new biomarkers in environmental monitoring: effect of cadmium and temperature, were observed. Such enzymatic activities (height, weight, fulton’s condition index) and digestive enzymes (trypsin, intestinal alkaline phosphatase and amylase) were measured at 15, 60 and 90 days interval after cadmium exposure. The results have demonstrated a significant decrease in biometric parameters, from the 3rd month, especially among groups subjected to a temperature of 21 °C. A decrease was observed in the important alteration of the activity of the different enzymes over time and especially for trypsin and alkaline phosphatase in individuals exposed to cadmium at 21 °C suggesting interactions between chemical and physical stresses on biological responses. For the first time, the digestive enzymes of the three-spined stickleback are reported as new environmental biomarkers.

TH126

Pesticide effects on stream fungi in a realistic apple-crop exposure scenario

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Significant amounts of pesticides applied in agriculture may reach surface waters via spray drift or runoff. However, the impact of pesticides on aquatic fungi has rarely been investigated, although fungi in streams and other inland waters play a key role in the decomposition of organic matter and therefore as plant litter. Here we assessed the effects of pesticides on aquatic fungi in a stream mesocosm experiment conducted in a large indoor stream facility. The experiment was designed to mimic a realistic apple-crop exposure scenario involving multiple low-dose pulses of pesticides. Our specific goals were to assess whether (i) quantitative polymerase chain reaction (qPCR) is a useful method to monitor the aquatic fungi abundance; (ii) aquatic fungi are affected by pesticide pulses at low doses; and (iii) individual fungal species can serve as indicators of pesticide impacts. Dried alder leaves (Alnus glutinosa) were inoculated with suspensions of homogenized mycelium obtained from two strains of aquatic hyphomycetes, Cladosporium herbarum and exposed in eight stream mesocosms. Four mesocosms were served as untreated controls. The other four were dosed seven times with six different fungicides, once with two herbicides and twice with two insecticides. ‘Regulatory Acceptable Concentrations’ (RAC) of these pesticides were applied on a total of eight days within two months. Up to four different fungicides were applied on a single day. Endpoints included abundance (qPCR) of the three target fungi, ergosterol as an indicator of total fungal biomass, and fungal spore production. Rates on leaf litter breakdown were also determined. Results are discussed in relation to their implications for risk assessment of pesticides.

TH127

Combined toxicity effects of binary pesticide mixtures on luminescence bacteria Vibrio Fischeri

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Pesticide have been mostly used as plant protection products (PPP) for agricultural and non-agricultural purposes. Various pesticides released to the aquatic environment have been found in the form of mixtures. In this study, luminescence marine bacteria Vibrio fischeri was used as a target living organism to measure toxicities of 7 single pesticides (chlorophrom, dimethoate, diquat, dibility monohydrate, acetamiprid, clomazone, dazomet, and dodine) which were selected in a list of PPP and their 21 binary mixtures. Additionally, to classify the combined effects at equitoxic ratio, toxic units (TU) was calculated with concentration of single chemical in mixture divided by EC₅₀ value of their single chemicals. Theoretically, the concept of 1TU is assumed as additivity, but the combined effects were classified as additivity when TU ranges from 0.8 to 1.2 considering human errors in this result. Based on TU values, 10% of total mixtures showed synergistic effect and 38% and 52% of mixtures indicated additive and antagonistic effects at EC₅₀ level, respectively. As single chemicals, dichlothyl, chlorpyrifos, and acetamiprid, clomazone and acetamiprid seem to be closely related to synergistic effect, while the most number of dodine and dazomet were found in antagonistic combinations, suggesting that dodine and dazomet are strongly involved in antagonistic mechanisms. Interestingly, dodine was much more involved in combinations having antagonistic effects, though it had considerably higher toxicity than six pesticides at EC₅₀ level.

TH128

Preliminary approach for the study of the effects of multiple stress factors on honeybees

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Bee decline is a problem of high concern all over the world. Since the late '90s, a complex pathology (Colony Collapse Disorder: CCD) brought to widespread evidence of honeybee disappearance especially in the U.S. and in Europe. It was not always possible to relate them to a specific cause. Many adversities may be responsible of CCD events and of the general honeybee decline: recrudescence of old and new pathologies, environmental stresses, including climate change, contamination from pesticides and emerging contaminants (e.g. nanoparticles) and electromagnetic factors that may interact among them and show synergic effects or multiple exposure ways. This work is a preliminary attempt to evaluate the effect of combined stress factors (pesticide mixtures and electromagnetic fields) on honeybees. Three experimental sites where selected in northern Italy: a control site, far from agricultural field and from any significant human settlements; an agricultural site, located in a farm where different crops (orchards) are present with a complex and controlled situation of pesticide application; a semi-natural site, far from agricultural areas and close to an electric line generating a strong electromagnetic field. In each site some experimental hives were posed. The sites were sampled monthly from May to October 2015. At each sampling date a suitable number of bee (both workers and standing) were sampled for biochemical (acetylcholinesterase, lipid peroxidation, antioxidant enzymes) and genetic ( Comet assay) biomarkers. Some observation were made on the health status of the colony. Moreover, electromagnetic field measurements were performed and samples of environmental matrices were collected for quantifying pesticide exposure and for calibrating suitable exposure models.

Molecular biological markers were set from the meteorological station closer to each sampling site. The effects of different stress factors are evaluated and compared. The work will be used as pilot project for the development of a more extensive research project on multi-stress effects on bee decline.

TH129

The sublethal effects of epoxicozalone and α-ocypethrin toward Daphnia magna reproduction and neonates’ size

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Azoic fungicides and pyrethroid insecticides, two classes of pesticides commonly used in agriculture, can reach surface water due to spray drift and run off after rain events. Because of the opportunity of both azoles and pyrethroids to enter surface water, and since azoles have been shown to synergize the effect of pyrethroids, the effect of their mixture is of concern. In particular, the sublethal effects of low concentrations are of interest, since these are the concentrations most often occurring in the environment. Beside the commonly described adverse effects of pesticide exposure on mortality, growth and reproduction, it is also generally recognized, that exposure to low doses of insecticides may result in hormetic responses. Hormesis is defined as a biphasic response, where the effect of exposure to low doses is opposite than the effect at high doses. One of the proposed explanations for hormesis due to low concentrations of insecticides is an initiation of the induction of detoxification enzymes such as cytochrome P450. The aim of the present study was to investigate the effect of sublethal doses of an azole fungicide (epoxicozalone) and a pyrethroid insecticide (α-ocypethrin) and their mixture, on Daphnia magna P450 activity and the aquatic crustacean D. magna. The study was designed to investigate the following hypotheses: 1) azole fungicides act as synergists of pyrethroid insecticides also at sublethal endpoints such as growth and reproduction 2) a possible trade-off between size and number of neonates may be present for the organisms exposed either to single compounds or to the mixture and 3) low doses of epoxicozalone stimulate cytochrome P450 activity in vivo thereby a) increasing the detoxification of α-ocypethrin resulting in less harm to reproduction or b) increasing metabolic costs of detoxification leading to lower growth and/or reproduction. The experiment was designed to separate and count neonates over time, while measuring length and protein content of mothers and neonates as well as cytochrome P450 activity of mothers over time. The cumulative reproduction of D. magna varied according to time and exposure. The reproduction observed in the different treatments will be compared and associated with length and protein content of both mothers and neonates and with cytochrome P450 activity of the mothers in order to address the three hypotheses.
Due to the wide use of surfactants, in personal care product, pharmaceutical, accumulation compartment. surfactants are likely to sorb on surface. Previous work has shown their presence and result in notably high hazard quotients. Due to their amphiphilic properties presence of a local impact resulting from the discharging contaminated Surfactants homologues profiling by non-target screening in sediment and aquatic ecosystems. Indeed, surfactants are often produced together with other environmental monitoring could underestimate the presence of surfactants in addition, the traditional approach to focus on a few target surfactants for help to estimate the exposure and risks of the respective aquatic ecosystem. In gradient in order to assess the impact of multiple stressors on a groundwater-fed ecosystem health, many biological indices tend only to reflect the ecological quality of water surface, rather than of the sedimentary zones where the accumulation of pollutants is often highest. To address this issue, we monitored meiohentic (i.e. nematodes) and macrohentic invertebrates along a pollution gradient in order to assess the impact of multiple stressors on a groundwater-fed stream, and thus, to quantify the link between chemical and ecological status. Physical conditions were comparable among sites. The studied stressors included point source pollutants coming from contaminated groundwater and aquaculture, and diffuse source pollutants originating from conventional agriculture and urban areas. The results indicate a change in community composition for both meio- and macrohentic fauna, pointing towards the presence of a local impact resulting from the discharging contaminated groundwater, which extends downstream along a dilution gradient of the groundwater contaminants. Ecological impacts could be linked to xenobiotic compounds coming from groundwater (both chlorinated solvents and pharmaceuticals), as well as the presence of trace metals (particularly copper and aluminum).

Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (P)

TH133 Disentangling ecosystem stressors along a river continuum covering a pollution gradient U.S. McKnight, Technical University of Denmark (DTU) / Environmental Engineering; A.T. Sonne, Danish Technical University / DTU Environment; J.J. Rasmussen, Aarhus University / bioscience; W. Traunspurger, University of Bielefeld / Department of Animal Ecology; S. Hös, Ecossa; P.L. Bjer, Technical University of Denmark DTU / Environmental Engineering Environmental pollution is intrinsically linked to the way humans live. Many of the environmental problems we face today have existed for decades; what has changed is our understanding of the key drivers, processes and impacts. In 2009, the environmental by Monitoring and Assessment Systems (MS) across the European Union (16-36 yr cycles) on the status of their water bodies found that rivers and transitional waters were often in worse condition than lakes and coastal waters. This is not surprising considering that streams integrate all of the diverse stressors found within a catchment (e.g. contaminated sites; diffuse source pollution; water abstraction). The chemical status of a water body is relatively straightforward to assess, defined in part by environmental quality standards on priority substances and in part by additional regulations imposed by individual MS. However, the biological quality elements used for the classification of ecological status are only loosely defined, leaving MS free to develop their own assessment tools. Although useful for the individual MSs, it impedes methodological standardization across different countries, thus contributing to inconsistencies and data gaps across Europe. Moreover, despite the unambiguous importance of benthic habitats for overall ecosystem health, many biological indices tend only to reflect the ecological quality of surface water, rather than of the sedimentary zones where the accumulation of pollutants is often highest. To address this issue, we monitored meiohentic (i.e. nematodes) and macrohentic invertebrates along a pollution gradient in order to assess the impact of multiple stressors on a groundwater-fed stream, and thus, to quantify the link between chemical and ecological status. Physical conditions were comparable among sites. The studied stressors included point source pollutants coming from contaminated groundwater and aquaculture, and diffuse source pollutants originating from conventional agriculture and urban areas. The results indicate a change in community composition for both meio- and macrohentic fauna, pointing towards the presence of a local impact resulting from the discharging contaminated groundwater, which extends downstream along a dilution gradient of the groundwater contaminants. Ecological impacts could be linked to xenobiotic compounds coming from groundwater (both chlorinated solvents and pharmaceuticals), as well as the presence of trace metals (particularly copper and aluminum).

TH134 Surfactants homologues profiling by non-target screening in sediment and Chadophora extract from freshwater river. H. Byers, L. Krauskopf, M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; M. Schmitt-Jansen, UFZ - Helmholtz Ctr Ecol Res, Research Department of Biotechnology/Ecotoxicology; W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis Due to the wide use of surfactants, in personal care product, pharmaceutical, pesticides, their presence is rather ubiquitous in the environment. Previous studies have shown that surfactants may be found in the aquatic environment at concentrations, which are below the limits for other micropollutants and result in notably high hazardous. Due to their amphiphilic properties surfactants are likely to sorb on surface. Previous work has shown their presence in sediment, but few have been investigating Chadophora as potential accumulation compartment. Chadophora are often found in nutrient rich river waters downstream of WWTP output, a known emission source of surfactant in the environment. The monitoring of chemicals like surfactants, in these algae will help to estimate the exposure and risks of the respective aquatic ecosystem. In addition, the traditional approach to focus on a few target surfactants for environmental monitoring could underestimate the presence of surfactants in aquatic ecosystems. Indeed, surfactants are often produced together with other homologues having different chain length, these surfactant profiles can as well be found in the environments. Therefore non-target screening of surfactant homologues would bring a better understanding of the fate of surfactant in aquatic ecosystem. In a case study, Holtemme River in Saxony-Anhalt (Germany), which is impacted by WWTP effluents, has been sampled for sediment and Chadophora. Non-target screening with special focus on surfactant homologues was performed on in several compartments, such as sediment and Chadophora. The algal toxicity of the environmental fraction was performed too. The method used for this study includes sequential extraction by Pressurized Liquid Extraction, combined with further clean-up steps; the chemical analysis was performed by LC/MS-MS/Obtritrap-MS. Longitudinal Surfactant homologues in the Holtemme River and several of its tributaries have been identified. Point sources of surfactants have been confirmed hot spots for subsequent in-depth investigations have been identified.

TH135 A non-target screening method for contaminants in fish F. Yang, Helmholtz Centre for Environmental Research UFZ; B. Deutschmann, RWTH Aachen University / Institute for Environmental Research Biology V; M. Krauss, T. Schulze, W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirectedAnalysis Compared with target analysis, non-target screening provides more comprehensive information on the chemical burden of fish tissues involving potentially pervasive but unknown compounds. In this study, a non-target screening method was developed to detected contaminants in fish muscle. Tissues were homogenized and then extracted, purified by QuEChERS and subjected to non-target screening in two steps: first, a high purity positive mode with UPLC-Q-Orbitrap-MS. Method development was based on 105 compounds covering a wide range of physiochemical properties and including chemicals that have been found in fish tissue before. The recoveries ranged from 6-167% for all the compounds and 70-110% for most of the compounds. The matrix effects were also in acceptable levels, mostly varying from 85-120%. The method was applied to assess the chemical exposure of contaminants in the fish collected from River Holtemme, Germany. Several contaminants were identified in the fish including naphthalene, benzo(phenanthrene, fluorenanthene, cypermethrin, 4-octylphenol, dibenzy ether, carbazole, toluidole and triclosan-methyl. Further quantitative analysis revealed that cypermethrin and 4-octylphenol were the predominant contaminants in the ng/g dry weight range. Moreover, the accumulation patterns of contaminants in the fish identified by the approach were well in accordance with the input of known contamination sources. The preliminary application indicates that the non-target screening method is a promising tool to disclose the chemical exposure and identify the predominant contaminant/s in fish.

TH136 Biomarker response analysis in fish within the SOLUTIONS project - Case study Holtemme B. Deutschmann, RWTH Aachen University / Department of Ecosystem Analysis ESA; T. Seiler, RWTH Aachen University / Ecosystem Analysis; F. Yang, Helmholtz Centre for Environmental Research UFZ, N. Markert, RWTH Aachen University / Ecosystem Analysis ESA; M. Velki, Josip Juraj Strossmayer University in Osijek, Department of Biology / Department of Ecosystem Analysis ESA; W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis Close collaboration with several case studies is one of the main advantages in terms of the realization of biomarker response analysis within the 7th EU RTD Framework Programme project SOLUTIONS (Solutions for present and emerging pollutants in land and water resource management). By using the infrastructure of these studies, biomarker monitoring approaches can be performed easily. Under the lead of the Helmholtz-Centre for Environmental Research the small river Holtemme was investigated in an interdisciplinary approach. The stream has its source in the Mountain Harz region (Germany) and flows through a number of small medium-sized cities in Saxony-Anhalt. Due to its low water quantity and several anthropogenic point sources the Holtemme is a very valuable model site to assess the impact of pollution induced biomarker responses in aquatic organisms. 10 individuals of brown trout (Salmo trutta) were collected at each of 4 different locations including a “natural” reference site. Locations were downstream waste water treatment plants (WWTPs) and one location in a more agriculturally influenced river section. The utilized biomarker battery covered a broad range of proven endpoints which have been employed in several previous studies: genotoxicity in peripheral erythrocytes (micronucleus test), biotransformation enzyme activity (EROD, GST, CES), oxidative stress (TBARS, CAT), and marker of oxidative stress (acetylcholinesterase (AChE)). To reduce time and sample consumption all common cuvette analysis for these endpoints were adapted to 96-well plate assays. For most of the investigated biomarkers significantly elevated/lowered values for the urban and agriculture-affected sampling sites could be demonstrated in comparison to the reference site. However, the results indicated no similar trend for all biomarker responses along the longitudinal profile of the River Holtemme, but the individual biomarkers gave “hot-spots” of response. Correlation analysis with chemical data...
might help to identify presumed stressors related to the different observed effects.

**TH137**

Simultaneous determination of PAH metabolites in fish bile by gas chromatography-random mass spectrometry

K. Kim, Suwan Noowongsanit, N. Suwanrasmi, J.-E. Löfstedt, National University / Department of Civil and Environmental Engineering

Recently, there have been frequent oil-spills along Korean coastal areas. So many studies have been conducted to assess the marine environment contamination and impacts on the ecosystems. Among the numerous oil originated contaminants, many of the studies have focused on parent pollutants. However, after organisms are exposed to pollutants such as PAHs, xenobiotic enzymes are released in response for oxidation, reduction, hydroslysis and other processes leading to the breaking down of the parent compound to metabolites. The focus of most pollution assessment is often on the parent pollutant not the metabolites though the parent compound has been partially or completely transformed. Therefore, in this study, we aimed to develop an analytical method for PAH metabolites in fish bile samples to enable us to understand PAHs metabolic pathways and their distribution in the marine ecosystem. For this study, ten PAH metabolites were investigated using GC/MS/MS A fast and efficient extraction procedure was developed in order to quantify the ten metabolites of napthalene, fluorene, phenanthrene and pyrene in fish bile. Detection and identification of the analytes were achieved using gas chromatography coupled with tandem mass spectrometry. Deuterium-labeled hydroxy PAHs were used as an internal standard. The established method includes enzymatic hydrolysis, liquid-liquid extraction and derivatization. The recoveries were obtained from flatfish (79.8%-104.2%), marble sole (85.7%-106.6%) and rockfish (88.3%-115.8%) in this developed condition.

**TH138**

Used of fish immune response in environmental risk assessment

A. Hennies, INERIS, I. Porcher, INERIS / INERIS UMR 7 SEBIO ECOT

Growing awareness of the value of ecosystems is linked with the increasing demand by citizens and environmental organizations for cleaner rivers and lakes, groundwater and coastal beaches. This citizens demand is one of the main reasons why the Commission has made water protection one of its priorities. This explains the restructuring process of the Water Framework Directive (WFD 2000/60/EC) which concerns the general protection of aquatic ecology, specific protection of unique and valuable habitats, protection of drinking water resources, and protection of bathing water. Two elements are integrated in the ecological protection of surface waters: the "good ecological status" and the "good chemical status". Thus, the environmental objectives concern long-term sustainable water management based on a high level of protection of the aquatic environment and the introduction of the principle of preventing any further deterioration of the ecological and chemical status. In fact, the introduction of several environmental pollutants in freshwaters induces adverse effects in aquatic ecosystems by the disturbance of several physiological processes of aquatic organisms. This trend of the ecosystem could be avoided by increasing our knowledge on the chemical injuries induced on the main physiological functions, such as reproduction, maintenance, survival and behavior. In this way, we want to demonstrate the interest of fish immune response in environmental risk assessment using different river samples.

**TH139**

Miniaturization of the salmonella/microsome assay in microsuspension

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The Salmonella/microsome assay is the most used mutagenicity test both for evaluation of chemicals and environmental samples. There are several versions of protocols available in the literature. Miniaturization of toxicological tests has been a tendency in compliance with the concept of the 3Rs (Replacement, Reduction and Refinement). MFP is a successful miniaturized version of the Salmonella/microsome assay, uses liquid medium and has a limited window of response. When quantification of the mutagenic response is important and strains with very different spontaneous reversion rates are used the assays has some limitations. Recently, a protocol that uses equal volumes per microplates was developed and similar results were obtained when compared to the regular Ames test (plate incorporation version). The objective of this study was to miniaturize of the microsuspension Salmonella/microsome assay using agar microplates under the concept of the 3R and different strains with spontaneous revertants rates (low, TA98, medium, TA100, high, YG94). Following the same procedure of the test Salmonella/microsome in microsuspension. Overnight cultures are concentrated 5-fold by centrifugation and resuspended in 0.015M sodium phosphate buffer. A volume of 50µL of cell suspension and 2µL of the sample are added in a tube and mix, then 13µL of this mixture are transferred for each tube containing 12.5µL of 0.015M sodium phosphate buffer or 35 mix and are incubated at 37°C for 48h. After this new assay are added and 200µL are poured into each well containing minimal agar. Colonies were counted after 66h incubation at 37°C. Results were expressed as minimum effective concentration (MEC2), which is the concentration that provides the double of the spontaneous revertant rate. We are presenting data for TA1538 and TA98. Experiments with YG1041 are still being performed. Both protocols MPA and MIR show a good precision and are appropriate for studies with animal liver microsomes.

**TH140**

Chemical and effect screening of sediments in European estuaries: River basin specific pollution versus Europe-wide priorities

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In the frame of the Water Framework Directive (WFD) a good chemical status needs to be achieved in European rivers. The rivers are subjected to continuous discharge of various organic pollutants due to diffused and point sources (i.e. wastewater treatment effluents, agriculture and surface run-off). However, there is a gap between the lack of knowledge on their occurrence and potential ecological effects in different river basins. Disparate regulations of different countries and emission sources can also result in unpredictable scenarios in different European rivers. Therefore, a systematic monitoring of many emerging pollutants is not routinely applied and their environmental fate and ecotoxicological impact is still unknown. In order to assess the contamination patterns and the drivers of site- or estuary-specific effect responses, sediment samples of the estuaries of eight European river basins were collected. Sediments were selected due to their well-known role as a sink for hydrophobic persistent organic pollutants such as PAHs, PCBs or brominated flame retardants. However, in recent studies, it was suggested that also more polar compounds accumulate in sediments and significantly contribute to the general toxicity. The sediments were targeted at chemical screening with high resolution LC- and GC-Orbitrap-MSMS of polar and non-polar organic compounds including for example classical POPs, pharmaceuticals, personal care products, surfactants, pesticides and biocides. Effect patterns were recorded using Danio rerio embryos applying a set of developmental endpoints together with specific biomarkers of toxicity including acetycholine esterase activity, cyto1a and aromatase and behavioral assays such as locomotor response. Passive dosing was applied in order to simulate the exposure of fish embryos to the sediments. Preliminary chemical analysis of 15 pyrethroids and 19 PAHs show that there is a different distribution in different river basins. Also the bioassay results show different toxicity patterns according to the sampling area. This approach will allow for multivariate analysis of contamination and effect patterns in order to group sampling sites and river basins and to identify the drivers of site- or estuary-specific response and thus supports the identification of River Basin Specific Pollutants.

**TH141**

Determination of Pesticide residues in sediment samples from Büyükk Menderes River and Possible risks for agriculture

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Pesticides are widely used because of the intensive agriculture. Runoff from areas treated with pesticides can pollute water. Pesticide residues in surface water can harm plants, animals and also ground water. Great Meandros River is very important for irrigation of surrounding fields in valley and also water system is used for delivery of river water in large area for ensuring irrigation in many crops. Meandros river was diveded in 8 part in the same distance and 8 sediment samples were collected along the river. The samples were analysed by GC/MS-MS and LC/MS-MS. The most prevalent pesticides were detected 44 DDE, Diflumethane, Formamid, Fenvalerate, Carbafuran and Pyrimethanil. A long term contamination of this pesticides could be expected by using them as irrigation water to the food web.

**TH142**

Effect-directed analysis (EDA) of Danube River water samples receiving untreated municipal wastewater from Novi Sad, Serbia

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The presence of endocrine disrupting chemicals (EDCs) in municipal wastewater has raised great concern regarding environmental health of aquatic ecosystem. Biologically active substances may cause harmful effects to human health and environment due to their potential to disrupt the endocrine system. The city of Novi Sad is one of the hot spots of pollution in the Danube River emitting untreated municipal wastewater into the river. The objective of this study is to identify EDCs and compounds causing oxidative stress responses in Danube river water influenced by Novi Sad wastewater through effect-directed analysis (EDA). Water samples from Danube River were extracted on-site using large volume solid phase extraction (LVSPE) into three different fractions i.e., neutral, acidic & basic with chromatobad HRX sorbent (hydrophobic polysyrene-divinylbenzene copolymer). These extracts were pre-screened on GeneBLAzer bioassays (mammalian cancer cell lines) for agonistic and antagonistic hormonal activity for three nuclear hormone receptors (estrogen, androgen, progesterone) and one for (mammalian cancer cell lines) for agonistic and antagonistic hormonal activity for phase extraction (LVSPE) into three different fractions i.e., neutral, acidic & basic with chromatobad HRX sorbent (hydrophobic polysyrene-divinylbenzene copolymer). One of the major challenges that environmental toxicologists and risk assessors are facing is the identification of exact toxicants in the complex exposure scenarios that are typical for many environments which we wish to protect. For that purpose, a linkage of effect data and hazardous compounds is required. In this study, bioassays, software simulation and chemical analysis were integrated to evaluate the potential ecological risk in the Danube River. The results indicated that samples from all three hotspots that were confirmed by fish embryo test (FET) induced significant genotoxic effects. According to the simulation of QSAR Toolbox and ChemProp, pesticides and PAHs were predicted to be the most genotoxic chemicals. Similarly, chemical analysis demonstrated that pesticide, especially 2,4-dinitrophenol, is the main genotoxicant in the Danube River. Ecological risk assessment by risk quotient (RQ) method illustrated that 2,4-dinitrophenol caused medium level risk in the Danube River.

TH144

A snow/fern two-century record of polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) at GV7 (Antarctica)

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A two-century record of fourteen polycyclic aromatic hydrocarbons (PAHs) and seven polychlorobiphenyls (PCBs) was obtained from two snow/fern cores gathered at GV7 (Antarctica) in order to assess the contribution of human activities to global environmental pollution. The total concentrations of 2PAH14 and 2PCB7/8were 1.2 ng/l and 0.09 ng/l in the deepest fern sample analyzed, and 3.4 ng/l and 0.22 ng/l in surface snow samples invested over the last few years, respectively. Our data highlighted the presence of PAH maxima in correspondence with the major volcanic eruptions in the period 1800–1930. Between 1930 and 2002, PAHs showed an overall 50% increase, with a slope of above 0.013 ng/l year–1. This trend can be predominantly attributed to the emission of incomplete combustion processes of organic matter related to anthropogenic activities. PCBs show a much higher increase (+200%) with a slope of 0.0034 ng/l year–1 in a very limited period (1930–1980) which is almost totally due to the massive industrial production and use of PCBs, here named “industrial PCB excess”. The slight tendency of PCBs to a constant level from 1980 to 2012 might be attributed to the reduction in the industrial production of PCBs and the restricted use only in totally encloosed systems which started in many countries in the late 1970s.

TH145

Priority of Pharmaceuticals in the Natural Environment in Kazakhstan

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University / Management and Engineering in environmental protection Pharmaceuticals are readily available in Kazakhstan with most of them being freely available for purchase over the counter. Wastewater treatment systems in Kazakhstan are also old and employ old technologies so the treatment may not be as effective as in western countries. Consequently, emissions of pharmaceuticals to the natural environment in Kazakhstan are expected to be high and impacts could be greater than elsewhere in the World. However, unlike contaminants such as metals, oil and rocket fuels, which have been heavily studied in Kazakhstan, there are no data on the concentrations and impacts of these substances in rivers and streams in the country. In this poster, we present a desk-based study to prioritize active pharmaceutical ingredients of most concern in surface waters in Kazakhstan. Initially data were collected on the number of products and active ingredients for different therapeutic classes in use in Kazakhstan and on the typical doses and treatment durations. These data were then used alongside simple exposure modelling approaches to estimate concentrations of active ingredients (+/- uncertainty) from around 1800 products) in surface waters in Kazakhstan. For each of the active ingredients, the potential hazard to aquatic organisms was predicted based on human pharmacology data and also using quantitative structure-activity relationships. Risk quotients were then calculated for each pharmaceutical based on the exposure and hazard predictions and the substances ranked in order of risk quotient. In the future, experimental studies are planned in Astana, the capital of Kazakhstan, to characterise the concentrations of the top priority compounds in surface waters and to monitor whether impacts of these could be occurring in reality.

TH146

An integrated approach to assessing exposure and ecotoxicological impacts in the State of Qatar

C.S. Warren, S. Saeed, ExxonMobil Research Qatar / Environment and Water Reuse Program; S. Prakash, V. Kolluru, Environmental Resources Management Exposure science and ecological risk assessment continues to develop and evolve in the State of Qatar. Efforts have been taken to verify the literature, validate methodologies developed in North America, Europe as well as other relevant hot and arid countries. In many cases, however, the extreme environmental conditions (e.g., high water temperature and salinity) and differing ecosystem structures (e.g., warm water species such as corals) require adaptation of the typical approaches and tools. At EMRQ we are developing an integrated approach to assess discharges, fate, exposure and ecotoxicological impacts in the marine environment. Our initial focus has been on the Liquefied Natural Gas (LNG) industry which is important to Qatar and other global regions; and increasingly so for North America and Europe. We have worked closely with our industry partners to robustly quantify discharges and model transport/fate using a spatially and temporally resolved hydrodynamic model combined with laboratory-based data. We also incorporate measured field data for evaluation of these tools and to assess ecological impacts. In the laboratory we have developed ecotoxicology protocols for reference species applicable to Qatari waters as well as dose-response relationships across various trophic levels and key contaminants. Current efforts include the development of an EcoRisk modeling framework which ties together these multiple components and environmental stressors into a tool that can be used by industry and government to assess ecological risk related to current discharge and exposure scenarios and also for future predictive and planning purposes.

TH147

Incorporating diffuse emissions in a load-impact based prioritization approach for metals

P. Van Sprang, ARCHE, F. Van Assche, IZA; H. Waetenschoot, Eearometaxa

In an effort to enhance public access to information, to examine progress in reducing emissions and, possibly, to help setting priorities for reducing emissions that may cause risk to man and environment, national and regional Pollutant Release and Transfer Registers (PRTRs) have been established. Currently, a series of metals are included in the reporting requirements that in first instance focus on point sources and fugitive sources. In industrialized countries, EMEC and EMEC debated about extending the PRTR schemes to include product and non-point source releases in order to make the pollutant registers possibly more relevant for risk prioritization and regional risk assessment. Indeed, PRTR registers are increasingly used for risk priority setting schemes under chemical management programs to ensure a more risk-based rather than a hazard-based approach. Currently, a main issue is that substances registers only include emissions (loads) making comparisons for prioritization less relevant if no correction is applied for impact (toxicity) potential. This poster will provide the outcome of a large exercise conducted by the metals sector (including Cu, Co, Cu, Mo, Ni, Pb, Sb, V and other) under the EU EL. The exercise involved an EL scale analysis of data on releases from products and other sources were assessed using up to date information and methodologies. Load-impact ratios were calculated as the ratio between the loads (t/y) and the metal specific chronic environmental toxicity reference values (e.g. HC5). The relevancy of these load-impact results for risk-based prioritization approach will be further discussed.
Degradation of Acetamiprid by esterase from aqueous solution

M. Yalçın, Adnan Menderes University / Faculty of Agriculture; C. Turgut, ADU Esterases are a group of enzymes which are the most important in the metabolism of xenobiotics including many pesticides. They protect the target site (acetylcholinesterase) by catalyzing the hydrolysis of pesticides. Acetamiprid is a systemic insecticide that is used to control many pests (e.g. Hemiptera, especially aphids) by soil and foliar application on a wide range of crops. The residue of acetamiprid is a big issue in many crops and countries. The aim of this study was to remove Acetamiprid in aqueous solution by addition of esterase enzyme. The process of acetamiprid removal from aqueous solution using esterase enzyme has been analyzed. Experiments were carried out at different time, different dose and pH to determine the optimum removing conditions. Analyses of acetamiprid residues conducted by LC/MS/MS. The performance of acetamiprid removal was found to be highly dependents on these conditions. Maximum removal was observed by esterase when compared by lipase and cellulase. Moreover our results will create opportunities for engineering degradative enzymes but more concentrations and mixture pesticides should be tested for further studies.

Identifying and regulating PBT and vPvB chemicals: Requirements, challenges and policy implications (P)

TH149 Bioaccumulation and PBT/vPvB assessment of organic substances: the inherent and unavoidable flaws in the current REACH approach

O. Warwick, Peter Fisk Associates Limited; P.R. Fink, Saxion House Mandatory EU requirements for in vitro bioaccumulation properties is essential for identification of substances of very high concern. The assessment of the B criterion has challenges since it is currently based on relative rather than absolute metrics. This review focusses on the inherent flaws in some of the regulatory metrics for B, which are rooted in the low relevance of laboratory studies to environmental fate. Such a criticism is much less applicable to the measures of persistence and toxicity. Laboratory studies of bioconcentration/magnification factor (BCF and BMF), investigate minimal aspects of real environmental behaviour i.e. only uptake and elimination in very simple studies. More fundamentally, it cannot be avoided that these metrics depend strongly on exposure concentrations (e.g. high exposure in the diet can overwhelm the ability of the organism to tolerate and confound meaningful measurements). The expression of, for example, BCF as a ratio of concentrations without reference to exposure, generates an illusion of steady state and a fixed metric. However, BCF considered as a ratio of rate constants is more useful. Therefore, the current system is internally flawed because a supposedly intrinsic property actually depends on exposure concentration, both in the lab and the environment. In reality, it is necessary to have a proper understanding of exposure route when considering uptake in the food chain. Yet the regulation is expressed without reference to exposure. Various authors have suggested metrics that are more robust measures of B’ than BCF or BMF; only those which refer to rate are really useful. As far as possible, elimination rates should be based on environmentally-relevant exposures. What is the consequence of the flawed understanding of bioaccumulation metrics? Paradoxically it leads to the inconsistency that exposure has to be discussed when determining the hazardous properties! A properly-functioning regulation is needed because the regulatory consequences are serious: rigorous control of exposure and possible withdrawal from the market. The anomalies are particularly severe for ‘V’. Some ways forward are suggested, based on meaningful measurements of elimination rates under relevant conditions; this would put B on a more solid basis, more consistent with P and T which have meaningful absolute units.

Monitoring, but for what? - Designing contaminant monitoring on a National scale

E. Farmen, M.V. Dimmen, B. Nordbo, S. Andersen, A. Sundbye, Norwegian Environment Agency Whereas PBT and vPvB focused contaminant monitoring may serve to collect important field data for regulation of chemicals, the purpose of environmental monitoring is commonly also to inform about the state of the environment. The environmental monitoring run by the Norwegian Environment Agency are currently trying to do both, but are we doing it right? Traditionally, National contaminant monitoring programmes in Norway were designed to assess time trends in order to detect alarming levels and changes in levels of contaminants. In recent years, increased effort has been put in addressing how contaminants biomagnify in both aquatic and terrestrial Norwegian food webs. Such measurements potentially inform regulators on real world occurrence and fate of chemicals, and have shown to be a useful component of monitoring programmes. Although valuable data already have been generated with this approach through Norwegian monitoring programmes, food webs fit for purpose has proven to be far from off-the-shelf. Whereas the model for fresh water biomagnification appears to work well, tools for marine and terrestrial food chains are more challenging. Moreover, other requirements for contaminant monitoring, such as implementing mixture toxicity risk assessment, evaluation of biological effect, and an ever-changing list of emerging contaminants for monitoring, leaves design of National monitoring a perpetual task. In the end, the question for regulators is therefore to what extent National contaminant monitoring should be focusing on emerging PBT and vPvB chemicals, compared to assessing the state of the environment.

Chemical policies, regulations and conventions in protection of European waters - Overlaps and gaps

J. Lesèt, T. Skårman, E. Brorström-Lundén, M. Rahmberg, T. Rydberg, IVL Swedish Environmental Research Institute; J. Munthe, IVL Swedish Environmental Research Institute Ltd Several policy instruments (Regulations, Directives and Conventions), which regulate emissions of chemicals and use of chemicals in preparations and articles for reducing risks to the environment and human health by chemical pollution, have been reviewed with respect to their potential for increased coordination, harmonisation and cooperation: EU Regulations - REACH, Plant Protections Products Regulation, Biocidal Products Regulation; EU Directives - Groundwater Directive, Marine Strategy Framework Directive, Drinking Water Directive, Urban Wastewater Treatment Directive, Sewage Sludge Directive, Industrial Emissions Directive, RoHS Directive, Toy Safety Directive; Multilateral environmental agreements - Stockholm Convention, CLRTAP, PRTR. The evaluation of potentials for synergies as well as risk for conflicts is carried out by a literature review, based mainly on information from the web sites of the issuers and from the legal documents and conventions, comparing how the following key aspects in WFD are treated in the other studied instruments: General characteristics; a) Scope: inland surface waters, transitional waters, coastal waters and groundwater; b) Reporting level: river basin; c) Substances: Implementation; d) Characterisation of water bodies; e) Identification of pressures and impacts (emissions from point sources, emissions from diffuse sources, establish inventories of emissions, discharges and losses); f) Monitoring programmes (concentration monitoring); g) Risk assessment of water bodies (Ecological status, Chemical status, Environmental Quality Standards; h) Programme of measures; i) Reporting (river basin management plans) Early results indicate the potential for increased coordination and cooperation between e.g. WFD and REACH, for the safe management of chemicals, such as better sharing of information between the different instruments. Further analysis will address, e.g., procedures for including new substances, efficiency in implementing restrictions or bans, sharing of compiled information on occurrence, effects, properties and abatement options, specific sectors of society where chemical legislation needs strengthening. Future instruments development will be discussed to protect European waters against chemicals of emerging concerns and mixture effects – an innovative legislative framework also embracing novel risk assessment frameworks such as chemical footprints and planetary boundaries for chemicals.

Biodegradation Test No. 309 (Aerobic Mineralisation in Surface Water) not applicable for a persistence assessment of strong adsorbing plant protection products with low water solubility

J. Hassink, B. Jene, BASF SE / Environmental Fate; D. Ebert, BASF SE The OECD 309 study (Aerobic Mineralisation in Surface Water) is a new data requirement under EU regulation 1107/2009 for plant protection products, where compounds are incubated in nutrient media and results are provided for 90 days with no contact to sediment. All experiences gained so far confirm that hydrolytically stable compounds will not degrade in this study because microbiological activity under such artificial conditions is not sufficient to mediate significant degradation. The majority of the substances are required to be hydrolytically stable to be used by the farmer in spray application. In contrast to the OECD 309 study, the water/sediment study (OECD 308) is designed to provide at least a minimum of microbial diversity as existent in natural water bodies. It represents the most relevant worst-case exposure scenario of plant protection products, i.e. the exposure via spray drift, run-off or drainage of a small freshwater body with sediment at the edge of an agricultural field. The OECD 309 study might provide additional information for the risk assessment of a water-soluble compound in coastal or oceanic water compartments but it is not appropriate to determine P-criteria for strong adsorbing substances with low water solubility by this study type, which is agreed by SANCÖ and ECHA guidance. The whole system DT₅₀ of the OECD 308 is the only reliably estimated degradation half-life in a water containing system and therefore more suitable. Furthermore, photolitical degradation of a compound should be considered when relevant. As an example for low soluble and strong adsorbing substance, experimental data for the herbicide pendimethalin are outlined and discussed. Pendimethalin is hydrolytically stable and under the artificial conditions of the OECD 309 study (no light) would elute a DT₅₀ of 80 and a P-trigger of 40 d for freshwater. However, in addition to the fast degradation in water by photolysis (DT₅₀ 3.5 d), pendimethalin partitions within a few hours from the water into the sediment where it is rapidly degraded. Therefore, sediment is the only relevant compartment for pendimethalin in water/sediment bodies to be considered for the P-value. The total water/sediment DT₅₀ for pendimethalin can be gained for 90 days is far below the P-criterion of 120 d for sediment. Aquatic mesocosm studies with
pristine sediment confirm the rapid partition of pendimethalin into sediment and its fast degradation.

**Standards - an essential link between environmental science and regulation (P)**

**TH154**

The Validation of Analytical Methods used in Ecotoxicological Studies

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The validation of analytical methods used in support of ecotoxicological studies has become a vitally important aspect of the registration process for plant protection products. The validation and reporting of analytical methods used to determine pesticide concentrations in ecotoxicological studies conducted for registration purposes are currently regulated by the SANCO/3029/99 rev 4 guidelines. These guidelines have been approached within the Dir 91/414/EC since revision 4 of SANCO/3029/99 was issued in July 2000. However, because directives such as these are generally not legally binding, in the past the guidelines were often either only partially followed or simply overlooked. When Regulation (EC) No. 1107/2009 came into force in 2011, adherence to the SANCO/3029/99 rev 4 guidelines became obligatory. This has resulted in serious implications for the registration process because ecotoxicological studies that had previously been accepted may well now be rejected on the basis of inadequate analytical methodology or incomplete analytical data. This is evidenced by the recent rejection of a number of residue studies by the authorities on the grounds of incomplete or inappropriate methodology and/or data. It is therefore likely that ecotoxicological studies that fail to meet the analytical criteria required by SANCO/3029/99 rev 4 may not be accepted anymore. It is the purpose of this poster to give an overview of current requirements for analytical methods used in support of ecotoxicological studies. We also provide an example of a checklist that can be used to evaluate analytical methods according to SANCO/3029/99 rev 4. Simple tools such as this can be used to elucidate the potentially complex and time-consuming process of assessing the validity of analytical methods used in ecotoxicological studies.

**TH155**

Normalizing Data to the Control Creates Large Errors

J.W. Green, DuPont / Applied Statistics Group

A technique common in the analysis of some ecotoxicity studies is to normalize data in treatment groups to the control. In the case of quantal data (e.g., mortality, emergence, hatching), the purpose is to adjust mortality data for background incidence and a method for doing this dates from 1925 called Abbott’s formula. It has been known since at least 1985 that Abbott’s formula can give biased results. Better ways to take background mortality into account have been known for several decades and software is widely available to carry out these alternative methods. This same idea has also been used for continuous responses, where the object is to estimate the concentration at which a specified percent effect relative to the control mean response occurs. The primary purpose in normalizing a continuous response to the control is to modify the data so that a probit analysis (which assumes quantal data) software package can be used. Good models are well known that do not require such data manipulation. It will be proved not only is the analysis of normalized data theoretically sound and unnecessary, but can produce estimates as much as 6X too large or too small in several types of guideline studies while other readily available approaches provide accurate estimates. Theoretical problems with normalized data are first made clear. Second, data from fish early life stage, non-target plant, and fish sexual development studies are analyzed using probit models fit to normalized data and more appropriate models to indicate the difference in both EC50 point and interval estimates. Third, Monte Carlo simulation studies based on experimental designs, variability and dose-response shapes typical of these studies are used to develop distributions for ECx estimates from these models. In the simulation studies, the true ECx is known, so these distributions can reveal the quality of estimates to be expected from the different modeling approaches. Both quantal and continuous data are considered Readily available software allows the use of statistically valid regression models to analyze quantal and continuous data arising from ecotoxicology studies. Analysis of normalized responses is unreliable, with both under- and over-estimation of ECx values by large factors occurring with little way to determine in a particular study that whether such min-estimation has occurred.

**TH156**

COMPARISON OF NOEC VALUES TO EC10/EC20 VALUES, INCLUDING CONFIDENCE INTERVALS, IN AQUATIC AND TERRITORIAL ECOTOXICOLOGICAL RISK ASSESSMENT

F. Galimberti, International Centre for Pesticides and Health Risk Prevention; F. Marchetto, ICPs; L. Menaball, S. Ullucci, G. Azimont, International Centre for Pesticides and Health Risk Prevention; A. Moreto, State University of Milan; W. de Boer, H. van der Voet, Alterra Wageningen University and Research Centre Ecotoxicological studies performed for the authorization of plant protection products (PPP) usually result in the reporting of endpoint values in terms of effect concentration (EC) affecting a percentage x of test organisms or where a x percentage of an effect is observed (ECx). The new Regulation (EC) No. 1107/2009 for the authorization of PPPs and the related data requirements provide that ecotoxicological endpoint data from chronic or long-term studies submitted by the Applicant are reported as EC10 or EC20 values together with the NOEC. NoEC values have been criticized since their values strongly depends on the experimental study design, whereas ECx values take into account the whole concentration-response curve and are therefore considered more appropriate. The aim of the project is to investigate the comparability of the ECx approach to the current NOEC approach on a larger data sets in view of the new Regulation requirements. Ecotoxicological data gathered from 70 active substances’ approval dossiers were collected and stored into a MS Access database. All the extracted ecotoxicological data were analyzed in order to derive NOEC and calculate EC10, EC20, EC50 with confidence intervals, using statistical models from the exponential and Hill families for continuous data, and logistic, log-logistic and complementary log-log models for quantal data. The optimal models for each parameter were selected using likelihood ratio tests and the Akaike Information Criterion. EC/NOEC ratio distributions were calculated considering the whole set of data and model outputs; data were grouped in different categories to remark any differences in the ECx/NOEC ratio distributions.

**TH157**

Robust data analysis to derive 17β-estradiol equivalent concentrations in in vitro bioassays

E. Simon, Centre Ecotex / Aquatic Ecotoxicology; C. Kienle; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; E. Vermeiren, Eawag-EPFL / Aquatic Ecotoxicology

Several different reporter gene assays that assess the activation of the estrogen receptor are currently being developed as ISO standards. Data analysis of these bioassay results is not a trivial issue and requires consideration to allow for comparable results between different assays or for the same assay but between laboratories. Typically the assays require sigmoidal dose-response curves when a reference compound (or positive control, PC) or an environmental sample is applied in increasing concentration. Subsequently, dose-response curves of the reference and sample are used to derive equivalent concentrations for the sample. However, curves generated for the samples are often not parallel to those of the reference, or do not achieve minima or maxima values that are similar to the reference. Non-parallel dose-response curves may occur when the compounds in the sample are different from the standard, when antagonists are present in the sample, or when sample matrix interferes with induction in the assay. Maximum induction values may not be reached because of low activity of the sample, sample solubility problems or cytotoxicity. Low activity may be expected, for example, for surface and/or drinking waters. In these cases the type of data analysis that is applied will influence the equivalent concentrations that are derived. This makes the comparison of studies conducted in different laboratories challenging. To circumvent these problems we suggest using the 10% effect level interpolation (“PC10 approach”) for in vitro data analysis. First, all data are normalized, where 0% refers to the response in the solvent control and 100% is the maximum response fitted for the reference dose-response curve. Then, the 10% effect level of the sample is interpolated from the reference dose-response curve and the 17β-estradiol equivalent (EEQ) concentration of the sample is determined. The requirements to apply the PC10 approach are: an effect level in the sample that is larger than 10%, or the data can be extrapolated the modelled sample response above the 10% effect level a limit of quantification (LOQ) that is below the 10% effect level. To discuss these specific aspects, we applied various calculation methods and show graphical examples of environmental samples that were assessed for estrogenic activity using different bioassays. The “PC10 strategy” appears to provide a harmonized solution for analysing in vitro screening data and a standardized data reporting practice.
concentrations a set of standard compounds with known estrogenic activity were tested in several defined dilutions with the yeast estrogen screen (YES) – a biosay that is currently being developed as an ISO standard. The selected compounds show specific concentration-response-relationships with e.g. different slopes. The assessment of the various methods for the derivation of equivalence concentrations is based on the assumption that e.g. a 1 to 2 dilution of a given sample should result in a biesected equivalent concentration after testing with the YES. In case of the non-linear interpolation, a common approach for the calculation of equivalence concentrations, the calculated equivalence concentration depended on the maximal iso-NP-concentration tested in the YES. If a maximal concentration of 500 μl iso-NP is tested the calculated EEQ is about 60 ng/l. If the same solution is diluted 1 to 2 and tested again, the resulting EEQ is about 50 ng/l – the apparent EEQ of the undiluted original sample would then be 100 ng/l. The derived EEQ increases with dilution of the iso-NP. The same data will be assessed by several approaches under discussion for the derivation of the EEQ aiming to contribute to the development of a guideline for the derivation of effects-equivalence-concentrations.

**TH161**

**Assessing the influence of part per billion variation of natural organic carbon levels on cationic polymer acute toxicity to D. magna**

E.R. Salinas, BASF SE / Experimental Ecotoxicology; J. Bozich, University of WisconsinMilwaukee / SFS; L. Peters, BASL SE; R. Lukas, BASF SE.

Cationic polymers (CPs) are surfactants which are widely used in industrial and commercial purposes, for example, as floccing agents in water clarification. CPs generally have a low environmental concern due to the presence of organic carbon in surface water that greatly reduces their bioavailability, even at low mg/l levels. Although dissolved organic carbon can be as high as 50 mg/l in European surface waters, regulatory aquatic testing guidelines (OECD and EPA) limit total organic carbon (TOC) in clean dilution water to <2 mg/l to determine the baseline toxicity of chemical substances. However the amount, source and quality of the organic carbon in dilution water can vary considerably between testing laboratories. To better understand the influence of minute TOC differences as a potential source of variation for clean water studies, we explored the impact of 0.2 mg/l and 1 mg/l of ORC as humic substances in artificial water (Elenz M4 medium) on EC₅₀ toxicity, all of which are considered by definition to be clean dilution water. We chose *Daphnia magna* to assess the acute toxicity of CPs with varying molecular weights and cationic charge density. Our results indicate that part per billion levels of TOC produced strong CP toxicity mitigation. These results suggest that background TOC in dilution water is a potential source of variation and should be considered when interpreting the results of aquatic toxicity studies. Therefore, we recommend that aquatic tests used to establish the baseline toxicity of cationic polymers should be standardized with added TOC in the dilution water at a minimally measurable amount (1 mg/l) to quench low level variability of background TOC and represent an environmentally realistic worst case exposure.

**TH162**

**Development of standardised methods in environmental toxicity assessment of nanomaterials**

J. Forkas, SINTEF Materials and Chemistry; A. Booth, SINTEF Materials and Chemistry / Environmental Technology; D. Altin, BioTrix; L. Stoten, SINTEF Materials and Chemistry.

In order to allow a reproducibly assessment of potential effects of pollutants a standardisation of ecotoxicity tests is necessary. While a range of standardised tests on environmental impacts of pollutants is available, their implementation in nano-toxicity testing is difficult and many of those methods are unsuitable for assessment of engineered nanomaterials (ENM). Small changes in environmental parameters or testing procedures can change the physico-chemical characteristics of the tested ENM and thereby influence the study results. In order to establish guidelines and standard procedures for ecotoxicological assessment of ENMs, the Nanoreg project aims to test, optimise and validate a set of suitable test methods for different types of ENMs. Preparation and characterisation procedures, a prerequisite for ENM toxicity evaluation, were established and validated for different ENMs in laboratories across Europe. Further, standard tests for toxicological assessments using the freshwater aquatic species *Pseudokirchneriella subcapitata* and *Daphnia magna* were evaluated and adapted to suit needs for ENM testing. A wide range of ENMs from various material classes are tested for their environmental stability and their potential ecotoxicological effects using the adapted ENM-standardised methods. The tested material include different nanobased ENMs (nano and modified single- and multi-walled carbon nanotubes, graphene and graphene oxide); a range of modified and unmodified titanium dioxide (TiO₂) based ENM, as well as metal ENMs. This large scale testing with suitable standardised methods will provide valuable ecotoxicological data and further show if a read across or grouping approach of similar ENMs is appropriate. Similarities between ENMs such as size, shape, material and surface for potential read across or grouping approaches in aquatic toxicity tests can be identified.

**TH163**

**Sparks from Hazardous and Noxious Substances (HNS) - Is standard toxicity data enough?**

M. Vannoni, J. Aldridge, D. Doran, S. Davis, Cefas; M. Kirby, Cefas / Ecotoxicology; T. McGowan, FERA; D. Sheahan, Cefas

Over the last decades shipping of chemicals at sea have increased significantly leading to the need to be prepared to respond to incidents involving hazardous and noxious substances (HNS). Typically during chemical spills the impact upon marine organisms is estimated using spill model predictions of time-weighted
average (TWA) or peak concentration compared to a predicted no effect concentration derived for constant exposure toxicity studies conducted in the laboratory. This study evaluates the toxicity of two chemicals, aniline, and benzalkonium chloride, comparing standard and brief exposures. Salinity and temperature were also taken into consideration to understand the behavior of these chemicals under different environmental conditions. Test toxicity was evaluated for the copepod *Tisbe batagillia*, the red algae *Ceramium tenuicorne* and the brown algae *Fucus vesiculosus*. Benzalkonium chloride and aniline showed EC₅₀ in a similar concentration range (1-2 mg l⁻¹) based on standard exposures. When brief exposures were considered, contrasting effects for the two test chemicals were shown on all the species tested. Exposure to benzalkonium chloride resulted in significant effects after 1 hour exposure to a concentration of 5 mg l⁻¹ but for aniline recovery of *Tisbe* (the most sensitive species to this chemical) occurred in exposures of a few hours at concentrations up to 300 mg l⁻¹. For variations in salinity and temperature toxicity decreased for some chemicals with the increase in salinity while increased temperature generally increased toxicity. Thus, a spill of a chemical with a specific mode of toxicity results in greater effects following relatively brief exposures, and exposed organisms show little or no recovery. In such cases impacts might be expected to be more extensive and persistent. In conclusion, for chemicals with specific modes of toxic action brief exposures, and possibly limited spill profile studies may be useful to support chemical spill risk assessment. Parallel studies on factors such as temperature and salinity are also valuable to help predict the likely scale of impact from chemical spills in different locations i.e. in the mouth of estuaries or further offshore or during different times of year or in different parts of the world.

**TH164**

**RHIZOtest - the first standardized laboratory bioassay designed to assess the environmental bioavailability of trace elements to plants in soils**

M. Bravín, CIRAD; L. Lernal, RHIOLAB; C. Chevassus-Rosset, M. Montes, CIRAD; D. Amal, P. Hinsinger, INRA, E. Doelsch, CIRAD.

A lot of laboratory bioassays were developed to assess the toxicity of trace elements on plants and among which some are widely used and even standardized at an international level. Surprisingly, there was no laboratory bioassay developed to assess the environmental bioavailability of trace elements to plants, that is to say the uptake of trace elements in plants. Considering the ecotoxicological relevance of the environmental bioavailability endpoint, we developed and tested a dedicated laboratory bioassay, the RHIZOtest, to allow its recognition and standardization it at an international level. The RHIZOtest is a plant-based test initially developed in the 1990s and used as a research tool to investigate the role of root-induced chemical processes as a driver of trace element dynamic in the rhizosphere and bioavailability to plants. The RHIZOtest is notably characterized by the small size of the system and by a physical separation between plant roots and soil that enables to collect easily and quickly both compartments separately. These characteristics led to evaluate its performance as a risk assessment tool. We first assessed the robustness, the repeatability and the reproducibility of the RHIZOtest via an international ring-test. The RHIZOtest was hence validated for the measurement of the environmental bioavailability of arsenic, cadmium, cobalt, chromium, copper, lead, nickel, and zinc to three target plant species (tomato, cabbage, and fescue). We secondly assessed the ability of the RHIZOtest to distinguish the environmental bioavailability of trace elements for different plant species. Using a scoring approach, we were able to classify 10 plant species as a function of the bioavailability endpoint. Finally, we assessed the ability of the RHIZOtest to distinguish the environmental bioavailability of trace elements for plants grown in different soils. We thus exposed tomato, cabbage, and fescue to 55 soil samples exhibiting very constrained physical-chemical properties. Armed with this knowledge, a draft describing the RHIZOtest tool and methodology was submitted to the international organization for standardization (ISO) and was validated as new standard, the NF EN ISO 16198, in February 2015. The development of the RHIZOtest is going on with its application to other contaminants such as nanoparticles and trace organic contaminants.

**TH165**

**The leaf fatty acid composition of plants: new biomarker as a standard for assessing soil quality**

M. LE GUEARD, LEB AQUITAINE TRANSFERT-ADERA / LEB AQUITAINE TRANSFERT; J. BESOULE, CNRS - Université de Bordeaux / Laboratoire de Recherche en Plénitude

The leaf fatty acid composition is proving to be a powerful biomarker to diagnose soil pollution. This biomarker is based on the fact that when plants are exposed to pollutants on soils, the reactive oxygen species (ROS) can increased dramatically and lead to cellular damages, including lipid peroxidation. The main target of lipid peroxidation is liquids containing polysaturated fatty acids, and more particularly lipids of the membrane. This is strongly associated with plant stress and a previous study involved numerous studies on the leaf fatty acid composition of lettuce grown under control conditions (ex situ approach) on contaminated and control soils and we showed that the $\text{C18:5(3,8,9,12,13):0 + C18:1 + C18:2}$ fatty acid ratio, called "Omega-3 index", decreased in leaves under stress. From these ex situ studies, it appeared that Omegas played a major bi-indicator than the standard tests existing in plant, such as emergence or growth inhibition. Moreover, we demonstrated that the Omega-3 index is a rapid, sensitive (metal and organic contaminants), robust, reproducible and repeatable biomarker of soil pollution which is then able to generate reliable data. These ex situ development of the Omega-3 index led in 2012 to the publication by the French general standardization body (VNFON) of a soil quality standard (NF V 31-233). We further studied this biomarker with plants grown on real sites during the French ADEME program "Biobindicators of Soil Quality". The results acquired during this program (among others) highlighted the efficiency of the leaf fatty acid composition of various plant species harvested on several areas of contaminated sites to provide an excellent biomarker of organic and metal contamination of soils. From these in situ data, we also developed and published a "mathematical" method with which it is possible in complex situations to analyze results provided by the use of the Omega-3 index in situ and to rank different areas of the same site according to their phytoxicity. Following these results, a standardization of the Omega-3 index as a tool to assess soil quality through the International Organization for Standardization (ISO) goes underway with a desirable applicability both ex and in situ.

**TH166**

**Use of Standardised Methods and Risk-Based Approach in the Oil Industry**

K. Wadhia, Opus / Environmental

A testing programme was implemented to investigate the management of produced waters using a risk-based approach. In employing this approach using standardised protocols, Whole Effluent Assessment (WEA) would potentially constitute an appropriate means of detection of unknown substances and has the advantage that each unique discharge is tested, rather than relying on predictive fingerprinting values. Whole Effluent Testing (WET) is also used in the UK offshore industry. Installations were chosen to give a broad coverage of location, type of production (oil, gas), stage of production (early, late) and environmental styles. Salinity and increased temperature generally increased toxicity. Thus, a spill of a chemical with a specific mode of toxicity results in greater effects following relatively brief exposures, and exposed organisms show little or no recovery. In such cases impacts might be expected to be more extensive and persistent. In conclusion, for chemicals with specific modes of toxic action brief exposures, and possibly limited spill profile studies may be useful to support chemical spill risk assessment. Parallel studies on factors such as temperature and salinity are also valuable to help predict the likely scale of impact from chemical spills in different locations i.e. in the mouth of estuaries or further offshore or during different times of year or in different parts of the world.

**A focus on research and education tools in environmental toxicology and chemistry (P)**

M. Masson, INRA Institut National de la Recherche Agronomique / USRAVE

Ecotoxicological studies need high quality measurements of chemical concentrations derived for constant exposure toxicity studies conducted in the offshore industry. Installations were chosen to give a broad coverage of location, type of production (oil, gas), stage of production (early, late) and environmental styles. Salinity and increased temperature generally increased toxicity. Thus, a spill of a chemical with a specific mode of toxicity results in greater effects following relatively brief exposures, and exposed organisms show little or no recovery. In such cases impacts might be expected to be more extensive and persistent. In conclusion, for chemicals with specific modes of toxic action brief exposures, and possibly limited spill profile studies may be useful to support chemical spill risk assessment. Parallel studies on factors such as temperature and salinity are also valuable to help predict the likely scale of impact from chemical spills in different locations i.e. in the mouth of estuaries or further offshore or during different times of year or in different parts of the world.
PLATINAAE works essentially as a partner for research programs. The main themes investigated are environmental contamination, biogeochemical cycle of elements in various ecosystems (crops, lands, forests...), soil quality, food chain or plant physiology. PLATINAAE performs also analyses on living organisms (invertebrates...) and wastes (sludge, compost...). In these scopes, the platform participates in numerous projects for environmental observation and experimentation, such as the ICOS Ecosystem program, the European program BioSoil, the RMQ network, RENECOFOR (monitoring of French forests)... PLATINAAE develops also innovative methods in analytical chemistry according to the needs of its partners (samples of very small sizes, trace and ultra-trace elements such as lanthanides or PGEs, mapping of plant organs...).

TH168 Biochem-Env, a platform of environmental biochemistry for research in ecology and ecotoxicology
N. Cheviron, V. Grondin, INRA Institut National de la Recherche Agronomique / UMR ECOSYS Platform BiochemEnv; S. Nélieu, O. Crouzet, M. Hedde, INRA Institut National de la Recherche Agronomique / UMR ECOSYS; C. Mougin, INRA (Institut National de la Recherche Agronomique / UMR ECOSYS) The consortium AnaEE-France (http://www.annee-s.fr) aims at understanding and predicting the biodiversity and ecosystems dynamics in a context of global change. It will allow improving the understanding of biotic processes/environment interactions, mobilizing experimental and modelling platforms devoted to the biology of continental ecosystems, both terrestrial and aquatic. In this context, the objectives of the platform Biochem-Env (http://www.biochemenv.fr) are to provide skills and innovative tools for biochemical characterization of soils, sediments, and microorganisms living in terrestial and aquatic ecosystems.

TH169 MOBYCITE: mobile cytomtery in environmental risk assessment
D. Rioult, UMR 02 INERIS-URCA-ULH SEBIO / MOBYCITE flow cytomtery core facility; A. Bado-Nilles, INERIS; J. Porcher, INERIS / INERIS UBIO SEBIO ECOT; S. Bouteille, URCA / URCA SEBIO Commonly used in biomedical science, flow cytometry is more and more involved in ecotoxicology and in environmental sciences on numerous cell types such as bacteria, yeasts, phyto / zooplankton and invertebrates and vertebrates cells. Environmental cytometry requires adaptations of techniques in cellular biology. Development of field methodologies are required in order to study cell organizations and functions closed to their interactions with environmental variables and environmental stress in wild contexts and with taking into account of samples quality preservation. In this context, the mobile environmental flow cytometry core facility named MOBYCITE is dedicated to applications in numerous scientific fields and particularly in environmental toxicology and ecotoxicology, but also in functional ecology and integrative ecophysiology. This core can be used by research teams as well as by public and private organizations working in environmental biomonitoring programs. Our mobile equipment is operable in the more isolated environmental field contexts (aqual and terrestrial), considering cell models from various animal species (algae, mollusk, fish, wild mammals...). Some studies using MOBYCITE platform will be developed in order to prevent new opportunities for biomonitoring the health status of wildlife organisms in health and environmental research risk assessments.

TH170 Analysis of 13-year soil monitoring data of Korean Soil Quality Monitoring Network
S. Jeong, Kansan National University / Dept of Environmental Engineering; J. Kim, S. Lee, Kansan National University; Y. An, Konkuk University / Department of Environmental Health Science The Korean soil quality monitoring network has been operated since 1987 and has yearly reported concentrations of soil trace elements for 1,521 observation points. The analysis of soil quality monitoring results (1997-2009) of Korean soil quality monitoring network. The monitoring results were reported with concentrations and land uses for 9 trace element concentrations (Cd, Cu, As, Hg, Pb, Cr $^{6+}$, Zn, Ni, and F). This study analyzed tolerance limits of the trace element concentration data for each observation point. This study classified 278 points as "variation occurred", showing that the upper 95% tolerance limit of the variation occurred point data was greater than the Korean soil quality standard. This study also evaluated anthropogenic contamination of those points by deriving background concentrations from the total soil quality monitoring data. Analysis results showed that fluoride levels were frequently varied in Korean soils and industrially-used sites showed significant changes in trace element concentrations. However, this study found that high nickel concentrations of soil monitoring data were attributed to naturally high background concentrations. This study also suggested better soil quality monitoring network strategies for achieving sustainable soil environment. The most important strategy for producing reliable soil monitoring data is to sample soils at the consistent points every monitoring time. (This study was supported by the GAIA project (2014000560001)) funded by Korea Environmental Industry & Technology Institute and Korean Ministry of Environment.)

TH171 "Métátox": a new one-year training program for engineering students on environment and management of toxicological risks for both human and ecosystem health
J. Faburé, INRA/AgroParisTech; L. Fechner, Inraea/AgroParisTech; F. Mariotti, V. Camel, INRA/AgroParisTech In recent years, the global contamination of our environment has generated a major challenge: understanding the long-term impacts of multi-exposures to a number of chemicals, both in the field and in the lab, to solve the increase of concerns regarding the impact of anthropic activities on both human and ecosystem health. Today, these concerns are essential for citizens, politicians, managers and firms. As a consequence, all industries have now to integrate their impact on the environment and the human health, since the assessment of the innocuousness of a substance or a product, towards both human and ecosystems is now a part of the evaluation of manufacturing processes and development of new products. In this context, AgroParisTech, the leading French Graduate School in Agronomy, Environment, Life science and Food technology, has created a new one-year training program for engineering students that develop a holistic approach of toxicological risks for humans (environmental and food toxicology) and ecosystems (ecotoxicology) health.

TH172 Petinca Science Center: A place for engaging science education in Southeastern Europe
T. D. Miščenkov, Petinca Science Center / Institute of Botany and Botanical garden Jevremovac; B. Mičić, N. Božič, Petinca Science Center Petinca Science Center (PSC) is an independent, non-profit organization for science education in Serbia. PSC's 35 years of expertise in organization of programs for gifted high school and university students, as well as educational and outreach programs for science teachers. Among the 17 programs at PSC available to students, over 40% are related to biology, chemistry and environmental sciences. Nearly 2,500 alumni from over 450 academic institutions and countries, together with the team of trained professionals, form our network of associates, lecturers and peer educators, all of whom are involved in course design and who help put on our numerous activities and programs. Each year, 1,500 students from Serbia and Southeastern Europe attend programs at PSC. Since a major reconstruction completed in 2012, PSC currently represents the most modern center for extracurricular science education focused on young people in Europe. PSC houses a fully equipped state-of-the-art laboratory facilities for biology, chemistry, and physics. PSC also houses a scientific library with 40,000 books and journal access. PSC also provides full board accommodation options for up to 170 participants at a time. A multidisciplinary approach is the hallmark of all PSC activities, which requires efficient integration of all our laboratories, instruments, clients, and associates. Specific facilities include a laboratory for cell cultures and molecular biology, a center for confocal microscopy, HPLC-MS...
and GC-MS equipment, a laboratory for analyses of trace elements equipped with an AAS and XRF, plant growth chambers, as well as several 3D printers and a 3D scanner, all of which are situated in one central laboratory building. The characteristics of PSC as a unique venue led to major changes in the concept of the most recent Young Environmental Scientists (YES) Meeting, organized by the Swedish governmental agency for sustainable development (Naturvårdsverket), in collaboration with the University of Exeter. The YES and the meeting are now hosted by PSC in March 2015, and for the first time in a YES meeting we offered hands-on short courses for 130 meeting participants. All 8 courses took place in one day simultaneously for all students. PSC’s laboratories equipped with cutting-edge instruments and our network of dedicated alumni and associates and partners make PSC one of the most convenient and engaging venues to host hands-on courses, summer schools, and scientific conferences related to current topics in environmental science.

How can we improve the link between academic research and policy-making in order to advance chemical risk assessment and management? (P)

TH173

Regular information of stakeholders and policymakers about a soil monitoring of persistent organic pollutants in Cuba

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Although Cuba has signed, ratified and put into force the Stockholm Convention on Persistent Organic Pollutants (POPs, 2008), there exists no systematic monitoring tool available for assessing the environmental and human exposure for chemicals. Cuba has been active in the Strategic Approach to International Chemicals Management (SAICM). In a project from 2010 to 2011 funded by SAICM, the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF) POPs were monitored globally (including Cuba) and passive air samplers were installed to evaluate the exposure. With our project, Soil-Q (Establishing a soil monitoring network to assess the environmental exposure to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the province of Mayabeque, Cuba), the investigation on POPs in Cuba has been reactivated by monitoring the soil. The newly established soil monitoring network in Cuba currently consists of 39 sampling locations in the province of Mayabeque, and additional 12 stations in the adjacent province of Havana. From these sites, representative soil samples were taken, using the rigorous protocol of the Swiss Soil Monitoring Network (NABO).

Analytical methods for the quantification of PAHs and PCBs have been established in the laboratories of the Cuban partner. Results indicated low rural exposure with these pollutants (max. 110 µg/kg of the sum of the 16 PAHs regulated by the EPA, max. 5 pg/kg of the sum of the 7 non-dioxine like PCBs). In contrast, concentrations at some places in Havana are considerable and mandate remediation. Workshops were held (initial and to present the above results) to decision makers (representatives of the agricultural, energy, environmental, and chemical safety sector). Present were also members from the Swiss Embassy and the Swiss development and Cooperation in Cuba (COSUDE). The echo and interest in the audience was high because Soil-Q covers the urgent demand of Cuban policymakers and stakeholders to know the POP pollution in their soils. The Cuban TV was also used for the dissemination of first results and an explanatory website about Soil-Q and its long-term aim is planned at the Cuban partner institute of agriculture (CENSA). Soil-Q is a promising start to advance in the chemical risk assessment and management and, as stakeholders and decision makers were approached from the beginning, a high potential for future research and related dialogues.

TH174

An Analytical Framework to Evaluate and Ameliorate Science-based Policy-Making in relation to complex environmental concerns using the latent constructs, Legitimacy and Effectiveness

J.-G. Gardener Le Gars, University of Exeter / Biosciences; C. Tyler, Biosciences College of Life and Environmental Sciences; R. Owen, The effectiveness and legitimacy of science based-policy making in Europe for the endocrine disruptor 17α, ethinyl-estradiol (EE2) is challenging to evaluate. Traditional policy performance measurement tools predominantly target regulatory compliance downstream of the policy development process. A death of tools to evaluate upstream scientific risk assessment and legislative proposal development hampers analysis of the legitimacy and effectiveness of policy-development processes. The use of science to guide policy decisions is hence rarely, systematically evaluated or monitored. Consequently, understanding of causes and potential solutions to effectiveness and legitimacy deficits is severely curtailed risking sub-optimal policy outcomes and loss of trust in both governing authorities and the scientific enterprise upon which they rely. The present study characterizes specific challenges of environmental risk policy interventions with respect to EE2 thereby facilitating recognition of the following issues confronting policy-makers: i) scientific & economic uncertainty in relation to adverse effects, their consequences and potential mitigation and management options, ii) low-consensus deliberative processes typified by values and interests divergence, iii) high complexity derived from social pluralism, institutional diversity and inter-regulatory dependencies. An analytical framework is derived pursuant to an extensive literature review and operationalized via a qualitative study in order to facilitate a systematic evaluation of variables impacting effectiveness and legitimacy in the case of EE2 policy interventions. The legitimacy of deliberative processes, the implementation thereof and concomitant participant discourse is evaluated and the effectiveness of resulting policy outcomes discussed. More specifically, the discourse of participants to deliberative processes at key decision points in the policy development process is analyzed. Variables influencing the development of policy outcomes (construct risk, the role of science therein and predominant cognitive mechanisms employed to transpose scientific evidence into decision outcomes are thereby identified. Understanding of the challenges of applying & using science and its relative influence on outcomes of policy-making processes is thereby greatly enhanced.

TH175

Socio-Economic Impacts of Copper Compounds Classification in Industrial Sites

L. Perez Simbor, European Copper Institute; S. Van Passel, Department of Engineering Management, Faculty of Applied Economics, University of Antwerp Regulation 1907/2006/EC concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), offers risk management measures to assure safe use of dangerous substances. Regulators use socio-economic and environmental considerations to assess impacts of applying those measures. However, it remains a challenge to properly assess the effects of risk management measures in several industrial sectors. Currently, the integration of the cost and benefits of applying harmonized classification on substances is not included in the decision-making process. The implementation of harmonized classification has direct and indirect consequences for industrial sectors and ecosystems such as the provisions to transport the substances. This research project aims to develop an economic-environmental analyses to assess the trade-off of applying harmonized classification in industrial sectors. It includes provisions for organizational and investment cost and, as a novelty, incorporates the external environmental cost and benefits, based on ecosystem services quantifications. The integrated method is applied in several case studies based on recent regulatory decisions that impact the European Copper industry. The results show that the environmental benefits of applying the measure cannot overcome the side-effects and the costs for industrial sites in a twenty years period.

TH176

MACRO-DB: A Risk assessment tool for management of pesticide use in water protection areas in Sweden

M. Gönczi, SLU / Centre for Chemical Pesticides; N. Jarvis, SLU / Soil and environment; J. Kreuger, Swedish University of Agricultural Science / Centre for Chemical Pesticides

In Sweden, farmers are not allowed to use plant protection products on agricultural fields located in water protection areas for abstraction of drinking water, without permission from the municipality. The risk of a pesticide leaching to groundwater or surface water is dependent on several factors and their combined effects cannot be easily predicted. There are 290 municipalities in Sweden and the assessment and the communication of risk is performed in many forms, sometimes resulting in a high degree of inconsistency in the outcome of the decisions. This causes differences in the level of protection of the drinking water in Sweden as well as unequal conditions for the farmers, which causes conflicts between farmers and the local authorities in many municipalities. The Centre for Chemical Pesticides (CKB) at the Swedish University of Agricultural Sciences has developed a risk assessment tool, MACRO-DB, to help the municipalities to assess the risk of pesticide contamination of the drinking water. MACRO-DB is based on the MACRO model which is a one-dimensional, process oriented, dual-permeability model for water flow and pesticide transport in agricultural soils. MACRO-DB is a simplified, user-friendly tool in two steps, mainly aimed for environmental inspectors, farmers and agricultural advisers. The first step is a very simple web application based on worst case scenarios. In the second step there are a limited number of parameters that the user needs to identify to run the model; site specific information on soil properties, crop, active ingredient, dose and application rate. The results of this risk assessment tool includes risk assessment of soil class, crop type and pesticide are provided in the tool, as well as a risk indicator for soil hydraulic properties. The results are given as a simulated concentration in the water, at 2 m soil depth, which will reach the groundwater and a simulated concentration in the water leaving the drainage system, which will reach the surface water. MACRO-DB was released in September 2013 and the tool is now used in many municipalities in Sweden. MACRO-DB makes it possible for the local authorities to base their decisions on best scientific knowledge. The results can also act as an independent, common foundation for discussions between the different stakeholders, which can help preventing unnecessary conflicts.

Cycle-based Sustainability Assessment (P)

TH177 Communicating results in a life cycle sustainability analysis
T. Ekvall, H. Ljungkvist, IVL Swedish Environmental Research Institute
We developed an approach to life cycle sustainability assessment (LCSA) where the research questions are case-specific and defined in a participatory process. The LCSA results are likely to be a mix of quantitative modelling output and qualitative, open discussions on widely divergent topics. Communicating such complex results is a challenge. We applied the LCSA approach to the case of a 50 km pipeline for transfer of residual heat from industries to a large district-heating system. The LCSA included 14 research questions on economic, environmental and social aspects. We communicated the results not only in a report, but also in a presentation prepared by the participants and one slide for each question. The content of the slides ranged from bar charts to bullet points. To make the presentation more uniform, we used a happy, neutral or sad smiley on each slide. The conclusions were summarised in a table with the smiley and a very brief comment for each question. We found this presentation efficient for communicating our interpretation of the results. However, it did not allow for communicating the methodological choices, assumptions etc. that are necessary for the audience to assess our results and conclusions. Instead, we saw a tendency for the audience to accept the smileys without further reflection. In conclusion, smileys are highly efficient for communicating conclusions on widely divergent issues, but they do not create a good basis for discussion of the results and analyses. This means they might close the door for debate over the decision at hand. This is a problem since our participative LCSA approach is particularly relevant for cases that greatly concern several different stakeholders, and debates are important to resolve conflicts of interest. Hence, communicating not only the conclusions of a LCSA study but also the complexity of the study remains an important challenge.

TH178 Sectoral analysis of the current state of life cycle sustainability assessment in the Chemical Industry: insights from the MEASURE project
D. Kräsch, Friedrich-Schiller-University Jena / Institute of Pharmacy; A. Manent, Evonik Technology & Infrastructure / Process Technology Engineering; E. Rother, Evonik Technology & Infrastructure GmbH / Process Technology Engineering; D. Schowanek, Procter & Gamble / Brussels Innovation Center
The Horizon 2020 SPIRE Program has developed a clear vision for the future of the European process industry for a more sustainable, competitive and growing economy. Ambitious reduction targets have been formulated for energy and raw material intensity and for greenhouse gas emissions. Cross-sectoral collaboration along the value-chain is key to achieve these objectives.
Harmonization of methods for life cycle sustainability assessment (LCSA) along the value-chain is necessary to allow a consistent quantification of the overall benefit of the alternative strategies developed within the SPIRE program. In this context, the European project MEASURE (Metrics for Sustainability Assessment in European Process Industries) was launched aiming to address critical issues and gaps in sustainability assessment. A sectoral analysis was conducted for several process industries. In this poster, special attention is given to the sector Chemistry and Consumer Goods and challenges for the implementation of LCSA.
Overall, a good degree of consistency is observed in this sector concerning life cycle indicators used. The WBCSD Guidance Document “Life Cycle Metrics for chemicals” (2014) is seen as a useful fundament. Nevertheless, some topics were identified as critical issues leading to remaining inconsistencies. Industries face many challenges for harmonisation (e.g. due to unsolved methodological issues, low data availability, results’ sensitivity and practical interpretation. Currently, toxicity issues assessed via LCA are rarely included in the decision-making process and play a minor role despite the recognized relevance of the indicator itself. The assessment of bio-based products remains also a critical issue: biogenic carbon is often inconsistently considered over the life cycle, the calculation of the emissions from Land Use Change are lacking harmonization, while other aspects such as the impact on biodiversity or land quality are poorly covered. Recommendations to tackle some of these issues will be presented to improve the implementation of LCSA. Finally, industries of this sector have gone along a challenging learning curve concerning sustainability assessment, the innovation process thanks to R&D adapted tools, and a staged assessment process starting with the use of screening tools based on selected indicators and ending up with a full Life Cycle Analysis. Such approach will be presented and recommended to assess projects and their innovations in the SPIRE context.

TH179 Development of Life Cycle Social Impact Assessment Method Using Covariance Structure Analysis
Y. Ichiugsu, N. Isubo, Tokyo City University
In recent years, Social Life Cycle Assessment has been paid attention. In 2013, UNEP/SETAC Life Cycle Initiative published a report about S-LCA, which describes a list of subcategories and candidates of indicator. However, there is very little information regarding cause–effect chain modeling indicating the relationship between inventory and impacts in society. The development of a method for S-LCA with clarification of cause-effect chain is critically important to improve the quality of assessment. ISO37120-Sustainable development of communities-was standardized in 2014. This provides a list of indicators for sustainable development in city service. There are a hundred of indicators with classified into seventeen themes. WWCD (World Council on City Data) published standard data that meet World Congress on City Data IS037120. Therefore, this study attempts to develop a framework of S-LCA method using statistical data which characterize social aspects of cities in the world. We use covariance structure analysis. This study draws Social Impact Pathway based on covariance structure analysis result.

TH180 Safe and Sustainable Chemicals, from question to method
S. Waaajers, University of Amsterdam/BIED Institute; M. Zijp, National Institute for Public Health and the Environment RIVM / DMG; M. Broeren, University of Utrecht / Copernicus Institute; A. Poganti, VU Amsterdam / Centre for Environment and Health; L. Shen, McGill University / Chemical Eng; E. Heugens, National Institute for Public Health and the Environment RIVM / Centre for Safety of Substances and Products
Developments such as depletion of resources and climate change urge a transition to a sustainable economy. But what is sustainability? A popular way to evaluate sustainability in terms of impacts on man and the environment is life cycle analysis (LCA). A drawback of LCA is the extensive amount of data needed and environmental, health and safety (EHS) aspects are generally not fully taken into account and are not compatible with substance regulations. Just as important is the necessity for defining the sustainability question to be answered. In many cases the choice for a method is based on habit or nice of expertise and not made explicitly. The aim of this study was to obtain insight in sustainability assessment methods currently used for or applicable to chemical products, biobased compounds, and production processes of substances, to structure these and organise it into a sustainability assessment identification key. We developed this identification key for manufactures, policy makers and those who perform sustainability assessments. The applicability of this approach is shown in a case study. Four cases were chosen, two feedstocks and two products: organic waste, sugar beet, chemical building blocks and bioplastics. Stakeholders were selected, interviewed and brought together within workshops to discuss their views on sustainability, the use of LCA methods, and knowledge gaps. An overview of the stakeholders’ questions regarding sustainability will be given and the application of the identification key is illustrated. In this way a transparent translation of theory and aims (definition of sustainability and promoting sustainable development) is put into practice, which is an often-missing link in current sustainability case studies in literature. Acknowledgement - This research is carried out in the framework of RIVM Strategic Programme (SPR), in which expertise and innovative projects prepare RIVM to respond to future issues in health and sustainability. We gratefully acknowledge the Athena Institute of the VU University Amsterdam for sharing their expertise and assisting in the stakeholder analysis and participation.
Identifying sustainability indicators through Open Space
T. Ekvall, IVL Swedish Environmental Research Institute
Sustainability is a broad concept with a large number of environmental, economic and social aspects. Any sustainability assessment can cover a selection of these aspects only. The choice of aspects and indicators is vital, since it can heavily affect the conclusions of the assessment. Different aspects will be crucial in different case studies. A case-specific approach that involves stakeholders in the selection of assessment indicators is likely to increase the perceived relevance of the results and the chances that the results of the assessment are accounted for in the decision process. The Open Space technology is well suited for discussing and deciding how to organise an activity where the structure is not known in advance. It has been used for, e.g., organising a conference, addressing a local societal challenge, and developing an action plan for a non-governmental organisation (NGO). The Open Space format for workshops is designed to create ample opportunities for meetings and discussions, where each individual participant has the freedom to join and leave discussions whenever he/she chooses. Individual deliberations and small-group discussions alternate with reporting and discussions in plenum in order to generate and preserve the energy in the meetings. Deciding on the topics and the schedule of the workshop is part of the workshop itself. We organised Open Space workshops at the initial stage of a life-cycle sustainability analysis (LCSA), inviting concerned companies, NGOs, policy makers, and researchers. We found the output from the workshop useful as basis for prioritising among the sustainability aspects and for formulating research questions for the LCSA, although this required an interpretation of the workshop results. We also found it necessary to add further research questions to make the LCSA sufficiently comprehensive and balanced. A key challenge in LCSAs as with Open Space workshops is to obtain a sufficiently large and representative group of participants in the workshop. This is likely to be possible primarily in cases that greatly concern several different stakeholders, such as policy decisions or investments in infrastructure.

Comparative life cycle assessment of alternative solutions for the management of medical equipment discarded: the case of a panoramic X-ray dental machine
G. Tomassetti, Università degli Studi di Brescia / Department of Mechanical and Industrial Engineering
In 2012 in the European Union there were 11,000 hospitals and 68,000 medical institutions (including long-term care facilities, clinics, specialized clinics, laboratories, etc.), with about 3,000,000 beds for acute illnesses. The supply of healthcare services is highly dependent on the use of medical equipment, whose employ is continuously increasing. Moreover, the use phase of the life cycle of medical equipment is very short. In fact, the adoption of new standards, the need to improve safety and functionality of the equipment and also marketing purposes contribute to a continuous renewal of the medical equipment: It is estimated that in European hospitals a medical equipment is averagely used only for 5 years. This brings to an increasing amount of medical equipment disposed. Once disposed, most of the medical equipment become a WEEE, i.e. a Waste of Electrical and Electronic Equipment, which represents both a serious risks for the environment, primarily due to their content of hazardous materials, and at the same time, if properly managed, a valuable resource. Considering healthcare institutions, scientific literature highlight that not enough attention is payed to medical WEEE, which are often neglected, stored in basements or in unused premises or donated to charity organization for their shipment in poor Countries without any warranty on their real destiny. Therefore, it is very important to promote a proper management of medical WEEE within healthcare institution. In this work, a comparative life cycle assessment (LCA) approach based on the Environmental Life Cycle Impact Assessment (Ecoinvent 3.1) methodology has been performed to evaluate the environmental impacts of different realistic solutions for the end of life of a panoramic X-ray dental machine. This work is part of the Life-MED project (LIFE13 ENV/IT/000620), funded by the European Union through the Life financial instrument.

Exploring feasibility of monetarization and water footprint
A. Gallice, Veolia / Research Innovation; H. Imbeault-Tétartaud, CIRAIG Polytechnique Montréal; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; J. Serre, VERI; S. Mehier, Veolia / Research Innovation
In a context where stakeholders ask for understandable results to support reliable decision making and communication, the subject of monetary valuation is prevailing. Moreover, companies began to notice increases in their exposure to risks (environmental and business risks) due to several reasons: access to resource, decision making and communication, the subject of monetary valuation is opportunities for meetings and discussions, where each individual participant has the freedom to join and leave discussions whenever he/she chooses. Individual deliberations and small-group discussions alternate with reporting and discussions in plenum in order to generate and preserve the energy in the meetings. Deciding on the topics and the schedule of the workshop is part of the workshop itself. We organised Open Space workshops at the initial stage of a life-cycle sustainability analysis (LCSA), inviting concerned companies, NGOs, policy makers, and researchers. We found the output from the workshop useful as basis for prioritising among the sustainability aspects and for formulating research questions for the LCSA, although this required an interpretation of the workshop results. We also found it necessary to add further research questions to make the LCSA sufficiently comprehensive and balanced. A key challenge in LCSAs as with Open Space workshops is to obtain a sufficiently large and representative group of participants in the workshop. This is likely to be possible primarily in cases that greatly concern several different stakeholders, such as policy decisions or investments in infrastructure.

The Ecological Scarcity Method (ESM), also called Ecosystem Point Method, is based on a distance-to-target approach: it defines critical flows which define a target level for selected physical flows to the environment, and compares these flows to the current respective flows, leading to so-called eco-factors. Critical flows and current flows are to be specified for defined system boundaries, usually the national level, which for the original ESM is Switzerland. The ESM has been applied in the framework of company environmental management of the Volkswagen AG (VW) since 2001. At VW’s German sites the ESM has been adapted to German framework conditions in the course of a research project. As a result, the ESM shall enable companies to determine which ecological goals and how to take them into account in the decision-making process. The Open Space technology is well suited for discussing and deciding how to organise an activity where the structure is not known in advance. It has been used for, e.g., organising a conference, addressing a local societal challenge, and developing an action plan for a non-governmental organisation (NGO). The Open Space format for workshops is designed to create ample opportunities for meetings and discussions, where each individual participant has the freedom to join and leave discussions whenever he/she chooses. Individual deliberations and small-group discussions alternate with reporting and discussions in plenum in order to generate and preserve the energy in the meetings. Deciding on the topics and the schedule of the workshop is part of the workshop itself. We organised Open Space workshops at the initial stage of a life-cycle sustainability analysis (LCSA), inviting concerned companies, NGOs, policy makers, and researchers. We found the output from the workshop useful as basis for prioritising among the sustainability aspects and for formulating research questions for the LCSA, although this required an interpretation of the workshop results. We also found it necessary to add further research questions to make the LCSA sufficiently comprehensive and balanced. A key challenge in LCSAs as with Open Space workshops is to obtain a sufficiently large and representative group of participants in the workshop. This is likely to be possible primarily in cases that greatly concern several different stakeholders, such as policy decisions or investments in infrastructure.

Th113
Exploring feasibility of monetarization and water footprint
A. Gallice, Veolia / Research Innovation; H. Imbeault-Tétartaud, CIRAIG Polytechnique Montréal; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; J. Serre, VERI; S. Mehier, Veolia / Research Innovation
In a context where stakeholders ask for understandable results to support reliable decision making and communication, the subject of monetary valuation is prevailing. Moreover, companies began to notice increases in their exposure to risks (environmental and business risks) due to several reasons: access to resource, increase in price, supply disruptions, lower quality of resource, competition for resource. It is therefore necessary to add further research questions to make the LCSA sufficiently comprehensive and balanced. A key challenge in LCSAs as with Open Space workshops is to obtain a sufficiently large and representative group of participants in the workshop. This is likely to be possible primarily in cases that greatly concern several different stakeholders, such as policy decisions or investments in infrastructure.

Public decision support & LCA: feedback from testing a simplified LCA tool for wastewater systems
L. Guérin-Schneider, National Research Institute of Science and Technology for Environment and Agriculture - IRSIA / UMR IRISA GESTE; M. Pradel, Irstea Clermont Ferrand / Ecotechnologies; L. Aissani, Irstea Rennes; V. Laforest, Ecole des mines de Saint-Etienne - Institut Fayol / UMR CNRS ENV/IT/000620), funded by the European Union through the Life financial instrument
The environmental efficiency of a wastewater treatment plant (WWTP) is usually assessed through its effluents quality. But this quality is achieved at the expense of other impacts occurring during the construction, operation and dismantling of the whole wastewater system (WWS) including sewers and WWTP. Many studies performed LCA of WWTPs already exist, but there is a lack of simplified and operational tools usable by non-specialists of LCA to perform LCA of entire WWS. The objective of this work was to develop a simplified software providing objective environmental indicators to be included in the decision making process along with other criteria when choosing among WWS options. To reach this goal, simplifications were done by adapting the software already available (ACV4E) and the software was implemented (namely ACV4E software). The potential users are public
wastewater services and engineering consultancies. The LCA results should be presented to local authorities for decision making and in some cases to citizens to justify the choices made. ACV4E was applied to real cases to test the software as well as the appropriation process and the effects on decision making. In addition a working group involving stakeholders was created. This group made a focus on the interpretation of LCA results. For the challenged makers making understandable and usable for non-specialists of LCA. The experiment suggests that the co-construction of the calculator with potential users is crucial for appropriation. It partially opens the LCA black-box and allows to better meet field needs. However, classical mid/endpoint bar charts proved to be inefficient for non-specialist interpretation and for communication to politicians and normalisation of the results almost always leads to misinterpretations. As a result of the working group process, these classical charts will be replaced by new types of representations. Local authorities that tested ACV4E were very interested in this tool even if the environmental criterion was often secondary compared to other criteria in the context of legal compliance with local standards and financial aspects. Finally, this experiment concludes that such a tool was more difficult to use directly by local authorities for which the need for LCA in decision making for WWs is choices is not frequent enough. Engineering consultancies could be the main users of the software because they have technical skills and will have more regular use of it.

TH187

Interpretation of LCA results for GRP pipe systems through normalization lenses

V. Vlaminck, HOBAS; M. Padzuro, REICHHOLD AS

HOBAS and REICHHOLD took a significant step in direct collaboration of both product R&D and life cycle assessment. The study evaluates the different choices of unsaturated polyester resins (UPR) for cc-GRP pipe systems, from a LCA perspective. To this purpose three types of resin have been used to manufacture HOBAS products and the environmental impacts were compared. The resins are: (a) Standard UPR resin containing contained PET material (denoted rPET-UPR) and (b) UPR resin containing bio-sourced material (denoted BIO-UPR). Based on EN 15804:2012 (Sustainability of construction works) the study compares fourteen impact indicators for pipe systems in the three scenarios. After the results were obtained and interpreted, normalization was applied in order to cross-check the first understanding of the indicators. CML data from April 2013 (Leiden University, CML-IA, Version 4.2, April 2013) was taken and the total emissions for Europe “E2U+3” (Wegener Sleswijk et al., 2008) were used as the reference scenario. For example, for a product manufactured with BIO-UPR resin in the first step increased variation of “renewable energy”, “water consumption”, “stratospheric ozone depletion” and “photochemical oxygen creation” were noticed from the calculation. The results of the second step, normalization, indicate that the impact category with by far the most relevance is the “depletion of abiotic resources”. Therefore, the issues which could be considered for further analysis may concern the use of crude oil (i.e. the formulation of the BIO-UPR resin includes intermediates made from crude oil). Thus, choice of BIO-UPR resin is a rather complex process and can hardly be reduced to the simple question of, for instance, which resin generates more or less CO2 eq emissions for the product. The use of normalization is of great help in identifying possible next steps in product R&D. The BIO-UPR formulation used in this study is one possibility of many. Based on the LCA results, focus can be put on improvements for the formulation. For the political decision making of alternative and renewable raw materials represents a key task. This study represents a step ahead on the path to sustainability/un

TH188

Allocating an environmental load to wastewater sludge when sludge moves from "waste" to "product", a methodological challenge

M. Pradel, Irstea Clermont Ferrand / Ecotechnologies; L. Aissani, Irstea Rennes; J. Villot, École des mines de Saint Etienne; J. Baudez, Irstea / TSCF research unit; V. Laforet, École des mines de Saint-Etienne - Institut Fайoil / UMR CNRS EN

Wastewater sludge is currently considered as a waste but with new industrial practices and European regulation (End-of-Waste directive) considerate will be soon a valuable product. This paradigm shift must be followed by the consideration of environmental constraints to introduce this new product on market. Assessing the environmental performances of these future ‘marketable sludges’ can be processed with Life Cycle Assessment (LCA) but it first implies to take into consideration the whole life cycle of this new product from the raw material extraction up to the product end of life. How this can be reasonably done when waste LCA consider that the waste is free of any environmental load when entering the system, assumption often known as the “zero burden approach”? Giving a environmental load to “product sludge” consists in the resolution of several methodological questions which arise as the wastewater treatment plant will provide several coproducts: the clean water and the sludge, both having a merchandable value. This paradigm shift implies that the wastewater treatment plant has several function and multifunctionality has to be resolved. Because sludge is a direct UPR and the sludge fraction from water treatment plant subdivision cannot be achieved. In the same basis, system expansion can be barely used as there is no known alternative to the “wastewater treatment” if system is expanded to only keep the “sludge production” one. Finally, allocating an environmental burden to these two coproducts appears to be the best way to solve the induced multifunctionality. As a consequence, allocation factors have to be developed to allocate an environmental burden by product to the whole wastewater released to the receiving media. This is quite challenging as it supposed (i) to model the sludge production both from primary treatment and biological treatment, (ii) to create new allocation factor based on these physical and biological processes as classical allocation factors cannot be used, (iii) to validate these allocation factors whatever the wastewater treatment plant configuration. This paper aims at presenting the allocation factors used to charge sludge with an environmental burden when sludge is from now considered as “product sludge”.

TH189

Integrating LCA and risk assessment for decision support

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In the last decades, the use of classic decision making theory has gained a lot of attention as decision support tool. Indeed, when stakeholders and governments face the problem of decision making over public safety and health or sustainable development, their judgment has to be justified to the community by objective proofs. In this perspective, sustainability and safety are the major issues to be considered in decision making. Decision makers always face the dilemma that the most sustainable solution may not be the safest one. Therefore, it is a challenge to optimize the system, ensuring sustainability and at the same time minimize risks. The Global Decision Support Initiative (GDSI) is a cross-disciplinary center established at the Technical University of Denmark (DTU) aiming to develop a new framework model for decision support with associated tools to solve this problem. Firstly a Life Cycle Assessment (LCA) is conducted to screen the studied system and identify the hotspots where emissions are largest and hazards are more likely to occur. Decision analysis is then applied to optimize the alternative that may reduce the consequences caused by those hotspots. To start with, we first identify and characterize the potential hazards and then quantify their associated direct and indirect consequences for each hotspot. A finite number of available actions are chosen among the ones that can reduce the consequences. For each action we apply decision analysis to evaluate the effect of the actions in mitigating the consequences, expressed in failure cost and damages to human health. Meanwhile, we apply LCA to assess the environmental impacts changes caused by the action. A common metric is developed to allow comparing RA and LCA results on a common scale and eventually integrate them into one result. The result is then used to rank the alternatives, taking stakeholder preference into consideration, and the optimal solution is identified. Here the traditional LCA is applied in the framework of decision making together with RA. It does not only identify the problem, but also provides ranking of possible solutions, where consequences from both potential risks and environmental emissions are considered. This framework is flexible and can be broadly used to support decision at different scales, e.g. national, sector or product level.

TH190

A normalisation and weighting procedure (ESA) to assists the decision making process: The case study of salt mining in the chlor-alkali industry

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Life cycle assessment (LCA) is a powerful tool to assess the potential environmental impacts throughout a product’s life-cycle [1]. However, LCA results comprises several impact categories that makes difficult the process comparison [2]. To reduce the LCA complexity, this work proposes a normalisation and weighting procedure to carry out the environmental sustainability assessment (ESA) of the extraction and purification of salt in the chlor-alkali process. The ESA is based on two main variables: natural resources sustainability (NRS) and environmental burdens sustainability (EBS) [3]. Different variables play an important role in the quantitative estimation of the NRS, mainly the materials, water, and energy usage need to be considered. Three main EBS are involved depending on the natural environment: air, water, and soil related to the emissions, effluents, and wastes leading to different environmental impacts. Nevertheless, these functions are rarely normalised thus represented as functions rather than variables. This methodology suggests the normalisation of EBS based on the threshold values proposed in the European Pollutant Release and Transfer Register E-PRTR, and a similar procedure based on the average consumption of natural resources of the process under study for the normalisation of NRS. Moreover, the normalised indicators can be subjected to a weighting procedure to obtain a global index that takes into account the different decision making process. However, the use of an absolute index could potentially mask certain results. For this reason, the comparison of several processes by means of a global index can be used to obtain an overview of the environmental performance of the system and to optimise the process. Nonetheless, individual indicators should be also assessed to determine the main environmental problems and thus provide opportunities for improvement of the critical points of the process.
TH191
Representativeness of environmental impacts in relation to Life Cycle Inventories
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Life Cycle Assessment is based on Life Cycle Inventory (LCI) of systems that identifies all emissions to the environment and consumptions of resources (1,800 referenced environmental flows in the Ecoinvent database). LCIs show many sources of variability due to the diversity of systems, the model uncertainties, the variations of the real world... Following the construction of LCIs, emissions and consumptions are translated in terms of environmental impacts through a set of impact categories. Different Life Cycle Impact Assessment (LCIA) methods (ReCiPe, TRACI, CML, Eco-indicator...) propose different ways of characterizing categories and each of them can be used to determine the results on the set of impact categories modeled. Then, LCIA methods reduce the complexity of systems comparison from a 1800-dimension space (i.e. the number of substances emitted or taken from the environment) into less than 20-dimensional problem (i.e. the impact categories) where each dimension has an environmental meaning. Each impact category represents, to a more or less precise extent, the original LCI. The aim of the present work is to determine how far impact categories are relevant. The method automatically organizes impact categories according to the information on LCIs that they gather. LCA results interpretation could be helped by this development by indicating the impact categories for which the LCI is the most correctly represented. The 11,332 LCIs of the Ecoinvent 3.1 database and a total of 692 impact categories were used in the present work. Both of them belong to a vector space of 1,769 dimensions where each dimension represents an environmental flow. After a data pre-processing (i.e., data cleaning and data normalization), cosine squared between directions of LCIs and impact categories were computed. The higher a cosine is, the more the impact category direction is similar to the LCI direction, and the more correctly the impact category considered represents the contribution of each environmental flow. Cosine squared results for each impact category were compared by using their median value. Grouping categories by their environmental meaning shows tendencies from eutrophication potential to land occupation/transformation for their ability to accurately represent LCIs from Ecoinvent.

TH192
Promoting Innovation & Technology in Europe: Sustainability Support and Information Network of Infrastructure
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In the roadmap to a resource efficient Europe, it is indicated that a sustainable and guaranteed supply of raw materials will be essential, together with an efficient transformation and use of the resources. The EIT (European Institute of Innovation and Technology) Raw Materials will contribute to this challenge, but to boost effective and efficient resource management in the EU, insight and support is required in sustainability of virgin resource extraction, recycling, resource conversion and environmentally, socially and economically sustainable technologies in general. The SSIC (Sustainability Support and Information Centre) and facilitates sustainable technological development and educational activities by making use of a state-of-the-art sustainability assessment toolbox. This Network of Infrastructure (NOI) project brings partners together with complementary expertise and therefore is the central contact point for industry and technology developers, from resource extraction to product design and recycling, when it comes to questions regarding input-output analysis, life cycle assessment, life cycle sustainability assessment, socio-economic assessment, sustainability of material flows and stocks, cost benefit analysis, recyclability benefit calculations, criticality analysis, supply disruption etc. It can be consulted in up-scaling projects, other NOIs, education, training etc. but will also be leveraged outside the EIT Raw Materials and will link up with existing initiatives at European level. Offering a fast and effective access to state-of-the-art datasets, methodologies and tools, the SSIC delivers a service package at the highest level for innovation growth in Europe. While looking for opportunities to strengthen the consortium by networking and linking to other initiatives, fostering collaborative projects with SSIC and external partners, the consortium will add through a multidisciplinary approach in making raw materials a major strength for Europe.
Contaminants of Emerging Concern in the Environment and their Management (PC)

MOPC01 Pesticide contributions of urban effluents and storm sewer on a French peri-urban river contamination

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Currently, pesticides are widely used by different users such as farmers, private consumers or public authorities. These compounds can be released into the environment and contaminate water resources, potentially impacting aquatic ecosystems. Several contamination routes are well identified: diffuse losses due to agriculture uses, or direct releases because of urban effluents. Storm sewers and wastewater treatment plants (WWTP) are known to be vectors of pesticides. Indeed, several studies have shown the presence of pesticides in wastewaters; moreover WWTPs are not able to remove all of them efficiently without adequate tertiary treatments. The purpose of this work was to study a continuum including a wastewater network, up to the collection natural aquatic ecosystem, a peri-urban river, receiving treated WWTP effluents and untreated inputs via storm sewers. The study allowed monitoring the main classes of pesticides as contamination. It has been shown that the major source of pesticides used in agriculture is the upstream of the studied system, as expected, mainly due to metabolites of metolachlor (0.2 g d\textsuperscript{-1}), one of the most used herbicide on the territory. On the contrary, WWTP effluent appeared to be the major vector for pesticides mainly in urban purpose such as fipronil (0.2 g d\textsuperscript{-1}), a veterinary used insecticide or diuron (2 g d\textsuperscript{-1}), an herbicide added in buildings for building protection against moscas and algae. Domestic wastewaters are suspected to bring more pesticides than industrial ones because some pesticides are classified as biocides and are mostly used by private consumers such as fipronil. Storm sewers have been shown to be also a source of contamination, but at a lower level than WWTP effluents. Such integrated studies by documenting the presence, sources, and fate of pesticides all along a continuum could give paths for reflection and in fine actions on sources, to reduce pesticide contamination of natural surface waters. Acknowledgement This study benefited from funds in relation with RESEAU and “Plan Micropolllutants” projects (associating as partners: Bordeaux Métropole, SUEZ, IRSTEA, Water Agency, CNRS, University of Bordeaux, CHU and Aquitaine Regional Council). This work was funded by the Aquitaine Regional Council and SUEZ. This study has been carried out with financial support from the French National Research Agency (ANR) in the frame on the Investments for the future programme, within the Cluster of Excellence COTE (ANR-10-LABX-45)

MOPC02 Pollutant fluxes in small catchments: Impact of urbanization, flow dynamics, and pollutant degradation

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Urban areas are responsible for the release of numerous emerging contaminants into the environment. Wastewater-related compounds are continuously introduced into aquatic systems by wastewater treatment plants (WWTPs). Hydrophobic chemicals such as PAHS are mainly released during flood events and the related combined sewer overflow. Within catchments, rivers act as transport pathways. While some pollutants undergo attenuation, others are very persistent. In this study emerging and particle related pollutant fluxes are assessed for the Steinlach and the Ammer rivers, two tributaries of the River Neckar in SW-Germany. For dissolved pollutants (e.g. pharmaceuticals and personal care products as carbamazepine, diclofenac or triclosan), fluxes have been estimated based on various sampling campaigns including monthly samplings and high-resolution sampling during base flow and flood conditions. By use of Lagrangian type sampling at consecutive control sections, also the persistence of these pollutants could be assessed. In water samples collected during event samplings, a correlation of total concentrations of hydrophobic pollutants (PAHs) with turbidity and/or total suspended solids was found, which allows for the calculation of pollutant concentrations on suspended solids. For both catchments, such correlation coupled with continuous monitoring of discharge and turbidity enabled the calculation of particle-bound pollutant fluxes. The results showed that fluxes of wastewater-related pollutants are preferably influenced by consumption patterns and WWTP efficiency. During flood events, these compounds tend to get diluted, revealing a source limitation of their input into the environment. Among these compounds diclofenac shows the highest fluxes in the monitored rivers. However, it must be reminded that dissolved compounds can undergo natural attenuative processes. In that regard, their fluxes were checked with distance from the source. Carbamazepine – as a counterexample – also shows high fluxes but behaves conservatively and may spread along farther distances. Results of on-line turbidity monitoring showed that high turbidity/discharge events account for the majority of particle-related pollutant loads. In contrast to soluble compounds, the mass fluxes are positively correlated with discharge, yet practically cease during base flow conditions. On the long run, however, both types of pollutant fluxes may be relevant and should be monitored to a sufficient level.

MOPC03 Modified-zoetites as potential sorbents for removal of per- and poly-fluoroalkyl substances from aqueous environments

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Perfluoralkyl substances (PFASs) are a class of stable chemicals used in fire-fighting foams, industrial surfactants, insecticides and surface treatments for paper and textiles. Perfluorooctane sulfonate (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF) were added to Annex B of the Stockholm convention on persistent organic pollutants (PDPs) in May 2009. A number of sites, such as landfills, agricultural sites contaminated with PFAS are also known to have been contaminated by PFASs. PFASs have also been found in groundwater. Limited work has been done on PFAS removal from aqueous environments. Sorbents (e.g., activated carbon, zeolite, peat etc.) for conventional organic pollutants might not be effective for remediation of PFAS. Knowledge of sorption processes and appropriate materials (i.e. sorbents) for PFASs has been the most recent studies have only focussed on PFOS and PFOSF. In our study, we modified a natural zeolite (clinoptilolite) to provide four sorbent materials and investigated their sorption potential for nine PFASs. The results based on the 24-h batch adsorption test were promising. CPC-zeolite and DDEMA-zeolite are the most effective among the four modified-zeolites in removing PFASs from aqueous environment. The sorption ranks were PFOS > PFNA > PFHxS > PFOA > PFHpA > PFBS > PFHxA > PFPA > PFBa. The modified sorbents had higher concentration removal for long-chain than for short-chain PFASs. For the same number PFAS carbon atoms, sorbents had higher sorption of PFASs than PFCCs. This research is on-going. We continue to investigate sorption kinetics, temperature effects, maximum sorption capacity, sorbent dosage effects, particle-size effect, PFASs competition effect, as well as the stability of these sorbents and the leachability of sorbed PFASs. Keywords: Perfluoroalkyl, sorption, modified-zeolite, aqueous. Acknowledgment – Thanks to the Natural Sciences and Engineering Research Council of Canada Grant # EGP477520-14.

MOPC04 Human exposure to plasticizer chemicals - correlation between indirect and biomonitoring exposure estimates in a Norwegian human cohort

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Plasticizers used as additives in numerous consumer products have a continuous release into the environment (pseudo-persistent), leading to consecutive human exposure which might cause adverse health effects (e.g. endocrine disruption). Indoor air quality and house dust may have significant impact on human health because people spend most of their time indoors, where the concentrations of plasticizers are comparatively high. Inhalation and dermal exposure through air, transdermal exposure through the skin, oral exposure through dust ingestion (hand to mouth contact) and diet have been considered as major exposure pathways. On the other hand, daily intake of plasticizers can also be determined through analysis of urinary metabolites. In the present study, indoor stationary air, personal ambient air and house dust (floor and elevated surfaces) samples were collected, by using solid phase extraction (SPE) and/or microwave assisted extraction and analyzed by GC-MS/MS. Based on the resulting concentrations of the diesters in these external media, we calculated the daily human intake rates for the Norwegian cohort, and compare them with reference tolerable daily intakes (TDI) for each analyte. Alternatively, the human biomonitoring approach allowed us to assess to which extent plasticizers entered the bodies of 61 adults living in the urban area of Oslo. Finger nails and three intra-dermal skin spots were collected from each participant in order to
monitor their internal concentration. The analysis of nine phthalate metabolites, together with two DINCH metabolites in urine and naphthalene was performed by LC-MS/MS and used to back-calculate the initial total exposure to the parent compounds. Among phase I metabolites, high levels of the monooesters of DEP and DiNP were observed in naphthalene (median concentrations of MESP 31.2 ng/g and 18.3 ng/g, respectively), while in urine the median concentrations of both the more specific biomarkers of secondary metabolic oxidation for DEHP was OH-DEHP 4.5 µg/L and 5-oxo-MEHP 4.7 µg/L, and for DINCH were cis-OH-MINCH 0.5 µg/L and cis-cis-MINCH 0.5 µg/L. The correlation of indirect exposure estimates and human biomonitoring exposure estimations is under investigation.

MOPC05
Method development for non-target screening of sewage sludge using comprehensive two-dimensional gas chromatography coupled to high-resolution mass spectrometry (GCxGC-HRMS)
C. Voermans, Utrecht University / Chemistry; P. Haglund, Utrecht University / Department of Chemistry
More than 100,000 chemicals are present in the technosphere and 30,000 of these are considered to be so-called “every-day chemicals”. Sewage treatment plants (STPs) are used to remove nutrients, but also some metals and organic chemicals, from urban waters and create a less contaminated effluent. Consequently, STPs form a link between the technosphere and the environment. A by-product of the sewage treatment process is sewage sludge – a solid product that contains nutrients as well as pollutants. These nutrients make the sewage sludge attractive for applications as fertilizer on agricultural fields, provided that the contaminant levels are not too high. In order to be able to investigate pollutants occurring in sewage sludge and to develop an analytical method for comprehensive non-target screening as needed. Up till now there is no method existing that allows a full screening of sewage sludge for a wide variety of compounds with different properties, which is the ultimate goal of this project. The current work has aimed at developing a validated non-discriminating sample preparation method for gas chromatography - mass spectrometry (GC-MS) analysis. Pressurized liquid extraction (PLE) was used for extraction, with in-line and/or off-line clean-up. In the in-line approach silica gel was added to the PLE cell for simultaneous extraction and clean-up, whilst the off-line approach involved clean-up by gel permeation chromatography. Analysis was performed by comprehensive two-dimensional GC (GCxGC) coupled to high-resolution MS in both cases. Good recoveries were obtained for all tested compounds with either one or both of the proposed sample preparation methods. Therefore the combination of both methods allows a comprehensive screening of sewage sludge contaminants by GC-MS.

MOPC06
Illicit drugs as new class of emerging contaminants in aquatic systems: high specific level of contamination in French West Indies compared to global monitoring results
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Since 2013, authors analysed each year illicit drugs (19 molecules and metabolites related to cocaine, cannabis, amphetamines, opiates and substitute consumption) in urban wastewater inlets of treatment plants (WWTPs) of Fort-de-France, the main city of Martinique island of French West Indies. Caribbean are the hub of cocaine trafficking), which also represent a hotspot of biodiversity, including several patrimonial species and ecosystems, on which the effect of emerging pollutant residues is unknown. Wastewater were daily sampled during a normal week, i.e. without festive event or other confusing factor, following a protocol framed by the European Union’s programme SCORE, gathering now worldwide similar initiatives. Samples were analysed (solid-phase extraction on Oasis HLB cartridges then LC/MS/MS quantification) after SCORE inter-calibration agreement. Results show very high concentrations of cocaine and metabolites in comparison to published results, exceeding 3 µg/L for benzoylcegonine (BZE) and 1.5µg/L for 1-14C-3-carbomethoxy-4-methylamphetamine (THC-COOH): these levels are ten-fold higher than those published for other cities. Opiates and their substitutes and amphetamine-like compounds were never detected. This data confirm a Caribbean-specific profile of drug consumption, partly explained by the place of this archipelago in the international market of drugs. Considering to temperate environment, source and human exposure to demonstrate the importance of European or North-American species at environmental concentrations, i.e. tenfold less than results from Martinique. Moreover, severe leakage of sewer and non-collective sanitation threaten surface water. Combining the small watercourse of the rivers and the local high population density, coastal and marine ecosystems could gradually be considered as exposed to such molecules, in combination with pharmaceutical residues. Authors will discuss the potential input of illicit drugs on the aquatic environment in Martinique Island, the predicted values regarding to actual data on ecotoxicological effect of the studied molecules, and the mean to ward off the urgent need for determining the stake of such emerging micropollutants on the accelerating biodiversity loss observed in patrimonial ecosystems.

MOPC07
MOPC07 of the biocide and plastic additive Triclosan to marine organisms: environmental risk assessment
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Triclosan is a well known biocide with the potential to disrupt hormone function in aquatic organisms. The 5-cloro-2- (dichlorophenoxy) phenol, or Triclosan, is a biocide of general spectrum widely used in multiple pharmaceutical and personal care products (including tooth paste) and also as an additive to different kinds of plastics. We have tested the toxicity of this substance by means of three standard marine biomarkers representing sensitive and phylogenetically distant biological models, including the microalgae Isochrysis galbana , nauplii larvae of the copepod crustacean Acartia and embryos of the echinoderm Paracentrotus lividus. In increasing order of toxicity, the E50 (µg/L) for sea-urchin embryos was 149 (95% CI: 140 to 158) the LOEC 120 µg/L and the NOEC 100 µg/L. For Acartia nauplii the EC10 was 943 (95% CI: 890 to 1000) and the NOEC 93.6 µg/L. For the microalgae the toxicity was even higher, with a EC50 of 34 (95% CI: 28 to 40), a LOEC 20 µg/L, and a NOEC of 10 µg/L. These results allowed us to quantify the risk posed by Triclosan on coastal ecosystems. The results also suggest that Triclosan concentrations should be surveyed in coastal waters, and water quality standards implemented for this substance.

Microplastics in the environment: Sources, Fate and Effects (PC)
MOPC08
Occurrence, composition and adverse effects of microplastics in native mussels collected along coastal and marine areas of the northern Adriatic sea
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In the recent years, the occurrence of microplastic particles in the aquatic environment has gathered a rising scientific interest. Several studies proved that the presence of microplastics may negatively influence the physiology of marine organisms of different feeding strategies, particularly in those species which can not discriminate the food source. Recent studies have posed the attention to the accumulation of these particles in the food web and the direct consequences to the human health as in even larger amounts of seafood are consumed nowadays. On the other hand, evidences also suggest the role of microplastics as vectors of chemical contaminants either used as additives during the polymer synthesis, or adsorbed directly from seawater i.e., PAHs, PCB, surfactants etc., However, models simulating physiological conditions in the gut suggest that both adsorbed pollutants and chemical additives of plastics might be released to organisms. Despite the importance of microplastics in adsorption and transport of hydrophobic pollutants, little is known about the distribution, accumulation in the marine food webs as well as their direct and indirect harmful effects. On this context, the Adriatic Sea represents a semi-enclosed basin characterized by a low water recirculation rate and an elevate anthropic pressure associated with unsustainable fishing and high inputs of environmental contaminants. Thus the primary objective of this study was to investigate the body burden accumulation, polymers characterization and ecotoxicological effects in native mussel (M. galloprovincialis) collected in various marine areas along the northern Adriatic sea. Results were compared to data collected from native populations of coastal areas. The early outcomes both show a significant site-, time- and oceanographic conditions-related distribution and support the need for a seafood safety monitoring program to better understand the actual human health related risk.

MOPC09
Microplastic particles analysis by Raman micro-spectroscopy and chemometrics: morphological and chemical characterization
L. Frère, LEMAR UMR 6539 CNRS; J. Moreau, Ifremer, Laboratoire Détection, Capteurs et Mesures - Centre Bretagne; I. Paul-Pont, C. Lambert, P. Soudant, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer; A. Huvet, Ifremer, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer; E. Rummel, Ifremer, Laboratoire Détection, Capteurs et Mesures - Centre Bretagne World production of plastics has been steadily increasing since 1950, reaching 311 million tons in 2014. This results in an accumulation of plastics in the marine environments. The global spread of microplastics requires the establishment of standard analytical methods to distinctly identify them and estimate their abundance in all environments (waters, sediment and biota). Most studies have used visual sorting to rank a particle as a microplastic but this method presents a high probability of a misidentification, especially with decreasing particle size. To date only a few studies on the detection and chemical composition analysis of microplastic particles have been published. Several methods have been developed to identify plastic polymers such as vibrational spectroscopy like micro-Fourier-transform infrared (FT-IR) spectroscopy and
Raman spectroscopy, which are non-destructive methods, and sequential pyrolysis-gas chromatography coupled to mass spectrometry (destructive method). The main disadvantage of these methods was the analysis of only selected subsamples and not the whole sample. In the present study, an alternative method was proposed to rapidly analyze all particles in a sample with Raman micro-spectroscopy. Three laser wavelengths (633, 691 and 785 nm) and three substrates (glass fiber filter, nitrocellulose membrane filter and gold coated microslide) were tested to increase the ratio between Raman intensity and fluorescence background. The analysis of particles was carried out using a combination of static image analysis of particles and automated Raman micro-spectroscopy counting the composition of a large number of particles. The optimal combination was the 785 nm-laser with the gold coated microscope slide, which guaranteed a good Raman signal for the fingerprint region (1400 – 600 cm\(^{-1}\)), allowing the identification of the most common polymers found in the marine environment (the “Big six”). The novelty of this study was also that spectra were processed with an algorithm programmed to remove the Raman spectral background in a similar way for all of the spectra while keeping the analytical signal intact. Spectral extraction and identification were achieved using the SIMPLISA algorithm model, which was modified to automate data processing. This analytical approach allowed a fast characterization and identification of a large number of microplastics particles, even when many polymers were combined.

MOPC11 Investigation of sorption properties and sorption kinetics of organic micropollutants onto microplastic particles
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For several years now, the pollution of aquatic ecosystems with microplastic particles, i.e. particles smaller than 1 mm, has been considered as a cause of rising concern due to their ubiquitous detection in the environment, their strong sorption capacity and their potential effects on all organisms along the food chain. Besides likely impairments of organisms due to the mere plastic material, microplastic particles are also suspected to act as vectors for contaminant transport. However, their function as pollutant carriers, which depends on material characteristics (e.g. plastic type and shape) but also on the substance properties, is still under discussion. In order to clarify this issue, sorption of six selected organic microplastics onto different types of microplastics (particles, fibers, fibres) is studied in batch experiments on equilibration sorption and sorption kinetics. Additional sorption experiments are performed in dynamic bench-scale approaches simulating natural flow conditions in water bodies to close the gap between laboratory data and the environment. Diffusion limited sorptive uptake is mathematically described using analytical solutions for film diffusion and intraparticle diffusion which are the predominant processes in regulating sorption of hydrophobic organic contaminants. Additionally, experimental results are simulated by a numerical model using data and parameter values from analytical fits. Our results from batch experiments indicate non-linear sorption behaviour with partitioning coefficients in the same order of magnitude as given in the literature for organic pollutants in PE, e.g. for benzo(a)pyrene, Freundlich coefficients are 3.76 and 4.21 L kg\(^{-1}\) for PE spheres and particles extracted from commercial facial cleaning products (peeling particles), respectively. Apparent diffusion coefficients for PE obtained from intraparticle diffusion were in the order of 10\(^{-9}\) and 10\(^{-11}\) cm\(^2\) s\(^{-1}\), whereas the rate constants for film diffusion were in the range of 10\(^{-8}\) and 10\(^{-9}\) s\(^{-1}\) for peeling particles and 10\(^{-5}\) s\(^{-1}\) for spheres. Numerical model simulations resulted in water film thicknesses of 1 to 100 μm. These data indicate that the kinetic uptake of benzo(a)pyrene into spheres is mainly determined by intraparticle diffusion (possibly due to incomplete wetting) while for the peeling particles a strong influence of film diffusion at early time points and of intraparticle diffusion at later time points could be observed.

MOPC12 First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia
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Microplastic contamination in the African Great Lakes is currently unreported, and compared to other regions of the world little is known about the occurrence of microplastics in African waters and their fauna. The present study was conducted in the Mwanza region of Tanzania, located on the Southern shore of Lake Victoria. The gastrophototactic trials of locally fished Nile perch (Lates niloticus) and Nile tilapia (Oreochromis niloticus) were examined for plastics. Plastics were confirmed in 20% of fish from each species by Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) spectroscopy. A variety of polymer types were identified with likely sources being urban waste and consumer use. Although further research is required to fully assess the impact of plastic pollution in this region, our study is the first to report the presence of microplastics in Africa’s Great lakes and within the fish species that inhabit them.
Development of a risk mitigation toolbox dedicated to pesticides in farmland in Europe: expected benefits for environmental protection and biodiversity and recommendations

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The registration process for Plant Protection Products (PPP) or pesticides in agriculture relies on a preliminary evaluation of the risks they may pose to human health and the environment in the farmland. Risk mitigation measures may accompany the registration in providing detailed conditions of use to reduce exposure of non-target organisms and / or residue transfer in human health and environmental compartment to acceptable levels and thus reach the expected protection goals. These mitigation measures range from the use of special application equipment to the implementation of landscape features such as buffer strips or recovery areas, which may be recommended on product’s labelling. Some of these measures are also recommended in the European Common Agriculture Policy (CAP). A two stage workshop was organized to inventory the knowledge and experience in risk mitigation measures throughout the EU. The workshop invited risk assessors and risk managers industry, academia and agronomical advisors/extension services within 21 European countries to develop a toolbox of risk mitigation measures designed for the more flexible management and use of pesticides for agricultural purposes, and contribute to promoting policy and practice harmonization within Europe. The workshop focused on a broad range of environmental risks: wildlife including vertebrates and invertebrates, flora and microorganisms, biodiversity as well as surface - and groundwater quality, identified as protection goals in the European regulation on pesticides. This poster will present the toolbox developed and illustrate the expected benefits of the measures recommended for the protection of wildlife in agro ecosystem.

INVENTORY OF POTENTIAL ECOLOGICAL FOCUS AREAS IN AGRICULTURAL LANDSCAPES IN THE CONTEXT OF THE COMMON AGRICULTURAL POLICY REFORM


According to the EU Commission, direct payments to farmers should be linked to ecological measures (CAP Greening). One component is the establishment of ecological focus areas (EFA) on the agricultural field. The aim of this study is to identify site-specific marginal areas within agricultural sites, which could be developed as potential ecological focus areas from an agronomic and landscape-ecological perspective. These areas are mainly characterized by perennial abiotic factors that cause plant stress (reduced water capacity, soil surface water over a cropping season. The PEC value is then compared to the toxicological sensitivity of wild pollinators will be collected. Based on this data, ecologically tolerable thresholds will be derived and the relative sensitivity of different groups of wild pollinators in crop-specific landscape scenarios will be determined. Using this information, criteria for the risk assessment scheme on wild pollinating insects as well as recommendations for the improvement of potential risk management options will be developed. The regulatory background and the aim of the project as well as first findings are presented in this poster.

Ecological enhancement of agricultural land in the Upper Rhine Plain

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The project “Ecological enhancement of agricultural land in the Upper Rhine Plain” aims to find a broadly implementable way to increase the biodiversity and especially to promote and support wild pollinators and honey bees in intensively used agricultural landscapes. Here, a matrix of 50 sites was selected to implement measures like flower strips or bee banks was implemented in the intensive farmland in order to increase species richness and populations of pollinators and other farmland wildlife. A mix of annual and perennial flowering plots was used, which are manageable by the farmers in their work routine and flexibly implementable in the farmland regime. Over a time period of five years (2010 - 2014) changes in landscape structure, pollinator biodiversity and ecological parameters of arable fields were recorded. After a baseline survey in 2010, flower strips and flower fields are cultivated on 10 % of arable land within 50 ha study areas on two farms since 2011 ongoing, which are complemented by unmodified control areas of the same size. Wild bees and butterflies were sampled on flowering plots in the enhancement areas and on flower-rich structures in the control areas. Wood blocks with nesting tubes were installed to facilitate sampling of the wild bee species, and bee banks (mounds of bare soil) were created in the enhancement areas as nesting structures for ground-nesting bees. Autumn and spring sowing was carried out and the seed mixtures were adapted year by year according to the results obtained regarding an overall good variability of plant species and flowering periods, their attractiveness for pollinators, their ability to suppress undesired weeds, and their affordability numbers increased greatly on the enhancement plots. The results show that both, a variety of different flower strips/fields and a long continuity of the measures contribute to an increase of species numbers and abundance of the species. Further results regarding species composition, effects on butterflies, seed mix composition and management options are shown and discussed. To sum up, the results indicate that a broad implementation of the approach could greatly contribute to the promotion of pollinators and help to increase the biodiversity in intensive arable regions.

Protection of wild pollinators in pesticide risk assessment and management

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Latest research on biodiversity in agricultural landscapes suggests that pollinating insects (especially wild bees and butterflies) are not sufficiently protected from the effects of plant protection products under the current risk assessment and risk management schemes. A result of uncertainties in existing risk assessment practice. Currently, only honey bees are tested in risk assessment and are assumed to be representative for all bee species. Other taxa of pollinators are not incorporated, so far. Moreover, the spatial dynamics of mobile arthropods (such as wild pollinators) which move between non-target area (e.g. buffer strip) and target area (field) are not taken into account. Becoming aware of these uncertainties European Food Safety Authority (EFSA) published a scientific opinion in 2015 addressing the state of the science on risk assessment of plant protection products for non-target arthropods. The preparation of a respective EFSA guideline is planned for 2017. The research and development project Z 6 – 9311.660 (FKZ: 3715 64 409) funded by the German Federal Environmental Agency (Umweltbundesamt), aims to provide a basis for the revision of risk assessment and risk management schemes for the protection of wild pollinators from the effects of plant protection products. As part of this project, the current EFSA scientific opinions and draft guidance for the risk assessment of bees and terrestrial non-target arthropods will be reviewed for deficits with respect to the protection of wild pollinators. Furthermore, data on ecology, exposure and toxicological sensitivity of wild pollinators will be collected. Based on this data, ecologically tolerable thresholds will be derived and the relative sensitivity of different groups of wild pollinators in crop-specific landscape scenarios will be determined. Using this information, criteria for the risk assessment scheme on wild pollinating insects as well as recommendations for the improvement of potential risk management options will be developed. The regulatory background and the aim of the project as well as first findings are presented in this poster.

Plant Protection Products: Risk Mitigation Measures for Birds

R. Gauch; O. Daniel, Agroscope / Institute for Plant Production Sciences IPS

To ensure a safe use, measures can be disposed along with the authorisation of plant protection products to mitigate the risk to non-target organisms. Such risk mitigation measures (RMM) should be quantifiable and generally applicable to a broad range of crops. For off-field non-target organisms, the risk can easily be mitigated by applying buffer zones to surface waters or biotopes, for example. For non-target organisms occurring in the field it is somewhat more challenging to find pollution RMM. Currently, oil and gas exploration for extraction of petroleum is represented in the European Union are presented. Further possible RMM for birds are evaluated and discussed. The pros and cons of the RMM are evaluated and discussed and their potential to be used within the regulation of plant protection products.

Evaluation of ecological sustainability of Italian rice cultivation: identification of regulation for environmental protection and analysis of existing voluntary or mandatory agri-environment measures

C. Gargiulo, D. V. Vargiu, N. B. Mattafire, M. V. Vitali, D. M. Viglietti, E. Di Tulio, NOMISMA; S. Cerioli, M. Tabacchi, ValOryza; C. Panigada, A. Finizio, University Milano - Bicocca / Department of Environmental and Landscape Sciences and Earth Sciences

Italy is the European leading rice producer (accounting for 48% of the EU production quantities) and exporter (representing 38% of the EU rice exports). Rice cropping is almost exclusively located in the Po Valley (Piemonte and Italy is the European leading rice producer (accounting for 48% of the EU production quantities) and exporter (representing 38% of the EU rice exports). Rice cropping is almost exclusively located in the Po Valley (Piemonte and
that make them a special case study, having both a high potential for expanding hydrocarbon extraction and also a region to assess associated cumulative environmental impacts. A key issue in the oil sands area is discrimination natural versus anthropogenic sources of oil sands related with contamination of surface and groundwater and the effects on aquatic ecosystem structure and function. The lower Athabasca River and its tributaries flow through natural bitumen deposits and consequently, it is a scientific challenge to distinguish between types and levels of hydrocarbon-associated contaminants that occur naturally in surface and groundwater from those arising from anthropogenic activities such as oil sands mining and upgrading of oil. The main objective of this study was thus to evaluate potential endocrine disrupting potential in alkylated analogues were observed. In this study, an oilfield-ecotoxicity datasets, and chronic ecotoxicological effects associated with the slumping of river bank material that is comprised of oil sands deposit that naturally enters the river systems through fluvial processes. For that, ecotoxicological tests were conducted using parental geological material collected from the river banks near the oil exploitation area (Alberta, Canada), in 4 different locations in 5 rivers. Similarly, each river bank. Further results regarding species composition, effects on butterflies, seed attractiveness for pollinators, their ability to suppress undesired weeds, and their regarding an overall good variability of plant species and flowering periods, their

Oil and Gas Extraction: Ecological Effects and Science-Based Management (PC)

TUCP08
AN ECOLOGICAL APPROACH TO OIL SANDS
D. Nunes Cardoso, CESAM, University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; J. Pestana, CESAM & University of Aveiro / Department of Biology of Biogeography and CESAM; S.F. Gonçalves, Department of Biology & CESAM - University of Aveiro / Department of Biology CESAM; F. Wrona, University of Victoria / Department of Geography; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Biology

The oil sand deposits of Athabasca River area, in northern Alberta (Canada), are the world’s largest oil reserves with several specific and special characteristics that are taken into account. Becoming aware of these

TUCP07
Swiss agri-environmental indicators: Development of pesticide usage and aquatic risks over five years
L. de Baan, Agroscope / Institute for Plant Production Sciences IPS; B. Gauch; O. Daniel, Agroscope / Institute for Plant Production Sciences IPS

In Switzerland, a set of agri-environmental indicators are calculated on a yearly basis to monitor the effects of agriculture on the environment. Two indicators are directed towards pesticides (i.e. plant protection products): (1) indicator on pesticide usage and (2) indicator on aquatic risks. These national indicators can serve as important data source for better understanding the exposure of agricultural landscapes to pesticides. In this poster, we present the development of these indicators over the last five years of this agri-environmental monitoring program (2009-2013). The input data for these agri-environmental indicators are collected through a network of more than 300 reference farms. These farms annually provide their field calendars, i.e. they report for each pesticide application the applied product, crops, date and amount of applied pesticide. Based on this data, several key figures are calculated for the indicator on pesticide usage, such as number of interventions and amount of active ingredients applied per crop group. For the indicator on aquatic risks, the pesticide usage is fed into the model SYNOPS (Gutsche & Strassmeyer, 2007), which calculates the fate and the final “predicted environmental concentration” (PEC) in edge-of-field surface waters over a cropping season. The PEC value is then compared to the ecotoxicity data of each active ingredient and the risk is expressed as Exposure–Toxicity-Ratio (ETR). Risks are then aggregated over different taxonomic groups (water plants, invertebrates and vertebrates) and over entire spray-application-sequences. The risks to aquatic environments are finally displayed as aggregated values per crop group. For the indicator of aquatic risks, there are large differences between crop groups, but inter-annual variation is rather small. For the indicator of aquatic risks, there are large variations between crop groups, within crop-groups and also between years. These agri-environmental indicators provide a key information for monitoring the long-term development of the environmental impacts of the current agricultural praxis in Switzerland and help to evaluate policy instrument to reduce these impacts. In addition, the indicators can help to understand and visualize the pesticide-intensity of different crop groups within agricultural landscapes. Oil and Gas Extraction: Ecological Effects and Science-Based Management (PC)

TUCP10
Quantifying the Role of Photochemical Processes in Aquatic Risk Assessment of Petroleum Substances
T. Parkerton, ExxonMobil Biomedical Sciences Inc. / Toxicology & Environmental Science; A.D. Redman, Exxon Mobil Biomedical Sciences / Toxicology and Environmental Science Division

Petroleum substances may contain polyaromatic hydrocarbons (PAHs) that interact with sunlight. These interactions can increase hazard, as a result of photo-enhanced toxicity, while reducing exposure, as a result of direct and indirect photodegradation processes. Currently, photochemical processes are not included in the STORISK modeling framework that has been developed and applied for conducting environmental risk assessments of petroleum substances as required
under the EU chemicals legislation (i.e. REACH). To quantitatively assess the role of light interactions on hazard and exposure assessment, available photodegradation and phototoxicity data were used to parameterize hazard and EUSES multimedia exposure models for a representative 3-ring (anthracene), two 4-ring (pyrene, fluoranthene) and 5-ring (benzo[a]pyrene) PAH. These models were then used to calculate risk quotients to aquatic life for various generic, regional exposure scenarios representing a range of sunlight exposures during winter and summer in both nutrient poor and rich natural waters. Risk quotients from these scenarios were then compared to the default case that ignored light. Results indicated that photodegradation rates were faster than biodegradation rates and of a similar magnitude for all PAHs except fluoranthene which had a ten-fold slower rate. Analysis of phototoxicity data indicated all PAHs exhibited a similar species sensitivity distribution when normalized to the combined PAH and daily ultraviolet exposure metric of micro W cm⁻² micro g L⁻¹. Resulting risk quotients for each PAH and scenario that included sunlight were similar to or lower than the no light case since the predicted enhancement in toxicity resulting from light exposure was effectively mitigated by reduced PAH water exposure due to photodegradation. This study indicates that neglecting light interactions in generic risk assessment for PAH containing petroleum substances does not preclude effective chemical management since risk quotients are not increased.

TUPC11
Integrated Environmental Mapping and Monitoring
I. Nilsen, StatOil ASA and Norwegian University of Science and Technology / Environmental Technology; G. Johnsen, Norwegian University of Science and Technology / Department of Biology; T.W. Natkemper, Bielefeld University / Biodata Mining and Environmental Modelling; S. Bub, NTNU and Norwegian University of Science and Technology / Environmental Technology

Technology development, including sensors and sensor platforms, has enabled a more holistic approach to environmental mapping and monitoring. To be able to meet the environmental challenges, interdisciplinary communication and collaboration is required. Therefore we are working on enhancing interdisciplinary communication and collaboration tools and methods. The vision is to provide solutions for stakeholder engagement and empowering the users of environmental mapping tools and systems.

Combining exposure and effects models and data for landscape based risk assessment in a regulatory context

TUPC14
Sustainable use of Veterinary Pharmaceuticals on the territory and groundwater resources quality
A. Di Guardo, Universita degli Studi di Milano-Bicocca / Department of Earth and Environmental Sciences; A. Finizio, University Milano - Bicocca / Department of Environment, Asta and Landscape Science and Earth Sciences

Emerging contaminants such as Veterinary Pharmaceuticals (VP) are becoming of increasing concern as consequence of their potential negative environmental impact. Due to their high consumption and the increasing presence of VPs residues in water, the scientific community has become increasingly interested in investigating the effects of these chemicals on aquatic environments, both in groundwater and surface water systems. This paper aims to address the problem of how to reach a sustainable use of VPs in the Lombardy area (North of Italy) and try to fill the gap of knowledge on how to implement appropriate risk mitigation measures on the territory to protect groundwater resources quality. In an existing tool called VULPES has been used to help to plan and manage VPs use in an sustainable way in the Lombardia Region. VULPES is a GIS-based decision support system enabling local authorities to assess the aquifer vulnerability to leaching of chemical substances by linking predictive exposure models with regional and local environmental scenarios (using GIS systems).

TUPC15
Towards a model- and landscape-based regulatory aquatic risk assessment of pesticides: state-of-the-art and recommendations for improving fate and effect models
A. Feocks, M. ter Horst, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; P.I. Adriaanse, Alterra Wageningen University and Research Centre / ERA team; E. van den Berg, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; J. Boesten, Alterra / ERA team; A. Rico, Wageningen University / Environmental Risk Assessment Team; H. Baveco, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; L. Wipfler, Alterra Wageningen UR

Ecological modelling approaches have been recently implemented in Environmental Risk Assessment (ERA) to overcome some of the limitations of experiment-based effects assessments, namely the extrapolation across different temporal and spatial scales, and the extrapolation across different levels of biological and environmental complexity (Schmolke et al., 2010; Forbes et al., 2011). In some early examples, ecological modelling approaches were applied to answer questions regarding the possibility for propagated effects and recovery of aquatic and terrestrial populations in a spatially explicit environment context. In most of these studies, exposure assessments were performed using standard exposure scenarios and linked to population models that used ecological scenarios not necessarily matching those used in the exposure assessment. Recently, EFSA proposed to define and use environmental scenarios including ecological and exposure aspects as an umbrella under which consistent representations of spatial and temporal boundary conditions and input parameter ranges can be collected. The idea of the definition of environmental scenarios is that the scenario selection procedures for the ecological and exposure scenarios and their parameterisation are based on common principles, allowing an integrated evaluation of fate and effects modelling results for regulatory ERA. In this presentation, we will give examples of early studies that integrate fate and effect modelling approaches at different landscape scales for aquatic (eco-)systems (Van den Brink et al., 2007; Galic et al., 2012, 2013; Focks et al., 2014, Baveco et al., 2014), and summarise a proposal for a structured approach to define ecological scenarios (Rico et al., 2015). Furthermore, we will summarise the state-of-the-art of fate models for the aquatic environment with respect to their applicability in ERA, balancing between the precision of model simulations, as needed for risk assessment, and the incorporation of large-scale systems, as required for more ecologically relevant landscape evaluations. In this presentation, the consequences of the development of ecological scenarios for the selection procedures for exposure scenarios and for the scale of the fate models to be used are identified. In addition, we will summarize the main challenges resulting from coupling fate and effect modelling approaches for pesticide registration and will propose some possible solutions as a way forward.

TUPC16
Validation of the GIS-based model SYNOPS to assess environmental risk of pesticides using four years of monitoring data in the small Lamme catchment
J. Strassmeyer, ral Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment; A.R. Dominic, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment; B. Golla, JKI, S. Lorenz, M. Stähler, A. Stüß, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants / Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; J. Tecklenburg, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment

The use of pesticides in agriculture causes environmental risks which must be managed carefully. On the EU level, specific Directives and National Action Plans (NAPs) have set reduction of agriculture-related pollution on the agenda. Within the German NAP, the risk indicator model SYNOPS-GIS is applied for regional risk analysis and assessment of the effects of mitigation measures. SYNOPS-GIS assesses the risk of chemical plant protection products for aquatic and terrestrial organisms. It combines data on pesticide usage with their application conditions, the concentration and the toxicity values for five aquatic reference species are used as a basis and is usually undertaken in the context of comparative study of public opinion on pollution in a small European city (York, East Asian countries, such as South Korea and Japan, urbanisation stands at higher levels by 2020. With higher rates of urbanisation come greater levels of pollution. In urban areas, pollution- and environment-related attitudes of citizens within the context current pollution- and environment-related attitudes of citizens vary greatly depending on factors such as country of origin, social and cultural characteristics of cities, and vice versa. This poster presents the findings of a comparative study of public opinion on pollution in a small European city (York, East Asia and future challenges).
catchment. The sample site is located 1.2 km downstream from the spring. The fields in the catchment were used by one farmer growing winter wheat, sugar beets and oilseed rape. All pesticide application events in the catchment were monitored. In addition weather data (precipitation, temperature) were measured and data on landscape structures (e.g. buffer zones, field margins), soil and slope were derived from 50% of the field area on SAR-Datasets. We applied a landscape-level model over the time period of four years, 14 were detected in the weekly composite samples. These monitoring results are correlated with predicted concentrations of SYNOPS-GIS and the results are discussed.

TUPC17 Development and application of a modular landscape-scale vole model for pesticide risk assessment combining the models Xplicit and Polaris T. Schad, Bayer CropScience / Environmental Modelling; M. Wang, WSC Scientific GmbH / Dept Efate Modelling; T. Preuss, Bayer CropScience / Environmental Modelling; S. Bub, Bayer CropScience

Recently, the potential of conducting landscape-level risk assessments has been discussed to predict potential effects of pesticides on non-target organisms at landscape scales (e.g. EFSA 2015a and b). This requires on one hand (i) to develop landscape model approaches, and on the other hand (ii) to develop scenarios fulfilling requirements of regulatory risk assessment and management. Landscape-scale modelling comes with a range of disciplines, ranging from modelling of environmental conditions, data and knowledge on land and farm management, exposure and fate modelling, ecological modelling, and in particular risk characterisation according to protection goals. All of these fields are in dynamic development and require specific knowledge. On this background, and the tentative fact a variety of very specific models often exist, we adapted a modular approach which allows combining individual model components using standardised interfaces. This not only allows to flexibly combine of models and to increase the level of complexity step by step in the risk assessment, but it also helps risk assessors to efficiently evaluate risk assessments, because once modules are validated they can be reused without the need of re-validation. In this respect, this approach also facilitates harmonisation. In a first implementation of this architecture we combine modules from a landscape-scale model (Xplicit; environmental conditions, land use/cover, PPP use, exposure, fate) with a vole model (Polaris), as well as modules for risk analysis. Due to its openness, it is straightforward to introduce e.g., new landscape data. This approach is used to analyse relationships between landscape characteristics (composition, structure, management, scales) and vole population dynamics, with and without pesticide use, in order to identify realistic worst-case scenarios for risk assessment (EFSA 2015c, Wang and Lattik 2013). In this poster we present details on the modular landscape modelling concept and its implementation for a vole risk assessment. We show that the model can flexibly be used at different scales and different risk assessment tiers (complexity level). In addition, we illustrate how the landscape-scale modelling approach can be used to identify which factors determine the level of susceptibility of populations.

TUPC18 Comparing observed and predicted honeybee nectar-foraging at landscape level H. Basovec, A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; D. Belgers, Alterra Wageningen UR; J. Verhagen, J. van Steen, Alterra Wageningen UR; J. Horsfield, Alterra Wageningen UR

We applied a landscape-level nectar foraging model using the spatial settings associated with a field experiment on honeybee foraging. The model calculates foraging behaviour based on energy balance of honeybee foragers for a single day in complex landscape mosaics. The hive population is assumed to select the resource patch with highest net energetic efficiency and to have perfect knowledge of its environment. By using real-world GIS data, including semi-natural elements such as field margins and buffer strips, present within a radius of several kilometres, the model can quantify foraging costs and yields in terms of energy for the specific field situation, and based on these calculations the amounts of nectar from the different fields and other nectar sources in the neighbourhood arriving at a bee hive. In the experiment a 1 ha flowering field of oilseed rape, was sprayed with a non-toxic tracer. After application, during the remainder of the day, bees were collected approximately every hour in the sprayed field and at the entrance of the two beehives situated next to the field. Tracer residues differed between field and hive containing the foragers hunting towards increasing at the hive, which indicated the presence of other yet unknown factors. We used the model to test several plausible explanations for the observed experimental results. In particular, the hypotheses were tested that either the presence of attractive alternative nectar sources in the wider landscape could have resulted in less than expected numbers of foragers visiting the crop, or that a large fraction of what were considered foragers were in fact apprentices, learning to practice their foraging skills. Vice versa, the experimental results helped to perform a reality check for the foraging model by comparing observed to simulated nectar concentrations. In particular, the assumption was tested that at any time, only a single, optimal, resource would be exploited.

A bug's eye view - quantifying pesticide heterogeneity at micro-scales needed to model non-target arthropod behaviour in orchards J. Witton, University of York / Environment; G. Weyman, ADAMA Agricultural Solutions Ltd.; M. Reed, Chemicals Regulation Directorate; M. Pickering, M. Hodson, University of York / Environment Department; R. Ashauer, University of York / Environment

Pesticide exposure within fruit orchards has been widely studied, but rarely at more than one spatial scale; in addition it is usually undertaken in the context of human exposure. Here we take a different approach. We aim to quantify pesticide exposure at scales relevant to individual non-target arthropod behaviour. Such species are included in the regulatory risk assessment of pesticides, and may provide important ecosystem services. We assessed five spatial scales during a spray application of the fungicide penconazole in an apple orchard in the UK in August 2015 and used two residue analysis methods: pesticide extraction from apple leaves and analysis via GC-MS; and computer-based image analysis of pesticide deposition on water sensitive paper. We report residue levels focusing on the spatial scales most relevant to non-target arthropod species widely used in both biocontrol and pesticide risk assessment: the green lacewing Chrysoperla carnea, and the predatory mite Typhlodromus pyri. In addition we evaluate the potential for water sensitive paper to be used in residue studies instead of cost- and time-intensive analytical techniques. Our results feed into a wider project to produce a model that predicts the population-level consequences of lethal and sub-lethals effects of pesticide exposure, including potential avoidance at the micro-scale, on the two non-target arthropod species.

Communicating research findings and uncertainties: Strategies, tools, and new platforms for environmental sciences (PC)

WEPC01 Contrasting public perception of pollution in a small European city and an East Asian megacity - the case of York versus Seoul L.H. Yoads, University of York / Environment

Around the world, a shift towards urbanisation is being realised. Currently, over 50% of the world’s population live in cities and this is expected to increase to 66% by 2020. With higher rates of urbanisation come greater levels of pollution. In cities, this encompasses a diverse range of issues under noise, air, soil and water pollution themes. Pollution in cities is an important problem due to the effect that the variety of pollutants may have on human health and wellbeing and the health of the natural environment. The rate and extent of urbanisation varies considerably around the world, thus the extent and composition of pollution in different cities also display great variety. Urbanisation in Europe stands at 73.6%, whilst in some East Asian cities, such as Seoul in South Korea and Japan, urbanisation stands at higher rates of 82.5 and 91.3%, respectively. Although the extent of urbanisation in both regions is not wildly variant, the above figures hide more interesting information on the significantly different historical development of European versus East Asian cities and the impact that these development trajectories have had on cultural, economic, policy, and decision making. This poster will examine how different urban environmental, social and physical characteristics of cities, and vice versa. This poster presents the findings of a comparative study of public opinion on pollution in a small European city (York, UK) and an East Asian megacity (Seoul, South Korea). The findings are presented within the context current pollution- and environment-related attitudes of citizens within each city and will include measures of: citizen engagement in the use of innovative technology and education around environmental pollution; participants in public policy decision-making; and behavioural trends in terms of limiting personal exposure to pollutants and reducing environmental footprints. In addition, the historical developmental trajectory of each city will be discussed and the possible impact that culture, economic development, rate of urbanisation over time and governance may have on the varying attitudes and opinions of each city’s public. The findings of this study have significant implications for beginning to understand how different cities value, digest and respond to information on pollution problems in substantially different contexts.

WEPC02 Uncertainty avoidance in environmental risk assessment - current guidance and future challenges A. Hunka, Halmstad University / Biological and Environmental Systems; M. Miller, ADAMA; A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; P. Thorbek, Syngenta / Environmental Safety; S. Waara, Halmstad University; V. Forbes, University of Minnesota / Ecology Evolution Behavior

Uncertainty estimations are inherently built into any scientific process, not excluding environmental risk assessment (ERA). Although usually hidden behind safety factors and policy decisions in recent years recent development of new assessment methods, uncertainty has become more explicit. Despite this, most people, including risk assessors and managers, avoid uncertainty and often display uncertainty-averse behaviours when making decisions. Uncertainty avoidance describes the level of tolerance of uncertainty and ambiguity, where choices are
biased towards assigning greater weight to information about uncertainty in comparison to the rest of available data. As to date, we know little about the social and psychological factors contributing to that phenomenon. The aim of our study is to systematically analyse different ways of addressing uncertainty in ERA, using existing (or proposed) guidance and legal frameworks for ERA, focusing especially on uncertainty propagation and sources of uncertainty beyond statistical estimations. We identified gaps, challenges and suggested ways to incorporate, so far overlooked, psychological factors, especially uncertainty avoidance, into risk management. Our preliminary results show that both perceived uncertainty and uncertainty avoidance have been so far overlooked in ERA regulations. The most general decision, as to whether the precautionary principle refers to “unacceptable risks” and scientific uncertainty, but leaves them in the domain of political responsibility. Currently, only ESFA’s draft guidance document explicitly acknowledges perceived uncertainty in ERA. Whereas uncertainty analysis is a well-established part of ERA guidance documents, a systematic, empirical analysis of uncertainty in ERA and especially of uncertainty “unacceptable risks” and scientific uncertainty, but leaves them in the domain of political responsibility. Currently, only ESFA’s draft guidance document explicitly acknowledges perceived uncertainty in ERA. Whereas uncertainty analysis is a well-established part of ERA guidance documents, a systematic, empirical analysis of uncertainty in ERA and especially of uncertainty avoidance is lacking. Existing probabilistic models incorporating uncertainty in ERA do not take into account individual or social variables. Still, the awareness of these kinds of influences on decision-making processes is growing. To keep ERA ready for emerging risks, it is not enough to develop new assessment methods and more accurate predictions. Underlying reasons for uncertainty avoidance need to be addressed to facilitate informed risk communication.

WEPC03 Accounting for Environmental Recovery in Risk Assessment and Management
S. Mcacing, ENVIRON UK Ltd; A.E. Bartram, Ramboll Environ / Product Safety Ecology and Sediment Management; N.J. Eury, Ramboll Environ UK Ltd

The Energy Institute (London, UK) has developed a guide for risk assessors to determine the duration of environmental recovery following major accidents to the environment. The guide is designed to support site safety reporting for the UK Control of Major Accident Hazard (COMAH) Regulations, which implement the SEVESO Directive (2012/18/EU). The information gathered on environmental recovery may also have wider uses for predicting recovery in damage and compensatory assessments (Environmental Liability Directive 2004/35/EC) and evaluating risks as part of contaminated site assessments. The guide is currently undergoing final sign off for publication in 2016. The aim is to provide an easy-to-use guidance for defining environmental recovery based on major accident scenarios for releases of SEVESO substances, including petroleum based products. The guide provides a framework for the selection of appropriate recovery criteria for a range of temperate habitats using typical release scenarios for hazardous substances. The primary audiences for the guide will be managers and their advisors responsible for the preparation of Site Safety Reports by establishment of risk screening for refineries and chemical production facilities together with storage terminals. The approach includes a review of published literature on habitat and species recovery from accidental spills. In parallel, the psycho-chemical properties, environmental fate and ecotoxicity of chemicals captured by COMAH/SEVESO III were evaluated to identify persistent, bioaccumulative and toxic (PBT) substances with the potential for longer-term impacts/delayed recovery. Reliable studies are used to compile recovery trajectories resulting in recovery durations for a range of habitats. These are presented in a step-wise framework for assessment to identify an appropriate recovery duration for each of the chemicals of interest and types of habitats on and around their sites. Taking account of environmental recovery in an assessment is often neglected, but understanding the consequences of an accidental release is important to valuing the scale and severity of an impact. Recovery information could be used to direct appropriate management actions (as in COMAH/SEVESO) and avoid under- or over-conservative compensatory actions in dealing with environmental damage and liability.

WEPC04 ECHA and communicating scientific content
T. Bräutigam, European Chemicals Agency

ECHA is an Agency of the European Union with the responsibility to manage four pieces of European chemicals legislation: REACH, CLP, Biocidal Products Regulation (BPR) and PIC. The objective of all of them is to ensure a high level of protection of human health and the environment, as well as to enhance competitiveness and innovation in the industry. The Agency needs to raise awareness and support duty holders – chemicals companies - in complying with the legislation. ECHA also needs to use the results of the legislative processes to promote the safe use of chemicals among downstream users, workers, consumers and other general audiences. This task requires content which explains the scientific data in an easily understandable way and in the relevant context. In order to achieve its communication objectives, ECHA needs to be realistic, strategic and consistent in its communication. The main communication principles are the following: Accessible. Communication vehicles are easily accessible, transparent and use understandable language. Strategic. ECHA focuses on defined priority topics and targets the key audiences. Reliable. ECHA’s communication is accurate and prepared. ECHA’s communication channels and vehicles to optimise the outreach and targeted messaging. EU level, ...
stability of the fish population in a lake. For this purpose, each player has to find resources and to use them for different actions (e.g. reproduction). The perturbations of the ecosystem are illustrated by “events” designed to reflect abiotic (e.g. water temperature) and biotic (e.g. parasites, human) stressors. The current versions of the games include four players (fish species): roach, pikeperch, bleak and tench. The idea is that the interactions between players (predation and competition) and to illustrate various feeding and reproduction strategies. A prototype of each game is currently available for testing and refinements are expected while testing the games. In a short term, the next versions will integrate the aesthetic design (edge of a lake) and refined processes parameters (model calibration). In a long term, we aim to develop an online version of the computer game and to use crowdfunding platforms to diffuse the board game. The very first objective of our games remains to be entertaining, keeping in mind that the ludic rather than educative aspects are central in such game-based media. If players forget that it is about ecology, it would mean that the underlying scientific concepts are understood, exactly what we want.

WEPC07
Heavy metal assessment in agricultural soils contributes to a sustainable information of the public regarding soil quality in a developing country.

D. Sosa Pacheco, CENSA; D. Bürge, Agroscope ISS; I. Hilber, Agroscope ART; R. Faire Garcia, L. Aguilar Díaz, CENSA; T. Bucheli, Agroscope ART / Environmental Analytics Natural Resources and Agriculture; A. Escobar Medina, Cuba is mainly an agricultural country and healthy food production depends on the quality of the soils. Several studies in Cuba propose reference values of heavy metals (HMs) indicating the quality of different soil types with little human action. Nevertheless contaminants monitoring data are sparse. Heavy metals are prone to be taken up by plants, which may affect food safety. To inform the stakeholders (consumers, farmers, government) about the quality of Cuban soils, a sound monitoring of contaminant exposure forms the base for an appropriate and adequate risk assessment, and corresponding communication with stakeholders. Our project Soil-Q, jointly carried out by the Cuban agricultural research institute CENSA and the Swiss Agroscope ISS aims to establish a monitoring network to assess the contamination status of Cuban soils. So, 39 sample locations with agricultural and rural character in the province of Mayabeque were monitored according to the rigorous protocol of the Swiss soil monitoring network. The mean concentrations of HMs (cadmium 2.6, chromium 63, copper 64, nickel 48, lead 16, zinc 55, and mercury 0.1 mg/kg, w.w.) in these sites do not exceed the intervention thresholds according to the regulations of Germany and Switzerland. However, the values higher than the median appeared in agricultural soils. Additionally, Soil-Q is to raise awareness and awareness of the stakeholders. A film in Cuban and other short workshops for representatives of related research and governmental institutes were held. The message was to inform about Soil-Q and that first results do not show a risk for humans and the environment. It is crucial to inform the general public as well as decision makers that this is the only beginning of a monitoring process that should extend over the whole country and over decades because soil is an important archive about human activities. Especially in a country where future changes are very likely, a long-term monitoring catching industrial and other human activities is of utmost importance for the welfare of humans and the environment. It is a big challenge to maintain an information flow with projects that are finite. Public perception towards the quality of the environment, especially as it relates to soil and food safety, is a critical problem that is very difficult to keep active. We hope to therefore learn more about this subject at this session by communicating and networking with other experts.

WEPC08
When are risks assessments of endocrine disrupters scientifically sound and secure?

P. Mathiesen, Independent Consultant; A. Leopold, Independent Consultant/Researcher / Wildlife International Ltd.; J. Oudm, Regulatory Science Associates; M. Roberts, Independent Consultant / Chemicals Emerging Technologies The poster summarises the consensus that resulted from the SETAC Pesticide™ Workshop: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances (EHRA), held at Penacscula on 31 January – 5 February 2016. The primary aim of the workshop was to provide objective advice, based on the current level of scientific understanding, to enable regulators/policy makers to make considered, informed decisions on whether to select a hazard- or a risk-based approach for a given endocrine-disrupting substance (EDS) under review. The workshop additionally considered recent developments in the identification of EDSs. All guidance developed was based on experience obtained from working on case histories of six data-rich endocrine-active substances (EAS) - ethyleneestriol, perchlorate, propiconazole, trenbolone, tributyltin and vinclozolin. This is a controversial subject because it is claimed by some that environmentally safe concentrations/doses of (certain?) EDSs cannot be established due to certain inherent properties e.g. unusual concentration/dose response curves; long-term effects resulting from short-term critical windows of exposure; difficulties of extrapolating to effects in unstated life-stages and species etc. The implication is that such substances should be regulated solely on the basis of their intrinsic hazards. This poster summarises information on current best practice in the environmental risk assessment of EDSs which was developed using a cross-section of international expertise on this issue. The core advice to regulators and policy-makers faced with a confirmed EDS is to ask a structured series of questions, as follows: Is exposure prediction reliable? Have relevant taxa and life stages been tested? Are tests adequate to detect delayed effects? Are tests likely to have missed adverse effects at concentrations below those tested? These questions are underpinned by detailed guidance on how they can be addressed, and at each stage, users are invited to fill in data-gaps as appropriate. Only if the required additional testing is not deemed feasible should regulation on the basis of hazard alone be considered until such time as suitable methodology becomes available. The overall conclusion of the EHRA SETAC Pest Workshop™ was that the questions above are adequately addressed, and that continued rigorous risk assessment of EDSs is scientifically sound and secure.

WEPC09
Uncertainties in biological responses that influence hazard or risk approaches to the regulation of endocrine active substances.

J. Parrott, Environment Canada / Water Science and Technology Directorate; P. Bjerregaard, University of Southern Denmark / Department of Biology; C. Borrgert, Applied Pharmacology & Toxicology, Inc.; K. Brugger, DuPont Crop Protection / L.E. Gray, U.S. EPA / ORDNEERLRTLTD Endocrinology Branch; T. Iguchi, National Institute for Basic Biology / Molecular Environmental Endocrinology; S. Kaddouri, University of Minnesota – Department of Plant Pathology / Ecology and Sediment Management; N.J. Eury, Ramboll Environ UK Ltd / Biosciences; L. Weltej, BASF SE / Crop Protection Ecotoxicology; J. Wheeler, Dow Agrosciences

Endocrine Disrupting Chemicals (EDCs) may have delayed or transgenerational effects and display non-monoctonic dose response relationships (NMDDs) that require careful consideration when determining endpoints for risk assessment. The case studies evaluated for the SETAC Pesticide™ Workshop: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals and other key examples from the literature are discussed. EDCs can have specific and profound effects when exposure occurs over sensitive windows of the lifecycle (developmental, reproductive). This may induce delayed effects where the adverse effect is manifest at different lifestage(s). This underscores the need for testing in appropriate (sensitive) lifestages and full lifecycle designs that capture adverse effects wherever they occur in the lifecycle. Such tests are available in the toolbox and should be employed to derive endpoints that cover all life stage sensitivities. Similarly, the potential for effects to become manifest in subsequent generations (transgenerational) has also been raised as a potential issue in the derivation of appropriate endpoints for EDCs. However, the evidence for such effects as a general issue is limited. Indeed this is reflected in the design of new higher tiers to assess EDCs developed under the auspices of the OECD and US-EPA by the move to extended one-generation designs and away from multi-generational studies for fish and mammals. The occurrence of non-monotonic dose or concentration response relationships is also considered a limiting factor for reliable risk assessment of EDCs. Substantial data reviews are underway to inform on their occurrence and relevance. However, evidence to date indicates they are more prevalent in in vitro and in vivo mechanistic data, not often translating to adverse apical endpoints. As a result, the focus of the workshop was on the general issue of how to evaluate NMDDs in the context of endocrine hazard and risk assessment procedures is presented. If careful consideration of delayed, transgenerational and NMDD effects is made, it is feasible to assess environmental endocrine hazards and derive robust endpoints for risk assessment procedures ensuring a high level of environmental protection.
Disrupters to determine the relevance to adverse, population level effects. For some EAS like tributyltin, population relevance of mollusk impetus is well established. In fish, altered secondary sex characteristics by vinclozolin did not affect reproduction in a medaka extended one-generation study, and were judged to be not relevant for fish populations. Higher concentrations of vinclozolin decreased reproduction in fish, which is population relevant. The amphibian metamorphosis assay can detect altered stage distribution of tadpoles, which is predictive of increased time to complete metamorphosis, and might result in adverse effects for amphibian populations. In birds, population-relevant endpoints include egg production, hatchability, embryo viability, growth and egg condition. Decreased titanium dioxide exposure to birds was seen with the thyroid-active compound, perchlorate. In mammals, anti-androgens like vinclozolin can affect reproductive development (e.g., decreased anogenital distance, nipple retention), but the population relevance of these endpoints is uncertain. In longer-term studies, vinclozolin reduced male rat fertility and successful mating. Other endpoints downstream of the recovery processes also are important when evaluating the adverse effects of EAS on wildlife populations.

Lastly, new methodologies (e.g., adverse outcome pathways - AOP and modeling) will aid in our understanding of endocrine perturbations and adverse population level effects once our knowledge on key event relationships improves.

WEC11

Current limitations and a path forward to improve testing for the environmental assessment of endocrine active substances


To assess the hazards and risks of possible endocrine active chemicals (EACs) there is a need for robust, validated test methods that detect perturbation of endocrine pathways of concern and provide insights reliable information as to assess to potential adverse effects on apical endpoints. One issue of significant concern to toxicologists is the ability to identify ex vivo EAC screening/testing programs involves resources in regard such as to cost, time, trained personnel, and animal use. This is especially problematic when considering the number of chemicals that some regulatory authorities need to assess. One way to address this challenge is to prioritize chemicals for possible in vivo testing using in vitro high throughput (HTP) assays focused on a suite of endocrine molecular initiating events (MIEs) of concern. Additional challenges associated with the design and conduct of in vivo EAC screening and testing include selection of appropriate species (i.e., sensitive and amenable to laboratory testing), endpoints and life-stages. A component of this involves experience gained using existing tests to determine, for example, those that have been shown to be informative to the degree of MIE of concern. However, there is the opportunity for strategic use of HTP data and/or early screening level information to help guide the selection of existing assays that can further evaluate a given EAC modality. Further challenges for EAC screening and testing involve guidance and optimization in several areas, such as concentration setting, statistical power to detect biologically significant adverse effects, delivery and analytical measurement of test substances, availability of technical expertise, and study interpretation, including linking mechanistic and apical effects. Some of these areas can be addressed by the lessons learned and best practices developed through recent experiences conducting EAC screening/testing. Additionally, the strong translational relevance. Recovery of EAC screening and testing data (e.g., compilation of historical control data) can be leveraged to refine test designs and performance criteria to maximize the power and utility of EAC screening/testing. Finally, a number of recommendations are provided for longer term research to address areas of uncertainty, including identifying potentially sensitive species for which test results are currently non-existent (i.e., invertebrates) and key endocrine pathways in addition to estrogen, androgen and thyroid signaling.

WEC12

Challenges in Assigning Endocrine Specific Modes of Action: Recommendations for Researchers and Regulators

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As regulatory programs focus on evaluating substances for their endocrine disrupting properties, there is a need for careful study design and data interpretation to distinguish between endocrine versus non-endocrine specific responses. This is particularly important where specific criteria are under development to distinguish between adverse effects of a compound and potential for estrogen receptor activity. Irrespective of the regulatory process, most jurisdictions use the WHO IPCS definition of an endocrine disruptor (ED), requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism. Such a definition is broad, and might capture mechanisms that in general can be both, substance specific and substance non-specific endocrine responses. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, non-endocrine toxic mechanisms (e.g. hepatotoxicity, acetylcholinesterase inhibition) may operate secondarily or in parallel to impact the endocrine system (e.g., reduced, altered thyroid hormone levels). Furthermore, endocrine responses may be adaptive in nature and designed to maintain homeostasis rather than inducing an irreversible adverse effect. The likelihood of indirect effects is increased in (eco)toxicological studies requiring the use of maximum tolerated dose levels, which must produce some adverse effect. The misidentification of indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. A review, based on 6 case study substances, was conducted to evaluate scenarios that could complicate the assessment of whether or not a substance is an endocrine disruptor. In order to achieve this objective, a weight of evidence approach was used to evaluate available data for the case-study substances. In this approach, the weight of evidence was based on compiling the plausibility, temporal, and dose response data on the essentiality of key events in adverse outcome pathways. A process is recommended where indicative (endocrine specific and non-endocrine specific) and apical endpoints can be evaluated to investigate whether an endocrine mode of action can be conclusively assigned to the effects observed for a given substance. Two examples are discussed each with varying degrees of endocrine mode action specificity.

WEC13

Evaluating the Credibility of Histopathology Data in Environmental Endocrine Toxicity Studies

J.C. Wolf, Experimental Pathology Labs., Inc.; G. Maack, Federal Environment Agency / Ecotoxicological Assessment

Agencies responsible for environmental protection are tasked with developing regulatory guidance that is based on the best available scientific evidence. Histopathology is a common endpoint in toxicological biosassays, and advantages of this endpoint for ecotoxicological research include: (i) reasonably high degrees of sensitivity and specificity relative to other endpoints; (ii) the ability to identify and/or confirm novel or unanticipated treatment effects that may not be detected by other means; (iii) the ability to reveal the presence of subclinical (and potentially confounding) background disease; (iv) the ability to link effects obtained from molecular or in vitro experiments to more apical population-relevant endpoints and (v) in some cases, the provision of key mechanistic information pertaining to toxicity. However, because of the subjective nature of histopathology, and the advanced level of specialized training required for the effective utilization of this endpoint, the reliability of histopathology data can vary and/or be inconsistent. Consequently, mechanisms for evaluating such data and providing uniform agreement no. 603437.

BIO-MONITORING TO EVALUATE SURFACE WATER

SETAC-Queensland / National Research Centre for Environmental Toxicology Entox; B.I. Viberg, Umeå University / Department of Biology and Environmental Chemistry; M. Kajm, University of Oslo / Centre for Environment and Health; R. Gjerde, University of Oslo / Department of Environmental Science; E. Michie, Shell International / Shell Health Risk Science Team; C.V. Eadsforth, SETAC Europe 26th Annual Meeting Abstract Book SETAC Europe 26th Annual Meeting Abstract Book

There is a need to improve the risk assessment of offshore produced water (PW) discharges and find a rapid and cost effective alternative to logistically challenging WET testing. A simple battery of monitoring techniques, which can further evaluate a given EAC modality. Further challenges for EAC screening and testing involve guidance and optimization in several areas, such as concentration setting, statistical power to detect biologically significant adverse effects, delivery and analytical measurement of test substances, availability of technical expertise, and study interpretation, including linking mechanistic and apical effects. Some of these areas can be addressed by the lessons learned and best practices developed through recent experiences conducting EAC successful/testing. Additionally, the strong translational relevance. Recovery of EAC screening and testing data (e.g., compilation of historical control data) can be leveraged to refine test designs and performance criteria to maximize the power and utility of EAC screening/testing. Finally, a number of recommendations are provided for longer term research to address areas of uncertainty, including identifying potentially sensitive species for which test results are currently non-existent (i.e., invertebrates) and key endocrine pathways in addition to estrogen, androgen and thyroid signaling.
Norwegian offshore oil and gas industry. PW includes both formation water (seawater or freshwater trapped with oil and gas in a geological reservoir) and injected water (seawater, freshwater and brine water as well as added chemicals that are injected to enhance recovery of oil and gas, and operational safety) and as such contains components such as dispersed oil, aromatic hydrocarbons, alkylbenzene solvents, organic chlorinated and inorganic salts. The exploration of oil and gas takes place offshore in sensitive coastal environments and as such stringent monitoring regulation must be followed. The currently used water column monitoring strategy includes the use of passive samplers and caged organisms to monitor the effects of releases of PW. Passive samplers and caged organisms must be placed on all monitoring stations and passive samplers should be used to monitor dispersal of discharge to the environment. This work investigated the relationship between monitoring results obtained via the use of passive samplers and organisms using a real PW sample as a case study. Four passive samplers (polyethylene, polyoxymethylene, polyurethane), silicone rubber and polystyrene) were assessed for potential toxicology using 2) alkylated PAs by determining respective passive sampler-water partitioning coefficients. A small field deployment in the Oslo fjord was carried out to investigate ease of deployment. Polyethylene and polydimethylsiloxane were identified as the most optimal passive samplers monitoring chemicals in PW. Polyethersulfone did not function as an equivalent passive sampler, as expected, and was very difficult to deploy as a single membrane in the field. A battery of bioassays were then carried out in using the PW sample and also extracts from the selected passive samplers that had been exposed to the PW sample. In addition the PW was diluted to simulate increased distance from the release point and the bioassays were carried out again. The correlation between concentrations determined with the passive samplers and the ecotoxicological response to the organisms will be investigated.

WEPC17
Mobile passive sampling as a tool for toxicological profiling
J. Novák, Masaryk University Faculty of Science RECETOX / RECEPTOXY. B. Vrana, Masaryk University, Faculty of Science, RECEPTOXY / Environmental chemistry and modelling; J. Matyáš, Masaryk University, Faculty of Science, RECEPTOXY / Centre for toxic compounds in the environment; T. Rusina, Masaryk University Faculty of Science RECEPTOXY / Environmental chemistry and modelling; K. Okomski, Masaryk University / Faculty of Science RECEPTOXY / Toxicology; R. Grabc, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocarbons Vodnany Czech Republic; P.A. Peale, Griffith University / Smart Water Research Centre; M. Macova, The University of Queensland / National Research Centre for Environmental Toxicology Entox; B.J. Escher, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology, S. Ait-Aissa, INERIS / Ecotoxicology Unit; N. Crescent, INERIS; K. Hilscherova, Masaryk University, Faculty of Science, RECEPTOXY / RECEPTOXY Many components of complex organic pollutant mixtures in waters are often present at very low levels, posing a challenge for current analytical methods. Passive sampling is one of the perspective methods that could address this problem. In our work, we used a mobile "active" passive sampling system with two types of sorbent (EMPORRE discs, silicone rubbers), which preconcentrates a wide spectrum of polar and non-polar organic pollutants from the water column. Eight Danube river stretches were sampled and spatial distribution profiles were described for a comprehensive range of organic pollutants including pesticides, pharmaceuticals and industrial chemicals. Sampling rate and the original concentrations of the pollutants in water were estimated for pollutants with different physicochemical properties by a set of reference compounds. The employed mode of passive sampling with an active exchange of water in the sampling chamber proved to be about five times more effective than static exposure and in relatively short sampling times allowed quantification of chemicals in pg/L levels. Results of passive sampling were also characterized with a battery of in vitro bioassays sensitive to chemicals with selected modes of action. Toxic potentials for causing endocrine disruption (anti/estrogenic, anti/androgenic), dioxin-like activity, pregnane X receptor (PXR)-mediated activity and oxidative stress-associated response showed spatial variation along the river. Polarity of chemicals played an important role, while AhR-mediated effects were associated mostly with non-polar compounds (sampled by the silicone rubber sampler) the rest of toxic potentials was associated mainly with more polar chemicals sampled by the EMPORRE discs samplers. To link the toxic potentials with chemical analysis data, the detected pollutant levels and their relative toxicity. Biomimetic techniques such as in vitro EAC will also reflect the potential of a substance to bioaccumulate and thus provides more ecologically relevant data. Initial BCF testing has demonstrated bioaccumulation potential of PW to be of low concern. In due course, Microtox and SPME-GC screening will assist with other screens in PBTK determination, fulfilling Step 2 of the RBA by providing toxicity and bioaccumulation data.

WEPC16
Screening methods for fate and toxicity of produced waters
E. Michie, Shell International / Shell Health Risk Science Team; C.V. Eadsworth, Shell International / Shell Health; G. Whale, Shell Health / Shell Health; T. Halmans, Shell Global Solutions International / Analytical Department; A. van der Laan, Shell Global Solutions International / Analytical
There is a need to assess the potential fate of produced water (PW) discharges and find a rapid and cost effective alternative to logistically challenging WET testing. A simple battery of monitoring techniques, which closely mirrors the OSPAR Risk Based Approach (RBA), has been trialled in a wide range of facilities. This study has applied solid phase micro-extraction coupled with gas chromatography (SPME-GC), a biomimetic technique, and the in vitro Microtox assay (Vibrio fischeri) bioluminescence assay for the purpose of produced water toxicity assessment. These techniques will ultimately form part of a toolbox for early tier routine screening of produced water effluents. A collection programme of PW samples was coordinated from a number of offshore PW waste streams. Aliquots were simultaneously tested using Microtox to describe the toxicity of both known and unknown components, and SPME-GC to determine the nature of the toxicants, and thus bioavailable, hydrocarbon concentration. Components desorbed from the fibre were quantified as total dissolved petroleum hydrocarbon concentration, by calibration against hydrocarbon standards. Toxicity (assessed using Microtox) increases in line with dissolved hydrocarbons determined by SPME-GC suggesting hydrocarbon contamination to be a major contributor to toxicity. Biomimetic techniques such as in vitro SPME will also reflect the potential of a substance to bioaccumulate and thus provides more ecologically relevant data. Initial BCF testing has demonstrated bioaccumulation potential of PW to be of low concern. In due course, Microtox and SPME-GC screening will assist with other screens in PBTK determination, fulfilling Step 2 of the RBA by providing toxicity and bioaccumulation data.

WEPC15
Assessing the toxic potential of river water by combining passive sampling with bioassays
Bioanalytical tools can provide important information on the toxic potential of complex chemical mixtures of environmental samples and are, therefore, complementary to chemical analysis. The combination of passive sampling with bioassays is a promising approach for assessing water quality as 1) passive samplers accumulate a broad spectrum of chemicals while excluding most of the matrix and 2) chemicals with the same mode of action are detected in parallel. Up to now, the majority of studies investigated solvent extracts of polar organic integrated chemical samplers with the yeast estrogen screen (YES) to assess contamination of aquatic environments with chemicals causing estrogen-like activity. To study the toxic potential of river water, silicone-based samplers were exposed in rivers for five weeks, extracted with n-heptane in a Soxhlet-apparatus and then extracts were tested in bioassays. In preliminary experiments, extracts from passive sampler that had been exposed in the rivers Rhine, Danube, Elbe, Saale and Saar were tested in bioassays measuring mutagenic (Ames fluctuation assay) and endocrine activity (YES). Thereby, samples from the river Saar showed high mutagenic potential in the Ames fluctuation assay, in particular when using the strain YG 1041 that is sensitive to nitroaromatic compounds and, after metabolic activation by S9-mix to aromatic amines. Therefore, a more comprehensive monitoring program was conducted along the river Saar in 2015, whereby passive samplers were exposed at twelve sampling sites in the river Saar, its main tributaries, and in the effluent of a wastewater treatment plant. All samples showed mutagenic potential with highest effects in samples from sites located in the Saarland conurbation, an important old-industrialised region in Germany, as well as in samples from the main tributaries. In most cases, aromatic amines were calibrated for PAHs, mutagenic activation with S9 mix, whereas no or only minor mutagenic potential were found without metabolic activation. This indicates that the mutagenic potential might primarily result from aromatic amines and not from nitroaromatic compounds.\n\nJahnke, A. et al. (accepted): Combining passive sampling with biochemical profiling of microorganisms and 2) chemicals with the same mode of action. Toxic potentials for causing endocrine disruption (anti/estrogenic, anti/androgenic), dioxin-like activity, pregnane X receptor (PXR)-mediated activity and oxidative stress-associated response showed spatial variation along the river. Purity of chemicals played an important role, while AhR-mediated effects were associated mostly with non-polar compounds (sampled by the silicone rubber sampler) the rest of toxic potentials was associated mainly with more polar chemicals sampled by the EMPORRE discs samplers. To link the toxic potentials with chemical analysis data, the detected pollutant levels and their relative toxicity. Biomimetic techniques such as in vitro EAC will also reflect the potential of a substance to bioaccumulate and thus provides more ecologically relevant data. Initial BCF testing has demonstrated bioaccumulation potential of PW to be of low concern. In due course, Microtox and SPME-GC screening will assist with other screens in PBTK determination, fulfilling Step 2 of the RBA by providing toxicity and bioaccumulation data.

SETAC Europe 26th Annual Meeting Abstract Book
Grass sampling may be scarcely adequate to evaluate the impact on surface waters of pesticide used in agriculture because of its poor spatiotemporal representativeness compared to the high variability of the contamination patterns. Indeed, it is still not easy to link the effect of agricultural practice changes to water quality at the catchment scale. Furthermore, biological indicators may also be useful in addition to pesticide concentration determination to evaluate their impact on ecosystems. In this way, the contribution of new integrative tools has been evaluated. Passive samplers and biological indicators have been applied together at the catchment scale so as to identify their coherence and complementarity. The tested chemical tool is the Silicone Rod (SR) dedicated to hydrophobic to moderately hydrophilic compounds. In total, 22 pesticides (12 herbicides, 5 fungicides and 5 insecticides) were analyzed as well as potential confusing parameters (metal(loid)s, nutrients, temperature). In parallel the biological approach consisted in an in situ study of i) leaf litter decomposition processes, which are mediated by the combined action of microbial and invertebrate communities and of ii) the measurement of fungal biomass within leaf-associated microbial communities. These tools have been applied during June 2014 and June 2015 (corresponding to a period of pesticides application on vineyard) in Ardières river. The Ardières basin, situated in the Beaujolais region (France), is submitted to viticulture pressure and a marked pesticide contamination has already been highlighted. Three measurement stations have been chosen along the contamination gradient from upstream to downstream. Results showed the high coherence of chemical and biological tools responses. All of them were able to reflect the spatial contamination gradients as well as the specific contamination and impact associated. Thus, decomposition rates and fungal biomass decreased from up to downstream, in relation to the observed increase in fungicides and organophosphorus insecticides concentrations. This biological impact was observed during the two years studied, while pesticide contamination decreased in 2015 compared to 2014. Finally, their sensitivity, reactivity and integration capacity make these tools relevant and promising to assess agricultural pesticide impact on surface water in addition to grab sampling.

WEPC19
From in-situ passive sampling into bio assays - an innovative approach of combined chemical and biological analysis of HOCs
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The proposed project aims at the development of innovative indicators to allow for a spatially structured description and assessment of the pollution and the risk potential of sediment-bound contaminants in marine ecosystems. For the first time the project will obtain toxicity data for the pore water concentrations of pollutants with largely reduced uncertainty, directly correlate the results with chemical analysis and verify the findings using artificial mixtures of the target analytes. This will be achieved by adaptation of an in situ equilibrium sampler (passive sampler) based on solid phase microextraction (SPME) for the investigation of hydrophobic organic compounds in marine ecosystems. The PDMS hollow fibers carrying the sampled pollutant mixtures will then be directly applied as passive dosing phases in small-scale biotest systems. As hence no extraction of the fibers is necessary, the risk to alter the original substance composition is significantly reduced. Thus, resulting data are highly representative for the actual pollution on-site. Subsequently, the analysed mixture will be reassembled and tested with concentration series using passive dosing in the test systems to derive concentration-response curves. Together with results on the distribution and accumulation of the contaminants an estimation of the risk due to sediment-bound pollutants will be possible (mixture toxicity). Such realistic toxicity data in combination with knowledge on the distribution of the compounds and correlated with sediment parameters are a good foundation to derive reliable indicators for a good environmental state of Baltic Sea waters.
The proposed project aims at the development of innovative indicators to allow combined chemical and biological analysis of HOCs to reflect the spatial contamination gradients as well as the specific contamination potential of sediment-bound contaminants in marine ecosystems. For the first time, biotests were used to assess the potential of sediment-bound contaminants in marine ecosystems. Akaike's information criterion was used to choose the best model combining chemical and biological data. The contribution of new integrative tools has been useful in addition to pesticide concentration determination to evaluate their accumulation of the contaminants and estimation of the risk due to sediment-bound pesticides.

Procedures for the bioassays were performed in a sequential, two-step method. A preliminary step involved the desorption of the fibers from the sediment. In the second step, the pre-filtered pore water was dosed in parallel with the control at six different concentrations. As hence no extraction of the fibers was performed, it was necessary, the risk to alter the original substance composition is significantly reduced. This biological impact was observed during the two years studied, while pesticide contamination decreased in 2015 compared to 2014. Finally, their sensitivity, reactivity and integration impact associated. Thus, decomposition rates and fungal biomass decreased to reflect the spatial contamination gradients as well as the specific contamination gradient from upstream to downstream. Results showed the contamination of sediments containing pesticides does not affect the hydrophobic organic compounds in marine ecosystems. The PDMS hollow fibers have already been highlighted. Three measurement stations have been chosen along the river to obtain toxicity data for the pore water concentrations of pollutants together at the catchment scale so as to identify their coherence and impact on ecosystems. In this way, the contribution of new integrative tools has been useful in addition to pesticide concentration determination to evaluate their accumulation of the contaminants and estimation of the risk due to sediment-bound pesticides.
Chebbo, Ghassan. MO136
Chebbo, Ghassan. MO136
Chebbo, Ghassan. MO136
Cheli, Giulia. MO136
Chen, Bo-Ching. MO104
Chen, Guo. MO104
Chen, Guan-qiao. MO136
Chen, qiqi. MO076
Chen, Sau Soon. 108
Chen, Shan. 497
Chen, Shaoqin. WE096
Chen, Wei-Yu. MO104, TH058
Chen, Wen-qian. 139
Chen, Xiao. TU259
Chen, Yi. 281, TU164
Chen, Jean-Charles. MO104, TH078, TH083
Chen, Meiyang. MO136
Chen, Yuesi. TU022
Cherfoni, Jean-Francois. MO179
Chinarro, David. WE250
Chion, Beatrice. TH063
Chippari Gomes, Adriana Regina. WE118
Choi, Jin-Soo. WE013
Choi, Jinhee. WE190
Choi, Kyungho. TU216, TUPC09, WE083, WE127
Choi, Minkyu. MO005, MO006
Choi, Yeong-Joo Choi. WE095
Choi, Yeowool. TU013
Clevage, Karin. 514
CHONOVA, Teofana. 443
Chou, Wei-Chan. WE007
Chouloumi, Tiphaine. TU132
Christensen, Guttorm. MO223
Christel, Heino. MO262
Christoffersen, Line. MO033
Christofeletti, Cintya. TU016
Chung, Kang-Sup. TU136
Churro, Catarina. WE088
Ciacci, Caterina. TU036
Ciesielski, Tomasz. 219, MO174, WE144
Ciffray, Philippe. 40
Cinelli, Marco. 46
Ciroth, Andreas. 108, 42, 45, 56, 521, MO287
Cirpka, Olaf. 2
Civit, Barbara. TU264, TU264
Claessens, Sasja. MO255
Claessens, Michel. WE251
Claassen, Silke. TU021, TU021, WE159
Claus, Evelyn. 91, MO121, WE187, WE051, WE051, WEPCE15
Clausen, Lauge. 20
Clavé, Julie. MO097
Clemente, Zaira. TU038, TU040, TU044
Clerambaud, Christelle. 229
Clobert, Jean. 515
Clok, Mark. TU094, TU094
Clough, Jonathan. 392
Cloutier, Pierre-Luc. TH115
Cluzeau, Daniel. 470
Coady, Katherine. 361, WEPCE11
Cobelo-Garcia, Antonio. MO179
Coca, Fernando. TU237, WE250
Codd, Geoffrey. MO202
Coeo, Carla. TU255
Coeurdassier, Michael. WE146, WE146
Coell, Catrin. 16
Cojoa, Cristian. 321
Colle, Mike. 540
Colbourne, John. 346, 351, WE174, WE190
Colinet, Hervé. TU155, TU155
Coll Mol, Claudia. 93
Coll, Pierre. 104
Coll, Mary. WE232
Coll, Blanche. TU061, TU061
Collinet, Marc. MO408
Collins, Adrian. 248, 85, MO176
Collins, Chris. 65, MO064, TU218
Collison, Elizabeth. MO106
Collotta, Massimo. TH185
Coman Smid, Diana. WE175
Comber, Mike. MO143
Comber, Sean. 547, MO013, MO301, MO157, MO162
Combi, Tatiane. TH100, WE074, WE077
Companies, Encarna. 26, MO160, WE050
Comi, Rabel. TU268
Conesa Alcaraz, Hector Miguel. TU204, WE026
Conine, Andrea. 378
Connor, Peter. TU263, TU263
Constantine, Lisa. 414, WE219, WECPC10
Conseil, Lara. 572
Cooper, Christopher. WE251
Cooper, Jean-Francois. 35, TH019
Coors, Anja. 245, 477, 98
Cope, W. Gregory. MO122
Coquery, Marina. TU043, WE052, WE052
Coppola, Nathalie. MO139
Cornells, Cayo. MO053
Corcoll, Natalia. 445, WE196, WE196
Cordella, Christophe. TU005
Cordery, Katherine. 193, TU109
Cordier, Laure. 381, 437
Coria, Jessica. TU185
Cornel, Peter. TU245
Cornelis, Geert. 27, 325, 328, 37
Cornelissen, Gerard. 374, WECPC14
Cornelissen, Gesine. WE098
CORNELUS, MELISSA. 418
Cornillier, Claire. MO017
Corrado, Sara. 369, TU265, TU265
Corrales, Jone. 18
Correia, D. MO160
Correia, Jorge. TU016
Correia, Manuel. 339
Corry, Thomas. MO261
Corsi, Ilaria. 173, 184, TH056, TH073, TU036, TU054
Corson, Michael. TU251
Cortese, Katia. TU036
Corti, Claudia. WE230
Cosgrove, John. MO057, WE131
Cosio, Claudia. 483, TH035, TH036, TU152
Cosso, Eline. 401
Cossu-Leguille, Carole. 169
Costa, Falbemi. TU261
Costa, Gonzalo. MO042, WE192
Costa, Sara. WE229, WE230
Costill, Katherine. TH114, TH116, TH130
COTELLE, Sylvie. 517, WE015
Couce, Ivan. 214
Coulet, Eric. 553
Couliou, Evelyne. TH135
Coullomb, Bruno. 109
Zivanov, Miloš. TU004
Zivkovic, Igor. TH029
Zlabek, Vladimir. TU011, TU022, TU022, WE183
Zoh, Kyung-Duk. WE067, WE095
Zonja, Bozo. 3
Zorita, Izaskun. 89
Zouari, Mohamed. MO148
Zubrod, Jochen. MO094, MO096, MO108, TH118, TU118, TU120, TU121, TU187, TU211, WEP05
Zühlke, Sebastian. 19
Zupanic, Anze. WE175
Zwaan, Roelof. TU096
Zwarg, José Ricardo. TH139
Zwart, Nick. 426
Zwiener, Christian. 2, TH018

Ø

Øverjordet, Ida. MO177, MO182, TH067, TH068, TH069, TH071, TH072
Øxnevad, Sigurd. WE201
Øygarden, Lene. TH077
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