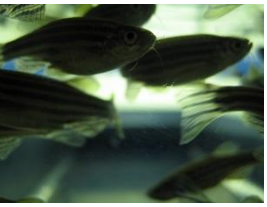
NORMAN workshop

Report

Mixtures and metabolites of chemicals of emerging concern



Organised by

NORMAN
RIVM - National Institute for
Public Health and the Environment
IVM – Institute for Environmental Studies
VU University
The Netherlands

18 - 19 November 2009
Amsterdam
The Netherlands

Meeting
VU University Medical Centre (VUmc)
De Boelelaan 1117
Room: De Rijn
Amsterdam, The Netherlands

rivm National Institute for Public Health and the Environment

norman

Vrije Universiteit amsterdam
Institute for Environmental Studies

“Mixtures and metabolites of chemicals of emerging concern”

Brief workshop report

Executive summary
The release of chemicals into the environment often causes the formation of transformation products which may not only be more toxic than the parent compound, but may also have a different environmental distribution pattern, and may be more persistent. The aspects of possible increased toxicity over time, deviating partitioning and increased persistence have so far been insufficiently taken into account in chemical regulation, and are also a topic of limited analytical awareness. On top of this, mixtures of parent compounds and metabolites are typically formed, possibly causing additional adverse effects due to aspects of mixture toxicity.

With this in mind, a workshop on mixtures and metabolites of chemicals was organised by RIVM – National Institute for Public Health and the Environment – and IVM – Institute for Environmental Studies – VU University (The Netherlands). The workshop took place in Amsterdam on 18–19 November 2009. The programme covered various aspects related to the formation of metabolites and degradation products of emerging substances and mixture effects – a topic of crucial importance for the assessment of the toxicity of emerging pollutants and products resulting from various treatment techniques.

About 70 participants attended the workshop with 16 presentations and 11 posters. The presentations were made available via the NORMAN website: http://www.norman-network.net/index_php.php?module=public/workshops/amsterdam2009_pdf&menu2=public/workshops/workshops

The first day of the workshop was dedicated to mixtures effects aspects. The co-occurrence of chemicals in the environment plays an important role in their overall environmental impact. The ecotoxicological assessment of mixtures is complex and there are still many knowledge gaps. However, checking compliance with environmental quality standards alone is obviously not sufficient: a number of modelling tools for prediction of mixtures effects exist already and they need to be integrated in future regular risk assessment protocols.

The second day was focused on the tools for identification and analysis of the metabolites and transformation products of chemicals of emerging concern. Here again the main tools available (*in silico* methods, analytical techniques, bioreactors, field studies) to study the chemicals’ transformation products were presented. However, knowledge gaps still exist in the analysis of transformation products and their fate, transport and toxicity. Moreover, most of the current studies address two main classes of compounds: pesticides and pharmaceuticals. The main questions that were left unanswered at the end of the day included: Is this the tip of the iceberg? How many transformation products should be covered additionally?

The theme of transformation products and mixture effects will be one of the topics of the TransCon2010 Conference. The TransCon2010 Conference is organised by EAWAG in September 2010 http://www.eawag.ch/medien/veranstaltungen/events/transcon2010_/index_EN. This conference will represent a follow-up to the NORMAN workshop on “Mixtures and metabolites of chemicals of emerging concern”. The NORMAN network is one of the honorary sponsors of the conference and NORMAN members have been encouraged to prepare contributions to the scientific programme.

In this brief report of the workshop, a general overview is given of the programme and the main findings. The workshop was divided into 4 sessions:

- Mixtures (bio)monitoring and modelling (Chair: Dr. Timo Hamers of IVM)
- Risk assessment of mixtures: approaches and practical experiences (Chair: Dr. Willie Peijnenburg of RIVM)
- Modelling, fate and risk assessment of metabolites and transformation products (Chair: Dr. Marja Lamoree of IVM)
- Chemical monitoring of metabolites and transformation products (Chair: Dr. Pim Leonards of IVM).

Two key-note presentations preceded Sessions 1 and 3:

- Mixing apples and oranges: problems, approaches and solutions in mixture risk assessment (Dr. Leo Posthuma - RIVM)
- Predicting biotransformation products: development and validation of a computer-based tool (Dr. Juliane Hollender on behalf of Dr. Kathrin Fenner – EAWAG, Switzerland).

DAY 1: 18 November 2010

Introduction to workshop

On behalf of the organisers of the workshop, a word of welcome was addressed by Willie Peijnenburg to the participants. These opening remarks included a brief outline of the workshop programme and a general introduction to the NORMAN network.

Keynote presentation

The first keynote presentation focused on the issue of mixture toxicity, and was presented by Dr. Leo Posthuma of RIVM. The title of the presentation was: “Mixing apples and oranges: problems, approaches and solutions in mixture risk assessment”.

Session 1 - Mixtures (bio)monitoring and modelling (Chair: Dr. Timo Hamers of IVM)

This session consisted of the following four presentations:

- Methods development to detect antibiotic activity in water samples (Dr. Stephan Kools – Grontmij /Aquasense) Appendix 1
- Toxicological relevance of emerging contaminants for drinking water quality (Dr. Merijn Schriks – KWR Watercycle Research Institute) Appendix 2
- Masking effect of anti-androgens on androgenic activity in European sediments (Dr. Marja Lamoree – IVM) Appendix 3
- Modelling and fate of mixtures (Dr. Jan Baas – Environ) Appendix 4.

Session 2 - Risk assessment of mixtures: approaches and practical experiences (Chair: Dr. Willie Peijnenburg of RIVM)

This session consisted of the following three presentations:

- Assessment of endocrine disruptive potential of complex pollutant mixtures in the river ecosystem affected by a major city – bioassays and chemical analyses (Dr. Ludek Blaha – RECETOX, Czech Republic) Appendix 5
- Risk assessment of mixtures of nine pharmaceuticals using multispecies biotests (Dr. Sureyya Meric – University of Cyprus) Appendix 6
- Toxicity profiling: an effect-based integrative tool for sediment quality assessment (Dr. Timo Hamers - IVM) Appendix 7.

In between Sessions 1 and 2, an interactive poster session was organised in which all posters were presented by means of a 3-minute presentation near the poster.

Main conclusions of Sessions 1 and 2

Following the presentations within Sessions 1 and 2, the main remarks were highlighted by Dr. Willie Peijnenburg and discussed with the participants. The following conclusions and recommendations were agreed upon:

- **The composition of mixtures is highly complex and variable over time. This requires specific approaches to be adapted to unravel their (adverse) environmental impacts.**
- **Various tools are available to assist in unravelling environmental impacts of chemicals. These tools include *in vitro* and *in vivo* testing and various types of *in silico* models for predicting fate and effects of (mixtures of) chemicals. Also, there is increased understanding of fate and effect modulating environmental factors. This allows for inclusion of these factors in quantitative exposure and effect models.**
- **Environmental quality standards for individual compounds are not suited for the assessment of mixtures of varying composition.**
- **"Mixture ecotoxicity" should be included in risk assessment of chemicals in either the first (generic) tier of risk assessment schemes, or in site-specific (second tier) risk assessment.**
- **In numerous cases we are NOT dealing with mixtures of known composition. A question that needs further attention is therefore: How to do risk assessment of co-occurring mixtures?**
- **A better understanding is required of the processes and underlying mechanisms leading to deviating patterns and adverse effects of complex mixtures of chemicals. In this respect it is questionable whether current research efforts cover 'all' modes of action and especially their biological interactions.**
- **We need to accept that we cannot know everything – we need to deal with the (few) data that are available, especially for grouping chemicals, prioritising them, etc.**
- **E(cotoxi)cology should be included as part of environmental monitoring programmes.**
- **Risk assessment is a critical issue regarding setting monitoring priorities and can thus assist in saving time, financial expenses, etc.**
- **Fate and effect models do exist! We should use them!**

DAY 2: 19 November 2010

Keynote presentation
The second keynote presentation focused on the issue of prediction of metabolites formation and was presented by Dr. Juliane Hollender on behalf of Dr. Kathrin Fenner (EAWAG, Switzerland). The title of the presentation was: "Predicting biotransformation products: development and validation of a computer-based tool".

Session 3 - Modelling, fate and risk assessment of metabolites and transformation products (Chair: Dr. Marja Lamoree of IVM)
This session consisted of the following four presentations:

- Modelling fate and effects of chemicals including their transformation products (Rosalie van Zelm – Radboud University, Nijmegen, The Netherlands) Appendix 8
- *In vitro* toxicity and formation of hydroxylated PBDE and NDL-PCB metabolites (Timo Hamers – IVM) Appendix 9
- Nitration processes of acetaminophen in nitrifying activated sludge (Serge Chiron – Aix-Marseille Universités-CNRS, France) Appendix 10
- The Risk to the UK Population of Pesticide Metabolites in Drinking Water (Chris Sinclair – The Food and Environment Research Agency, Sand Hutton, UK) Appendix 11

Session 4 - Chemical monitoring of metabolites and transformation products (Chair: Dr. Pim Leonards of IVM)
This session consisted of the following four presentations:

- Identification and quantification of transformation products in the aquatic environment by high resolution mass spectrometry (Dr. Juliane Hollender – EAWAG, Switzerland) Appendix 12
- Highlighting and identification technique of chlorination by-products of Ethinylestradiol in Drinking Water Treatment by untargeted profiling method by LC-HR(MS)n (Dr. Geal Gervais – École Nationale Vétérinaire de Nantes, Nantes, France) Appendix 13
- Analysis of polar organic chemicals in European rivers and ground waters by SPE-LC-MS-MS (Dr. Robert Loos – European Commission JRC, Ispra, Italy) Appendix 14
- Emission of pharmaceuticals from care units into wastewater: from identification of sources to monitoring (Dr. Barry Pieters - Grontmij /Aquasense) Appendix 15.

Main conclusions of Sessions 3 and 4
Following the presentations within Sessions 3 and 4, the main remarks were highlighted by Dr. Pim Leonards and discussed by the participants. The following conclusions and recommendations were agreed upon:

- **Various tools are available to study transformation products of parent compounds in the environment, ranging from *in silico* predictions, analytical techniques and bioreactors to field measurements.**
- **Modelling tools are now available for predicting biotransformation products, which could assist the search for TPs during experimental and environmental monitoring studies.**
- **Analytical techniques are very well developed and available to identify unknown TPs, but the process is time consuming. Therefore, we need interactive software tools between databases (direct links with the internet).**

- **Most degradation studies for TPs are limited to bacteria degradation. We therefore do not know much about the risk of transformation products/metabolites at higher trophic levels (e.g. invertebrates, fish, mammals, birds). Some TPs are more persistent than their parent compounds and have the ability to bioaccumulate.**
- **Comparing laboratory tests and screening studies of WWTPs would be a suitable tool to identify new TPs.**
- **Knowledge of the fate of TPs in drinking water treatment is limited: TPs should be studied at the level of drinking water sources and groundwater.**
- **Scientific knowledge gaps exist on various aspects of transformation products and metabolites in the fields of analysis, fate (pathways), exposure, toxicity and risk.**
- **The question is, how much of the TPs (mainly pesticides), do we cover, and is this the tip of the iceberg? We expect that with current new analytical techniques, that are more sensitive and have higher identification power, the future challenges will be at the level of processing and handling of the large datasets to discover TPs.**
- **An important challenge will be to combine models, software and databases to further investigate the fate, exposure and risk of TPs.**

Abstracts of all presentations are given in Appendices 1–15, and the presentations can be found on the NORMAN website.

Abstracts

METHODS DEVELOPMENT TO DETECT ANTIBIOTIC ACTIVITY IN WATER SAMPLES

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We cannot think of living without antibiotics. They are widely used to cure and prevent diseases for humans and animals. However, their ubiquitous use has lead to unexpected and unwanted emissions to the environment. Traces of antibiotics have been measured in several environmental compartments and this may cause a constant selective pressure on bacteria, resulting in resistance. Potentially, effects can occur mainly in the aquatic environment, because the main sources for antibiotics are due to run-off from farmlands (direct release to surface water) and human use (indirect release via wastewater). Therefore, monitoring of the presence and effects of these compounds is asked for. For these monitoring purposes, good, fast and reliable methods are needed. A promising low-cost high-throughput screening method based on microbial growth-inhibition was developed at RIKILT Wageningen to test food samples. Later, experiments by others showed that it can also be applied to test surface water samples using extraction techniques. Recently, different measurement and extraction techniques were tested and compared using eight antibiotics in pure form. Generally, good results were obtained with the standard measuring method. Some cross – reactivity between antibiotics was observed, giving more information about the sensitivity of the test. Comparison of the extraction techniques showed differences between methods. Further research has shown that this test is capable of indicating the presence and activity of several types of antibiotics in surface water and treated waste water. We will continue to develop this test to help water managers and others in signify the occurrence and sources of antibiotics in the environment.

TOXICOLOGICAL RELEVANCE OF EMERGING CONTAMINANTS FOR DRINKING WATER QUALITY

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The detection of many new compounds in surface water, groundwater and drinking water raises considerable public concern, especially when human health based guideline values are not available it is questioned if detected concentrations affect human health. In an attempt to address this question, we derived provisional drinking water guideline values for a selection of 50 emerging contaminants relevant for drinking water and the water cycle. For only 10 contaminants, statutory guideline values were available. Provisional drinking water guideline values were based upon toxicological literature data. The maximum concentration levels reported in surface waters, groundwater and/or drinking water were compared to the (provisional) guideline values of the contaminants thus obtained, and expressed as Benchmark Quotient (BQ) values. We focused on occurrence data in the downstream parts of the Rhine and Meuse river basins. The results show that for the majority of compounds a substantial margin of safety exists between the maximum concentration in surface water, groundwater and/or drinking water and the (provisional) guideline value. The present assessment therefore supports the conclusion that the majority of the compounds evaluated pose individually no appreciable concern to human health.

MASKING EFFECT OF ANTI-ANDROGENS ON ANDROGENIC ACTIVITY IN EUROPEAN RIVER SEDIMENT UNVEILED BY EFFECT-DIRECTED ANALYSIS

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This study shows that the androgen receptor agonistic potency is clearly concealed by the effects of androgen receptor antagonists in a total sediment extract, demonstrating that toxicity screening of total extracts is not enough to evaluate the full in vitro endocrine disrupting potential of a complex chemical mixture, as encountered in the environment. The anti-androgenic compounds were masking the activity of androgenic compounds in the extract with relatively high anti-androgenic potency, equivalent to 200 nmol flutamide equivalents/g dry weight. A two-step serial liquid chromatography fractionation of the extract successfully separated anti-androgenic compounds from androgenic compounds, resulting in a total androgenic potency of 3,820 pmol dihydrotestosterone equivalents/g dry weight. The fractionation simplified the chemical identification analysis of the original complex sample matrix. Seventeen chemical structures were tentatively identified. Polyaromatic hydrocarbons, a technical mixture of nonylphenol and dibutyl phthalate were identified to contribute to the anti-androgenic potency observed in the river sediment sample. With the GC/MS screening method applied here, no compounds with AR agonistic disrupting potencies could be identified. Seventy-one unidentified peaks, which represent potentially new endocrine disrupters, have been added to a database for future investigation.

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MODELLING AND FATE OF MIXTURES

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Risk assessment is typically based on single compound exposures. But "in real life there is no such thing as a single chemical exposure". And as the number of possible combinations is (almost) endless we need tools to understand but more importantly predict effects of mixtures. Within the framework of the European NoMiracle project a process-based method to assess effects of mixtures was developed. The method rest on the Dynamic Energy Budget (DEB) Theory and takes the organism as a starting point. Traditional methods take the effects as a starting point and link these to concentrations. These methods have severe limitations for the extrapolation potential and for the interpretation of experimental data. Therefore these methods are of limited use to predict effects of complex mixtures as we encounter them in our environment. Taking the organism as a starting point overcomes these disadvantages: effects of mixtures on different endpoints at different points in time can be interpreted within one single consistent framework. Having a theoretical framework also allows for extrapolation of experimental results to other points in time, to compounds that were not measured or even to different organisms.

The method will be introduced conceptually and its application will be illustrated by an assessment of the effects on survival of in situ exposed *Daphnia magna* to surface waters in "het Westland". In this region a cocktail of pesticides, metals, nutrients, PAHs and PCBs is encountered in the surface waters.

ASSESSMENT OF ENDOCRINE DISRUPTIVE POTENTIAL OF COMPLEX POLLUTANT MIXTURES IN THE RIVER ECOSYSTEM AFFECTED BY A MAJOR CITY - BIOASSAYS AND CHEMICAL ANALYSES

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There is increasing evidence that certain environmental contaminants have the potential to disrupt endocrine processes, which may result in reproductive problems, certain types of cancers and other toxic effects related to (sexual) differentiation, growth, and development in a number of living species. The aim of the presented study was the complex assessment of contamination of the aquatic environment, focused especially on the presence of pollutants with endocrine disruptive (ED) activity in the rivers of densely populated and industrial area and potential influence of large municipal waste water treatment plant (WWTP). The activities have been assessed in the region of city agglomeration (Brno, Czech Republic) spread in the basin of two rivers, with more than 400,000 inhabitants, variety of industrial activities and a large wastewater treatment plant. The presence of contaminants with ED-potential in water has been confirmed by the application of multiple types of integrative passive samplers (SPMD for persistent organic pollutants and POCIS for polar compounds and pesticides). At the same time, sediments were collected and analyzed. ED activity in the influent and effluent wastewater of WWTP was tested during a year-long study to obtain temporal variability and better characterization of the influence of the treatment process on the contamination. Organic extracts of sediments, passive samplers and composite samples of waste water have been tested with a battery of *in vitro* bioassays with recombinant yeast and mammalian cell systems for cytotoxic, dioxin-like, (anti)estrogenic and (anti)androgenic activities. Results documented significant estrogenic and androgenic activity in the influent waters to the WWTP and generally high removal of the bioactive compounds during the treatment process. The riverine samples showed significant dioxin-like, antiestrogenic and antiandrogenic potencies. The complex approach based on chemical analyses and biological assays in the series of samples enabled to assess the presence and distribution of bioactive contaminants in various components of the river ecosystem and to elucidate the character of ED-active compounds. The research has been supported by the Czech Ministry of Education projects 2B06093 and 2B08036 (ENVISCREEN).

RISK ASSESSMENT OF MIXTURES OF NINE PHARMACEUTICALS USING MULTISPECIES BIOTESTS

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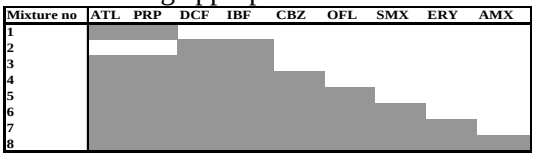
Keywords: pharmaceuticals, ecotoxicity, mixtures, modelling, risk assessment.

Regarding the assessment of the environmental risk of pharmaceuticals, acute effects have been reported to be unlikely due to their low environmental concentrations. As STP effluents have been shown to contain mixtures of pharmaceuticals, their metabolites and transformation products, it is now considered important to study possible combination effects of pharmaceuticals in chronic studies. There are few studies available in the literature on mixture toxicity of pharmaceuticals, and some examples are:

(i) the combined effect of ibuprofen, fluoxetine, and ciprofloxacin to *Lemna gibba* and *Myriophyllum* spp for 35 d (Richards et al. (2004);
(ii) mixture of atorvastatin, acetaminophen, caffeine, sulfamethoxazole, carbamazepine, levofloxacin, sertraline, and trimethoprim assessed on a variety of somatic and pigment endpoints in rooted (*M. sibiricum*) and floating (*L. gibba*) (Brain et al., 2004);
(iii) acute toxicity of three b1-selective blockers (acebutolol, atenolol, and metoprolol) and three non-b1-selective blockers (nadolol, oxprenolol, and propranolol) in mixture using acute 2 d *Ceriodaphnia dubia* immobility test (Fraysse and Garric, 2005);
(iv) a mixture of seven common pharmaceutical agents (acetaminophen, diclofenac, gemfibrozil, ibuprofen, naproxen, salicylic acid, and triclosan) using freshwater amphipod *Hyalella azteca* over three generations Borgmann et al., 2007).

Preferably, mixtures should be tested both at effective (high) dose levels and at realistic (low) dose levels (Richards et al., 2004). A pragmatic approach to whole mixture toxicity is to test toxicity without assessing the types of interactions (Brain et al., 2004). The concentration addition (*CA*) model seemed to be appropriate, providing a reasonable worst-case estimation of different mixtures as reported by Borgmann et al., (2007) for b-blocker mixture toxicity and by Cleuvers (2003; 2004; 2005) for carbamazepine and clofibric acid, diclofenac and ibuprofen, the mixture of diclofenac, ibuprofen, naproxen and acetylsalicylic acid and the mixture of three b-blockers (propranolol, atenolol, metoprolol).

Various mixtures as shown in the following matrix of nine pharmaceuticals were tested to four bioassays: *Daphnia magna, P. subcapitata, Artemia salina and Lepidium sativum* within the scope of the PHAREM project (AEIFO/0506/16) for three doses (0.25, 0.5 and 1 mg/L of each one in the mixtures). The results showed how the toxicity is varying among the groups of the pharmaceuticals and that the relationship '*species-response'* is more significant than the '*dose-response'* one. *P. subcapitata* was the most sensitive species for all the mixtures tested. That of D. magna followed this species's sensitivity. However, the model evaluation of our results presented hereby did not obey either to CA or Dissimilar action *(DA)* model (*antagonism)*. This can be due to i) the EC_{50} values obtained in this study may need to be re-evaluated using other models (e.g. Linear, Logistic, Gompertz, Exponential, Hormetic models) to fit the concentration–response relationships; ii) any risk model beyond CA and DA models which is a running study; iii) increasing model species in order to obtain accurate data sets for testing appropriate models.



ATL (Atenolol); PRP (Propranolol); DCF (Diclofenac); IBF (Ibuprofen); CBZ (Carbamazepine); OFL (Ofloxacin); SMX (Sulfamethoxazole); ERY (Erthromycin); AMX (Amoxicillin)

TOXICITY PROFILING: AN EFFECT-BASED INTEGRATIVE TOOL FOR SEDIMENT QUALITY ASSESSMENT

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A toxicity profile is a toxicological fingerprint of the complex mixture of contaminants present in the environment. For each environmental sample, a toxicity profile can be obtained by testing its integrated potency to cause a response in a battery of bioassays each representing a different mode of action. We explored the applicability of in vitro toxicity profiles for effect-based water quality assessment using a dataset in which sediment extracts from 15 different locations in the Rhine-Meuse estuary were tested in five different bioassays. Toxicity profiles were translated into hazard profiles, indicating for each mode of action the relative distance to the desired or acceptable water quality status. Using hierarchical clustering techniques, location-specific hazard profiles of four out of five harbor locations in the test set were classified together into a cluster of harbor locations. Apparently, the fifth harbor location could be identified as a harbor with a hazard profile deviating from "normal" harbor profiles. This deviation could be attributed to relatively high dioxin-like and estrogenic potencies and may be a reason to focus on this particular location for "in depth" research into the identity and possibly the source of the responsible contaminants. Toxicity profiling and subsequent hazard profiling seems to be a tool that is directly applicable for water quality assessment. However, it requires a very careful selection of a reference toxicity profile that is either measured at a reference location or is designated as a desirable or acceptable toxicity profile for that particular location.

PREDICTING BIOTRANSFORMATION PRODUCTS: DEVELOPMENT & VALIDATION OF A COMPUTER-BASED TOOL

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Many aquatic contaminants of current concern such as biocides, pesticides, and pharmaceuticals are known to be subject to biotransformation at their entry point into the environment, e.g., agricultural soils or sewage treatment plants. While for certain classes of pesticides the formation of stable and mobile transformation products during biotransformation is a well-known phenomenon, little is known about the formation of stable products for most other compound classes. In-silico methods that predict likely biotransformation products for a given organic compound are therefore valuable tools. They can be helpful in gearing chemical analysis towards identifying transformation products in environmental samples (Kern et al., 2009; Hollender et al., this workshop), and are also instrumental in developing an efficient procedure to include relevant transformation products into chemical risk assessment of diverse compound classes. The University of Minnesota Pathway Prediction System (UM-PPS) is a freely available web-based tool (http://umbbd.msi.umn.edu/predict/index.html) and consists of about 210 structure-based transformation rules used to predict possible biodegradation pathways of organic contaminants. Due to the broad applicability of some of these rules, UM-PPS typically produced a large number of possible transformation products and pathways when the rules were applied without further prioritization. In this presentation, we report on an approach that uses existing information on known biodegradation pathways as contained in the University of Minnesota Biodegradation/Biocatalysis Database (UM-BBD) to establish hierarchies between different transformation rules applicable to a given compound structure. These so-called relative reasoning rules, when tested for a set of xenobiotics, lead to a reduction in prediction space of up to 25% per generation predicted, without compromising the tool's power to predict known transformation products (Fenner et al., 2008). While UM-PPS so far was based on biotransformation data mainly derived from pure culture studies, ongoing work in our laboratory aims at testing its validity and applicability to more environmentally relevant situations. The general strategy of those studies is to combine biotransformation batch experiments using sludge-seeded bioreactors with product identification using high-resolution mass spectrometry. In one project, we use this setup to test UM-PPS rules with regard to their validity under activated sludge conditions and to further develop pertinent relative reasoning rules. First results allow differentiating the rate of enzymatic amide hydrolysis as a function of adjacent functional groups, which in turn helps to refine existing amide transformation rules and associated relative reasoning rules (Helbling et al., this workshop). In a second project, we apply UM-PPS to screen for biotransformation products of human pharmaceuticals both in the bioreactors and in sewage treatment plant effluents. In this way, we have identified eight so far unknown transformation products of five high consumption human pharmaceuticals.

Kern, S.; Fenner, F.; Singer, H.P., Schwarzenbach, R.P.; Hollender, J. (2009). Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry, *Environ Sci Technol*, pubs.acs.org/doi/pdf/10.1021/ es901979h.

Fenner, K.; Gao, J.; Kramer, S.; Ellis, L.; Wackett, L. (2008). Data-driven extraction of relative reasoning rules to limit combinational explosion in metabolic pathway prediction.

JOINT IMPACT: MODELING FATE AND EFFECTS OF CHEMICALS INCLUDING THEIR TRANSFORMATION PRODUCTS

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Current life cycle impact assessment (LCIA) of chemicals focuses on the fate and effects of the parent compound only, neglecting the potential impact of transformation products. We now assessed the importance of including the potential impact of transformation products in the calculation of characterization factors (CF), applying the method to freshwater ecotoxicity for 16 chemicals. Chemical-specific uncertainty was taken into account via probabilistic simulations. Including transformation products resulted in a typical increase in CF that differs seven orders of magnitude between the chemicals considered. This increase, however, can be highly uncertain,
particularly due to a lack of toxicity data for transformation products and a lack of mode of actionspecific data. We show in a case study that replacement of atrazine with other pesticides for application on corn results in a median impact score of almost two orders of magnitude lower when including fate and effects of the parent compounds only, whereas inclusion of transformation products leads to comparable median impact scores for atrazine and its replacing pesticides.

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NITRATION PROCESSES OF ACETAMINOPHEN IN NITRIFYING ACTIVATED SLUDGE

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This work is an attempt to elucidate the quantitative significance of acetaminophen (APAP) nitration in nitrifying activated sludge and to propose a reaction mechanism for this process. The link between nitrification and nitration of APAP was investigated at different scales. Results from field studies showed the occurrence of 3-nitro-APAP and to a lesser extent 3-chloro-5-nitro-APAP, a transformation product probably generated from the nitration of 3-chloro-APAP, at concentration levels in the 50-300 ng/L range in a full scale wastewater treatment plant (WWTP) operated with nitrogen removal. Batch experiments with nitrifying activated sludge confirmed APAP removal by nitration and suggested that nitrifying bacteria may play a role in this transformation process through the release of reactive nitrogen species. In vitro assays provided evidences that nitration through the production of nitrous acid is a very unlikely pathway. In contrast, nitric oxide ($^{\cdot}NO$) produced by nitrifying bacteria is probably involved in APAP nitration through the formation of peroxynitrite in presence of superoxide. Kinetic data as well as transformation mechanisms and pathways will be presented. A previous work from our group has shown the relevance of photonitration as a transformation pathway of phenolic compounds in shallow surface waters (Chiron et al. 2007). This work provides evidences that in systems such as nitrifying activated sludge, where biomass is exposed to dynamic changes in nitrite, ammonium and oxygen concentration, nitration of APAP is also relevant. The production of 3-nitro-APAP would account for a few percents of the total transformation rate of APAP in WWTPs. Even though nutrient removal strategies have improved the whole effluent quality of municipal WWTPs, there is a potential risk to generate harmful transformation products. The formation and occurrence of nitro-APAP derivatives in urban wastewater cycle is a matter of concern for the protection of aquatic organisms due to their potential persistence and increased hydrophobicity relative to APAP and due to the genotoxic properties of nitrophenols.

Keywords: nitration, nitrifying activated sludge, acetaminophen, reaction mechanism.

Reference

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THE RISK TO THE UK POPULATION OF PESTICIDE METABOLITES IN DRINKING WATER

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Once pesticides are applied following normal agricultural practice they can degrade via biotic and abiotic processes to produce a range of metabolites. Some of these compounds have been shown to be more mobile and more persistent than their parent pesticides and may move from the site of application to surface and ground waters. Subsequently these waters containing metabolites may be used as a source of water for human consumption. Therefore this desk-based study was performed to identify which metabolites (if any) could contaminate source drinking waters in the UK, quantify the anticipated levels in raw source waters, investigate the effect of drinking water treatment processes on those compounds and finally determine whether any may pose a risk to consumers.

Data on UK pesticide approvals (current and those recently revoked) were used as a starting point from which 485 potential metabolites were identified. The potential hazard (estimated using commercially available software), their potential to exhibit pesticidal activity and their potential to contaminate surface waters were used to select 53 metabolites for further study. Concentrations of these metabolites in three anonymous UK surface water catchments were estimated using an empirical relationship derived from statistical analysis of a large set of field data collected from literature (performed in a previous study). Catchments were selected on the basis that they either had metabolite monitoring data available or had previously been identified as a high risk for contamination with pesticides. Catchment scenarios were characterised by overlaying GIS data on pesticide use, cropping areas and soil types within each catchment. Concentrations in raw waters were then refined for potential removal during drinking water treatment. Metabolite properties were used to estimate the removal at each stage of the processes to ultimately derive estimated total removal.

Potential exposure to the metabolites arising from the consumption of drinking water were then calculated by combining the estimated concentrations in finished drinking waters with the average amounts of water consumed per day by adults and toddlers. For each metabolite, the daily intake values were then compared with an ADI where available. However for most metabolites, it was not possible to source an ADI and, in such cases, a Project Specific Derived Value (PSDV) was estimated; where used, justification for the level of estimated PSDV was given by generating a toxicological profile for each compound. The results of this risk assessment were judged to be generally reassuring, for all metabolites the estimated consumer intakes from drinking water based on realistic estimates were less than 10% of the ADI (or PSDV) for all sections of the population and all catchments considered.

IDENTIFICATION AND QUANTIFICATION OF TRANSFORMATION PRODUCTS IN THE AQUATIC ENVIRONMENT BY HIGH RESOLUTION MASS SPECTROMETRY

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The need to consider transformation products (TPs) of organic contaminants in the risk assessment for the aquatic environment is mentioned in several European directives but little concrete guidance on how to identify relevant transformation products is given. Liquid chromatography combined with mass spectrometry has become a key technique allowing the trace analysis of TPs in environmental matrices. However, analytical challenges are still the clear identification of known TPs without reference standards which are often commercially not available and the identification of previously unidentified TPs which have never been described. One approach to overcome these limitations is to employ hybrid tandem mass spectrometers which combine two mass spectrometric technologies including one high resolution technique. Full-scan chromatograms acquired with high mass accuracy and resolution allow selectively searching for the molecular ions of TPs based on their exact mass while MS/MS technology provides structural information of compound fragmentation.
In our studies, we carry out a targeted screening and quantification of known TPs using isotope dilution as well as a screening of suspects for which no reference standards are available. For both approaches the analytical procedure includes solid phase extraction, liquid chromatography, and electrospray ionization followed by linear ion trap Orbitrap mass spectrometry with a mass resolution up to 100,000 and a mass accuracy below 5 ppm. The procedure to positively identify target TPs without reference standards in a given sample consists of extracting the exact mass from the chromatogram, selecting peaks of sufficient intensity, checking for retention time correlation, and interpreting mass spectra.
Within a Swiss survey of groundwater from agricultural and urban areas we screened for 87 pesticide and pharmaceutical TPs and the respective parent compound. The results confirm the importance of pesticide TPs which was show in US groundwater (Kolpin et al., 2004). Triazine herbicide TPs were detected most frequently, the highest concentrations were found for chloridazon TPs which have not been included in monitoring campaigns until now.
A suspects screening procedure was developed for the identification of TPs of 52 pesticides, biocides, and pharmaceuticals in a set of seven surface water samples from different regions in Switzerland (Kern et al., 2009). A target list of 1800 TPs was compiled for these parent compounds using literature information as well as the University of Minnesota pathway prediction system, a computer-aided prediction tool for biodegradation (Fenner et al., this workshop). Nineteen TPs which partly have not been reported in the environment so far were identified by the procedure. Additional 10 low concentrated TPs were identified by reference compounds. Overall, the findings show that the presence of TPs in the aquatic environment is not negligible and more TPs should be included in monitoring programs.

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HIGHLIGHTING AND IDENTIFICATION TECHNIQUE OF CHLORINATION BY-PRODUCTS OF ETHINYLESTRADIOL IN DRINKING WATER TREATMENT BY UNTARGETED PROFILING METHOD BY LC-HR(MS)^N

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Endocrine disruptor compounds (EDCs) are chemical substances that interact with the hormonal system. The study of their fate and occurrence in the Environment and particularly in water (waste water, surface water and drinking water) has become a major issue for public health authorities and drinking water producers.
The Ethinylestradiol (EE2), a synthetic substance used in birth control pills, is an EDC. Some studies have demonstrated its estrogenic impact on the environment, especially its implication in fish's feminization [1]. As a consequence, its removal and transformation from drinking water have become of prime concern. Studies about treatments of waters containing amounts of EE2 demonstrate strong decreases of the substance during drinking water production processes. Though some EE2 by-products (BPs) have already been described [2,3,4], their formation in real conditions has never been clearly demonstrated. For this purpose, a method borrowed to the metabolomic science [5] is used, allowing an untargeted approach for EE2's chlorination by-products identification.
Water from a drinking water treatment plant has been spiked with EE2 at 5 µg/L and treated by chlorination (NaOCl) at 0.8 mg/L. Different samples (spiked and unspiked waters) have been extracted by Solid Phase Extraction. Separation is performed with High Performance Liquid Chromatography coupled to High-Resolution Mass Spectrometry detection (HPLC-(HR)MS). Chromatograms have been processed by XCMS (in order to extract / underline ions significantly different from EE2-spiked treated samples to other samples. This technique highlighted 70 ions corresponding to 10 unknown compounds. After characterization of these substances (retention time, exact m/z, isotopic patterns), a list of possible raw formulas for each compound has been emitted. Then, reducing both shifts between observed m/z and theoretical values, and shifts between isotopic patterns, it was possible to select for each compound a raw formula that fitted well. Afterwards, combining data from literature and molecular modeling [6],structures for each compound were proposed. These structures were confirmed or not, by mass spectrometry fragmentation data. Finally a structure for each unknown chlorination BP of EE2 is suggested and with a high level of confidence for 5 of them. Among those compounds, 3 of the by-products are described for the first time.
This metabolomic approach has proved to be a powerful tool for pollutants treatment BPs identification in real conditions. It would be applied to other contaminants.

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ANALYSIS OF POLAR ORGANIC CHEMICALS IN EUROPEAN RIVERS AND GROUND WATERS BY SPE-LC-MS-MS

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The Institute for Environment and Sustainability (IES) of the Joint Research Centre (JRC) in Ispra (Italy) has started an initiative on environmental European-(EU)-wide monitoring assessments. The first two campaigns focused on polar organic pollutants in river surface and ground waters. The objective is to produce pan-European data sets of environmental concentration levels for chemical contaminants to be used for continental scale risk assessments, environmental fate studies and further decision making.
In total, 122 sampling stations from European streams and rivers (in autumn 2007) in 27 European Countries, and 164 groundwater sampling stations (autumn 2008) were screened. Around 40 laboratories across Europe participated in these two sampling and monitoring exercises.
The river water samples were analysed for 35 and the ground water samples for 56 selected polar organic contaminants (by JRC). Analyses were performed at the JRC in Ispra by solid-phase extraction (SPE) of 400 ml water (950 ml for ground water) followed by triple quadrupole LC-MS-MS analysis using labeled internal standards for quantification.
Target compounds comprised selected pharmaceuticals (e.g. Carbamazepine, Ibuprofen, Diclofenac), the antibiotic Sulfamethoxazole, pesticides and their degradation products (e.g. Diuron, Bentazone, Triazines, 2,4-D), perfluorinated acids (PFOS, PFOA), Benzotriazoles (corrosion inhibitors), hormones (Estrone, Estradiol), and endocrine disrupters such as Bisphenol A and Nonylphenol, and DEET, Sucralose, and Triclosan.
In addition, Chloridazon-desphenyl, Chloridazon-methyldesphenyl, and N,N'-Dimethylsulfamid (DMSA) were analysed in all 164 ground water samples by IWW (Germany), and several antibiotics such as Erythromycin, Trimethoprim, Roxithromycin, Sulfadiazin, Sulfathiazol, Oxytetracycline, Chlortetracycline, Tetracycline, Enrofloxacin, Ciprofloxacin, Marbofloxacin, and Clarithromycin in selected 28 samples by Umweltbundesamt (Austria).
In the surface waters, the most frequently and at the highest concentration levels detected compounds were 1H-Benzotriazole, Caffeine, Carbamazepine, Methylbenzotriazole, and Nonylphenoxy acetic acid (NPE_1C). Caffeine, Ibuprofen, and Carbamazepine were among the compounds with the highest maximum concentrations: 40, 31, and 12 micrograms per liter, respectively.
A comparison with the ground water results gives important information on the chemical fate of the chemicals in terms of persistency, ground water infiltration potential, and thus environmental relevance.
Groundwater samples were analysed jointly by IES-JRC, IWW, UBA Austria, and Masaryk University. In these samples, the most relevant compounds in terms of frequency of detection and maximum concentrations detected were DEET (84%; 454 ng/L), Caffeine (83%; 189 ng/L), PFOA (66%; 39 ng/L), Atrazine (56%; 253 ng/L), Desethylatrazine (55%; 487 ng/L), 1H-Benzotriazole (53%; 1032 ng/L), Methylbenzotriazole (52%; 516 ng/L), Desethylterbutylazine (49%; 266 ng/L), PFOS (48%, 135 ng/L), Simazine (43%; 127 ng/L), Carbamazepine (42%; 390 ng/L), nonylphenoxy acetic acid (NPE_1C) (42%; 11 □g/L), Bisphenol A (40%; 2.3 □g/L), PFHxS (35%; 19 ng/L), Terbutylazine (34%; 716 ng/L),

Bentazone (32%; 11 □g/L), Propazine (32%; 25 ng/L), PFHpA (30%; 21 ng/L), 2,4-Dinitrophenol (29%; 122 ng/L), Diuron (29%; 279 ng/L), and Sulfamethoxazole (24%; 38 ng/L).
A subset of 95 samples was also analysed by Masaryk University for estrogenic and androgenic compounds by reporter gene biotests. No androgenicity was observed; estrogenic effects were determined in 4 samples (estradiol equivalents 0.2-1.2 ng/mL).
The chemicals which were detected most frequently above the European groundwater quality standard for pesticides of 0.1 micrograms per liter (□g/L) were Chloridazon-desphenyl (26 samples), NPE_1C (20), Bisphenol A (12), Benzotriazole (8), N,N'-Dimethylsulfamid (DMSA) (8), Desethylatrazine (6), Nonylphenol (6), Chloridazon-methyldesphenyl (6), Methylbenzotriazole (5), Carbamazepine (4), and Bentazone (4).

Chemical mixtures:

Our results show the importance of multi-residue analytical methods for analyzing chemical mixtures. In total, 77 different organic chemical compounds were analyzed in the ground water samples. The maximum number of compounds detected at any site was 29, and the median number of detections per site was 12. There was no sample free of organic chemicals; in five samples only 3 compounds were found.
Moreover, multi-compound analysis is mandatory for compliance with the European groundwater quality standard of 0.5 □g/L for the sum of pesticides (and degradation products).

Metabolites:

In our study many important metabolites or degradation products of environmental concern were identified. NPE_1C (nonylphenoxy acetic acid) is a recalcitrant degradation product of NPEO surfactants; it was among the most relevant compounds detected in ground waters, with a frequency of detection of 42%, and a maximum concentration level of 11.3 □g/L. Interestingly, this compound is degraded in surface water, but stable in anoxic ground water.
Other important metabolites detected were Chloridazon-desphenyl, Chloridazon-methyldesphenyl, N,N'-Dimethylsulfamid (DMSA), Desethylatrazine, Desethylterbutylazine.

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EMISSION OF PHARMACEUTICALS FROM CARE UNITS INTO WASTEWATER: FROM IDENTIFICATION OF SOURCES TO MONITORING

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First choice: oral presentation
Second choice: poster presentation

Pharmaceuticals have recently been identified as a group of emerging substances. Some pharmaceuticals occur in surface waters in concentrations high enough to be measured and possibly cause negative effects on aquatic organisms. Hence the identification of sources, their spread and quantification of individual substances seem essential to develop cost-effective measures and reduce emissions in the future. Pharmaceuticals can be divided into veterinary and human medicines. Emissions of veterinary pharmaceuticals usually originate from diffuse sources (run-off manure from live stocks). Human pharmaceuticals on the contrary are largely released from point sources such as outlets of wastewater treatment plants. Here, human pharmaceuticals enter the sewage system through excretion of both urine and faeces. Previously the release of pharmaceuticals from hospitals have been characterised in The Netherlands (Verg(h)ulde Pillen project). However, information is still lacking on the remaining care units such as nursing homes, mental health care, and care units for mentally, physically and sense disabled people.
The Dutch Foundation for Applied Water Research (STOWA) has therefore asked Grontmij | AquaSense to perform a literature study, an inventory of pharmaceutical prescriptions in care units and a large monitoring program of pharmaceuticals in wastewater in cooperation with seven different water boards in The Netherlands. The literature study included a characterisation of the different care units in The Netherlands, present use and future trends in use of medicines, excretion values and a desk study of environmental risks. The inventory of pharmaceutical prescriptions by drug stores in the different care units was used to estimate total emissions of (groups of) pharmaceuticals and identify priority substances necessary to be measured in the following monitoring program. Finally, analytical measurements of a set of pharmaceuticals will be performed on wastewater samples from effluents of several care units, effluents of suburbs, and in- and effluents of sewage treatment plants.

RELATIONSHIP OF OCCURRENCE OF PHARMACEUTICALS IN SURFACE WATER WITH CONSUMPTION

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To explain occurrence of emerging substances in surface waters it is of importance to understand the relationship between emission and surface water concentrations as found. The group of pharmaceuticals is a good group to study this, as detailed data on consumption and therefore emissions are available.

Pharmaceuticals are frequently monitored in the Rhine delta between the year 2002 and 2008. Average concentrations of X-ray contrast media were often above 0.1 µg/L, while the average concentration of carbamazepine was around 0.1 µg/L, and average concentrations of the other pharmaceuticals generally fell between 0.1 and 0.01 µg/L. These concentrations were used to calculate annual loads transported by the Rhine at Lobith. The loads are compared to the annual consumption upstream (France, Germany, Switzerland).

This mass balance approach shows that substantial fractions ranging up to 70% of the 20 most frequently observed pharmaceuticals consumed in the Rhine at Lobith. The average recovered fractions of the X-ray contrast media in the Rhine are the highest (48%). The average recovery of antibiotics are lower (18%) and show less variation than the X-ray contrast media. The average recovery of the beta blockers, lipid regulators, analgesics/anti-inflammatory drugs and carbamazepine are around 11%. The recovery of ibuprofen is lowest with 1.1%. The fraction that is recovered in the surface water is generally higher for the pharmaceuticals with lower hydrophobicity. This correlation can be explained by the fact that the hydrophobicity of a chemical is an important determinant for its behavior in the human body, waste water treatment and environment.

The observed annual loads were compared to estimated loads, based upon consumption, metabolism by humans and removal by waste water treatment. The observed and estimated annual loads were rather similar. The ratio of estimated and observed annual loads was smaller than a factor 2 for 15 out of the 20 pharmaceuticals. This illustrates the potential of using consumption data to predict concentrations in the acqueous environment.

Abstracts posters

INHIBITION OF GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION AND ACTIVATION OF MAPKS BY CYANOBACTERIAL EXTRACTS

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Cyanobacteria produce various biologically active compounds which might represent a cancer risk. We investigated effects of various cyanobacterial extracts and pure cyanotoxin microcystin-LR on inhibition of gap-junctional intercellular communication (GJIC) along with activations of mitogen-activated protein kinases (MAPKs) in normal rat liver stem-like WB-F344 cell line. Pure microcystin did not inhibit GJIC or activate MAPKs, but complex extracts of water blooms significantly inhibited GJIC and activated MAPK, independent from the content of microcystin. The most pronounced effects were systematically observed in extracts of the cultures of Aphanizomenon flos-aquae as well as in complex water bloom extract dominated by the same species, which does not produce microcystin. Microcystis sp. culture or water bloom had significant but less pronounced effects on both GJIC inhibition and MAPK activation.

We investigated inhibition of GJIC by cyanobacterial extract and effects of inhibitors of different signaling pathways (PC-PLC, Mek, Redox, protein tyrosine kinasis, pKa) on recovery of GJIC. Observed inhibition of GJIC caused by cyanobacterial extract goes most likely through activation of map-ERK kinases (Mek) in the cells. These are the first results indicating the involvement of cyanobacteria toxins in the regulation of GJIC and MAPK. Supported by NIEHS grant #R01 ES013268-01A2 and Center for Water Science (Michigan State University) to Upham and by project AVOZ60050516 of Institute of Botany (Czech Academy of Sciences) and by the GACR grant No. 524/08/0496 to Blaha.

NOVEL TOXIC METABOLITES IN CYANOBACTERIAL WATER BLOOMS WITH POTENCIES TO ACT AS TUMOR PROMOTERS

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Nutrient pollution of aquatic ecosystems is a global problem resulting in massive growth of cyanobacteria, development of water blooms that produce various toxins. While the toxicity of some cyanotoxins (such as cyclic peptide microcystins) has been extensively studied, several studies document presence of other metabolites that pose significant risks for environment and human health. Here we demonstrate that cell extracts as well as extracellular exudates of major cyanobacteria (*Microcystis aeruginosa, Aphanizomenon flos-aquae* and *Planktothrix agardhii* etc.) modulate established markers of tumor promotion. Our results show that cyanobacterial samples inhibit gap-junctional intercellular communication (GJIC) in rat liver stem-like cells WB-F344. Rapid (5-30 min) downregulations of GJIC were observed followed by later activations of ERK1/2 mitogen-activated protein kinases (MAPKs). The effects were independent from the presence of microcystins in cyanobacterial samples and no effects were observed after exposures to pure microcystin-LR. In summary, our results show that cyanobacteria produce hazardous yet unknown toxins with activities known to play promote growth and development of tumors. Our further research (supported by the grant 524/08/0496 from the Grant Agency C.R.) is focused on the further characterization of responsible compounds and exploration of the detailed mechanisms of their toxicity.

PAHS AND N-PAHS INTERFERING WITH RETINOID SIGNALING IN VITRO CAN ALSO MODULATE LEVELS OF PROTEINS IMPORTANT FOR CELLULAR DIFFERENTIATION

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Retinoids are dietary hormones important during proliferation, differentiation and apoptosis. They act mainly through nuclear receptors for retinoic acid, RARs and RXRs, which form heterodimers and activate retinoic acid response elements (RARE) and induce expression of several genes important for differentiation. Disruption of these signaling pathways by environmental contaminants can lead to several adverse effects especially during embryonic development. Using pluripotent P19/A15 cell line transfected with luciferase reporter gene under control of RARE we found that some polycyclic aromatic hydrocarbons (PAHs) and their N-heterocyclic analogs (N-PAHs) can modulate retinoic acid mediated response *in vitro*. To elucidate possible mechanism of this modulation and its consequences for differentiation, we focused our experiments on selected compounds with various effects on retinoic acid response– downregulation (1,7-Phenantroline), up-regulation (benz[a]anthracene and benz[c]acridine) or biphasic effects (phenanthrene). We found that protein levels of Oct-4 (pluripotency marker and important differentiation factor) were downregulated (40%) by 1,7-phenanthroline and also some other tested compounds slightly modulated Oct-4 levels. Besides effects on Oct-4, also RXR protein levels were affected by 1,7-phenanthroline. As many PAHs and N-PAHs are inducers of aryl hydrocarbon receptor (AhR), its levels were also analyzed after exposure in P19 cells and AhR protein downregulation was observed for some of tested compounds. This study thus found that some compounds (such as PAHs) interfering with retinoid signaling can also modulate levels of several proteins important for cellular differentiation and thus indicate possible mechanism of PAHs embryotoxicity or teratogenicity. This study was supported by projects ENVISCREEN 2B08036 and INCHEMBIOL VZ0021622412

DETECTION OF THYROID HORMONE RECEPTOR DISRUPTORS BY A NOVEL STABLE *IN VITRO* REPORTER GENE ASSAY

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Abstract
Environmental pollutants have been shown to display adverse effects on the regular function of the endocrine system. The thyroid endocrine axis is of remarkable importance as the biologic effects of thyroid hormones (TH), which are mediated through the thyroid hormone receptors (TRs), are essential for growth, development, cellular differentiation and metabolism.

Considering the large number of natural and manufactured chemicals that may disrupt the TH mode of action, legislation under the REACH framework demands risk evaluation of all industrial and non-industrial chemical products. A great effort has been put in the development of integrated and intelligent testing strategies for evaluation of the potential adverse effects of these compounds, particularly in the development of *in vitro* assays.
The high number of chemicals to be assessed within a few years (30.000 substances marketed at volumes greater than 1 ton/year) creates a paramount need for specific, functional and high-throughput *in vitro* screening systems. Although some promising assays have been developed for the interference with the TH transport, to our knowledge, there are no fast and specific tests enabling the detection of compounds directly acting on the thyroid hormone receptor (TR).

In this study, a stable reporter gene assay was developed using the TH-responsive rat pituitary GH3 cell line, that constitutively expresses both TR isoforms. Stable transfection of the pGL4CP-SV40-2xtaDR4 construct into the GH3 cells resulted in a highly sensitive cell line (GH3.TRE-Luc), which was further optimized into an assay that allowed the detection of triiodothyronine (T3) and thyroxine (T4) concentrations in the picomolar range already after 24 hrs of exposure. The 22.5-fold induction of T3 is illustrative of the high responsiveness. This assay also enabled the quantification of the agonistic effect of the pharmaceuticals triiodothyroacetic acid (Triac) and tetraiodothyroacetic acid (Tetrac), hydroxy polyhalogenated diphenylethers (OH-PBDEs), hydroxy polychlorinated biphenyls (OH-PCBs) and the antagonistic action of sodium arsenite (NaAsO2).

The greater specificity compared to proliferation assays proved to be valuable for the evaluation of the contradicting reports found in the literature about the putative TR-mediated mechanism of action of amiodarone, bisphenol A (BPA) and its halogenated derivatives (TCBPA and TBBPA).

The specificity, high reproducibility, small standard deviation and magnitude of responses confirm this assay as a valuable new high-throughput tool for *in vitro* assessment of potential TR-specific modulators and mixtures.

TEMPORAL CHANGES OF SPECIFIC ACTIVITIES OF POLLUTANT MIXTURES IN RIVER COMPARTMENTS – CONSEQUENCES FOR RISK ASSESSMENT

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Risk assessment is complicated by the presence of very complex pollutant mixtures in all environmental matrices. Specific problem for risk assessment represents the contamination of river ecosystems, which are by nature rather dynamic, especially when we are concerned with contamination of water and surface sediments from existing primary and secondary sources. Presented study targets the contamination of rivers Morava and Drevnice in south-eastern part of the Czech Republic. This industrial and agricultural area presents a suitable model ecosystem for study of pollutant accumulation, migration and distribution. The results of a year long monthly study characterize the temporal and spatial dynamics of contamination by compounds with specific modes of action of both sediments and water samples. Screening bioassays represent suitable tools which enable to integrate the overall potency of the mixtures of compounds present in the environmental samples for eliciting toxicity through important modes of action. Battery of *in vitro* bioassays has been used for assessment of AhR-mediated (dioxin-like) activity, anti/estrogenicity and anti/androgenicity of sediments and water samples. Our results have revealed relatively low dioxin-like, estrogenic and antiandrogenic activities in aqueous phase, while significant activities were associated with sediments. The composition of contaminant mixtures present in water and surface sediments exerts strong fluctuations in both space and time reflecting the dynamic character of the river contamination that needs to be taken into consideration in risk assessment of the contaminated river environments. This research was funded by projects ENVISCREEN - NPVII 2B08036 and INCHEMBIOL 0021622412.

PASSIVE SAMPLING AND *IN VITRO* BIOASSAYS AS TOOLS OF ASSESSMENT OF ENVIRONMENTAL IMPACT OF SEWAGE TREATMENT PLANTS SITUATED ON SMALL STREAMS

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Sewage treatment plants (STPs) have been widely discussed as the potential sources of complex mixtures of pollutant residues. The composition and levels of hazardous chemicals depend on the removal efficiency of the waste water treatment technologies. The presented study is concerned with the impact of municipal STPs on smaller rivers in the Czech Republic. Surface waters have been sampled by two types (pesticide and pharmaceutical versions) of Polar Organic Integrative Samplers (POCIS) upstream and downstream of 7 municipal sewage treatment plants situated on upper parts of streams in the Czech Republic. The extracts from passive samplers were tested by a battery of in vitro bioassays to determine (i) overall non-specific cytotoxicity, (ii) endocrine-disruptive, ED, potential (estrogenicity, androgenicity)and (iii) dioxin-like toxicity of the river waters downstream and upstream of STPs. The results document that all monitored STPs negatively affected water quality. The in vitro detection systems revealed significant presence of estrogenic compounds in the samples and showed increase of their concentrations in all downstream samples compared to the upstream ones. The bioassays provided important information not only about the presence of compounds with specific modes of action but provided also a comparison of ED potential of mixtures of compounds captured by different POCIS samplers (pesticide vs. pharmaceutical variants). This study was supported by the grants ENVISCREEN (No. 2B08036) and MSM 6007665809 of the Czech Ministry of Education and by the SP/2e7/229/07 grant from the Ministry of the Environment of the Czech Republic.

REMOVAL OF PHARMACEUTICALS DURING WASTEWATER TREATMENT AND ENVIRONMENTAL RISK ASSESSMENT USING HAZARD INDEXES

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In a long term study, which covered 4 sampling periods over three years, a total number of 84 samples, specifically 28 influent, effluent, from seven WWTP located in the main cities along the Ebro river Basin (North East of Spain), as well as receiving river waters, were analyzed to assess the occurrence of 73 pharmaceuticals covering several medicinal classes. Results indicated that pharmaceuticals are widespread pollutants in the aquatic environmental. Calculation of removal rates and compound half-lives, assuming that compound degradation followed first-order kinetics, suggested that conventional wastewater treatments applied at the seven WWTP were unable to completely remove most of the pharmaceuticals under study. The evaluation of compound degradability, in terms of half-lives, is an important task to discuss integrated solutions for mitigation of pollutants entry into the water cycle.
The wide spectrum of substances detected in receiving river waters indicates that WWTP outlets are major contributors of pharmaceuticals in the aquatic environment. However, municipal wastewater treatment represents an obligatory and final treatment step prior to their release into the aquatic media, since load of pharmaceuticals in outlets were considerably reduced after treatment.
Finally, hazard posed by pharmaceuticals in both surface and effluent wastewaters was assessed by calculating hazard indexes. According to EMEA guidelines, this quotient was calculated as the ratio between Measured Environmental Concentrations (MEC) and Predicted No Effect Concentrations (PNEC). The use of Measured Environmental Concentrations (MEC) instead of the estimated ones (PEC) by models has been widely used to evaluate risks posed by pharmaceuticals in a specific site. On the other hand, PNEC were extrapolated by dividing EC_{50} values from the literature (standard toxicity values) by an assessment factor of 1000.
If this ratio (MEC/PNEC) is higher or equal to one, suggests that this particular substance could cause potential adverse effects. In this context, risks towards algae, daphnids and fish, were evaluated in both river and effluent wastewaters, according to the water quality criteria fixed by the Water Framework Directive, which precludes the convenience of assessment using taxa of three different trophic levels of the ecosystem. The overall relative order of susceptibility was estimated to be algae>daphnia>fish. Results indicate that no significant risks could be associated to the presence of pharmaceuticals in those matrices, indicating that reduction of compound concentration after wastewater treatment as well as dilution factor once pharmaceuticals are discharged in receiving river water efficiently mitigate possible environmental hazards.

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MIXTURE OF NATURAL AND CHEMICAL COMPOUNDS: EFFECTS ON DETOXIFICATION AND OXIDATIVE STRESS PARAMETERS IN BIRD *COTURNIX COTURNIX JAPONICA*

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Combined exposure to both anthropogenic and natural stressors presents a common problem in the polluted environment, where the wild-living organisms have to face multiple stressors. The defense system of organisms can be attenuated and more susceptible to other stressors when interacting with single xenobiotics and moreover one chemical may modify the effect of the other by altering its kinetics and/or dynamics. Water contamination with cyanobacteria is becoming a serious animal health problem in many parts of the world since secondary metabolites of cyanobacteria have been shown to cause adverse effects in various organisms including birds. Also heavy metals contamination constitutes serious environmental problem because of their increasing concentrations and bioavailability by biota over the last decades. Among many negative effects of cyanobacteria and heavy metals in the environment, there is at least one shared mechanism of action – their ability to increase the generation of reactive oxygen species (ROS). Formation of ROS and oxidative stress is associated with the development of many pathological states and damages including immuno-pathological damage. The aim of this study was to estimate the influence of cyanobacterial biomass, lead and immunological challenge in single and combined exposure schemes on biochemical markers of oxidative stress and detoxification in Japanese quail (*Coturnix coturnix japonica*) in sub-chronic study. Significant differences between exposure variants were observed. Responses of biochemical parameters (glutathion, glutathion-S-transferase, reductase, peroxidase; lipid peroxidation) were more pronounced in the co-exposure groups of quails than in the individual exposures. The generation of oxidative stress combined with insufficiency of defensive mechanisms could result in effects on the health status, especially if multiple stressors are involved, which is often the case in the environment. The research has been supported by the Grant Agency of the Czech Academy of Sciences (Grant No. AV0Z60050516) and by the Czech Ministry of Education - projects INCHEMBIOL (MSM0021622412) and ENVISCREEN (No. 2B08036).

RETROSPECTIVE MONITORING OF METHYLTRICLOSAN IN FRESHWATER FISH COVERING THE PERIOD 1992 - 2008

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Methyltriclosan (MTCS) is a transformation product of the biocide triclosan (TCS) which is commonly used e.g. in personal care products and textiles. Via waste water TCS reaches freshwaters since its degradation in sewage treatment plants (STP) is not complete. Moreover, a fraction of TCS is transformed to MTCS during the STP process. To study levels of the lipophilic MTCS in aquatic biota, muscle of fish (bream, *Abramis brama*) archived by the German Environmental Specimen Bank were investigated. Standardized annual homogenate samples were analysed by GC/MS directly (MTCS) or after derivatisation (TCS). Fish originated from 17 different German freshwater sites including the rivers Elbe, Mulde, Saale, Rhine, Saar and Danube. The period covered for MTCS was 1992 - 2008. Since TCS levels were low it was only analysed for the period 1992 - 2003 and 2008 (maximum 69 ng/g TCS in Saar fish in 1998; lipid-based data). TCS and MTCS could not be detected in fish from a reference site (Lake Belau, Northern Germany). However, especially in fish samples from rivers influenced by STP effluents high MTCS were detected (e.g., in Saar bream up to 580 ng/g in 2005). For most sampling sites MTCS concentrations were highest in the period 2002 - 2005. Most time series revealed statistically significant increasing trends of MTCS over a decade until about 2003. However, afterwards levels stayed constant or even decreased at nearly all sites. It is assumed that fish body burdens of MTCS are linked to consumption patterns of TCS. Therefore, the decrease of MTCS is probably a result of a voluntary renunciation of the use of TCS in washing and cleaning agents by the member companies of the German Cosmetic, Toiletry, Perfumery and Detergent Association (IKW) as announced in 2001.

Keywords: environmental specimen bank, triclosan, bream, personal care products

MOVEMENT, ACCUMULATION AND REMOVAL OF A MIXTURE OF POLLUTANTS ORIGINATING FROM URBAN RUNOFF IN SMALL WATERSHEDS IN GERMANY AND LUXEMBOURG

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Urban runoff is one of the most globally-relevant examples of complex chemical mixtures, and its impact on water bodies worldwide is significant. Although the exact composition may differ in time, space and quantity, the general composition of urban runoff world-wide can be guaranteed to contain, among others, heavy metals, PAHs, pesticides, sulfate and nitrate. These compounds generally accumulate on roads, roof tops, and other surfaces until they are transported in runoff during rain events to storm water systems, retention areas, and water bodies. In this way the first flush of urban runoff acts as a large dose of a chemical mixture. During precipitation events, sewage overflows (may) lead to enhanced concentrations of (household contaminants) and pharmaceutically active compounds in receiving waters.

Heterogeneous catchments of the German Olewiger Bach and the Luxembourgish Pétrusse and Mess were selected as diverse small water study sites representative of Urban catchments. This poster focuses in the experimental area of the Olewiger Bach, which flows in its lower part through the suburbs and the City of Trier and drains into the Moselle. A constructed wetland, which is a small part of the Olewiger Bach catchment, is a semi-central rainwater retention system in a small urban environment. The receiving stream is heavily influenced by the outflow of the retention basin regarding to water volume und contaminant load. Calculation of pollution input and output will give further information about mixing processes and contaminant behaviour in retention areas.

The objective of this research is to evaluate the mixing processes (and thereby the sources within the catchment), accumulation, movement and/or removal of a group of organic and inorganic pollutants. The chemicals to be analyzed were selected as being typical for urban runoff worldwide, eco-toxicologically-relevant, and a few chosen with reference to local usage patterns. A number of metabolites are also to be examined.

The results will be evaluated by comparing them with further small water study sites, the Luxembourgish Pétrusse and Mess.