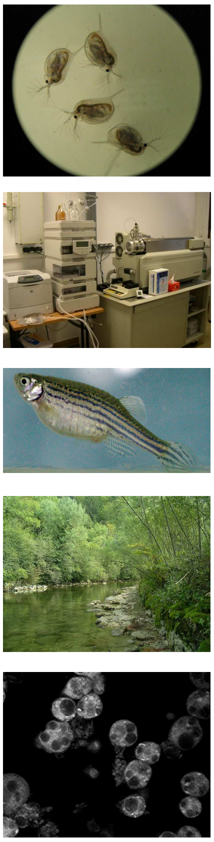
4th NORMAN workshop



“Integrated chemical and bio-monitoring strategies for risk assessment of emerging substances”

Organised by
FP6 NORMAN project
CEMAGREF – Agricultural and
Environmental Engineering
Research Institute
Lyon - FRANCE

17-18 March 2008

Trace Element –
Institute for UNESCO

LYON, FRANCE

ABSTRACT BOOK

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Cemagref

norman Network of reference laboratories for monitoring of emerging environmental pollutants

CLUSTERS DE RECHERCHE RHÔNE-ALPES ENVIRONNEMENT

Sixth Framework Programme

AQUAREF

Objectives of the workshop

The workshop will explore chemical and biological effects-driven strategies for risk assessment of emerging pollutants and the potential for their integration under legislative frameworks.

Within the Water Framework Directive, for example, the assessment of the aquatic ecosystem is based both on biological monitoring of ecosystems at the community level and on chemical monitoring of priority and river basin-specific substances. Nevertheless, the link between the two approaches is not straightforward.

Toxic effects in the field can be caused by complex mixtures of known and unknown pollutants. Thus, beside a chemicals-driven strategy for the ecological risk assessment of the pollutants in field, it is necessary to apply and explore new strategies, combining both biological responses and chemical analysis, to identify toxic spots, characterise chemicals likely to cause adverse biological effects and, finally, assess the ecological risk of the identified chemicals at relevant spatial scales

Specific topics

The aim of the workshop is to provide an overview of, and a basis for discussion on:

- existing protocols and experiences from the application of strategies integrating chemical and biological methods, under different policy frameworks
- the way in which these strategies should be used to complement the chemical-driven risk assessment approaches
- the advantages and the still existing limitations of these strategies (and associated research needs) from the point of view of the policy managers
- -proposals for concrete actions for harmonisation and standardisation of integrated chemical and bio-monitoring strategies for large-scale monitoring of ecological risk assessment of chemicals.

Scientific committee

J.Garric	CEMAGREF - Agricultural and Environmental Engineering Research Institute (FR)
M.Coquery	CEMAGREF - Agricultural and Environmental Engineering Research Institute (FR)
R.Tutundjian	CEMAGREF - Agricultural and Environmental Engineering Research Institute (FR)
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J.M. Porcher	INERIS - National Institute for Industrial Environment and Risks (FR)
P.Leonards	IVM - Institute for Environmental Studies, VU University Amsterdam (NL)
R. Owen	EA - Environment Agency (UK)
H. Budzinski	CNRS - National Centre for Scientific Research (FR)

Monday, March 17th

9:00 – Welcome breakfast & Registration (1st Floor)

10:00 – Introduction (Ground level)

Session I : Prioritisation of emerging substances for field monitoring and risk assessment

Chaired by: Valeria DULIO (INERIS-France) and Marina COQUERY (CEMAGREF-France)

10:15	Keynote speaker: Eric VINDIMIAN (Ministry of Ecology and Sustainable Development - France)	*Achievement of a good chemical and ecological status: existing challenges and a way forward to improve the risk assessment strategies*
10:45	Jean-PhillipeP BESSE (CEMAGREF – France)	*Implementation of a prioritization method for selecting human pharmaceuticals to survey in surface waters in France*

11:15 Coffee break (1st Floor)

11:30	Jaako MANNIO (SYKE – Finland)	*Screening of consumer and industrial chemicals and pesticides as priority substances in Finnish aquatic environments*
12:00	Keynote speaker: Alistair BOXALL (University of York – UK)	*Emerging and future environmental contaminants: What should we be testing and monitoring?*

12:30 Lunch & poster session (1st Floor)

Session II : Effects–driven approaches for field monitoring and risk assessment of emerging pollutants

Chaired by: Werner BRACK (UFZ – Germany) and Pim LEONARDS (IVM-The Netherlands)

14:30	Keynote speaker: Werner BRACK (UFZ – Germany)	*Toxicity identification in contaminated sediments*
15:00	Keynote speaker: Kevin THOMAS (NIVA – Norway)	*Effects-directed identification of potential emerging substances in effluents and surfacewaters*
15:30	Gregory LEMKINE (Watchfrog S.A.S – France)	*Adaptation of measurements on small model organisms to semi-robotised readouts*

16:00 Coffee break (1st Floor)

16:30	Keynote speaker: Concha MARTINEZ-GOMEZ (Spanish Institute of Oceanography – Spain)	*Biomonitoring strategy of chemical pollution along the Iberian Mediterranean Coast: Development of the MEDPOL approach*
17:00	Keynote speaker: Lars-Otto REIERSEN (AMAP – Norway)	*Arctic as the Sentinel for Environmental Processes and effects; Results from the Arctic Monitoring and Assessment Programme (AMAP)*
17:30	Heinz RUDEL (Fraunhofer IME – Germany)	*The German Environmental Specimen Bank Program as a Tool for the Retrospective Monitoring and Assessment of Emerging Chemicals*

18:00 End of first day

20:00 Riverboat Dinner to Barbe Island

Tuesday, March 18th

Session III : Integrated approaches within risk assessment strategies for monitoring risk of emerging pollutants at local and large scales

Chaired by (morning): Jeanne Garric (CEMAGREF-France) and Eric VINDIMIAN (Ministry of Ecology and Sustainable Development - France)
chaired by (afternoon): Dick VETHAAK (Deltares – The Netherlands) and Bo JACOBSEN (Avedoere Wastewater Services – Denmark)

8:30	Keynote speaker: Ketil HYLLAND (NIVA – Norway)	*Integrated chemical and biological monitoring in marine ecosystems – OSPAR approach*
9:00	Keynote speaker: Dick VETHAAK (Deltares – The Netherlands)	*Field monitoring and effect assessment of emerging substances in the marine environment: integrated approaches and future challenges*
9:30	Keynote speaker: Etienne VERMEIRSSEN (EAWAG – Switzerland)	*Passive sampling and combined biological and chemical analysis of estrogens and photosynthesis inhibitors*

10:00 coffee break (1st Floor)

10:30	Bo JACOBSEN (Avedoere Wastewater Services – Denmark)	*Combined chemical analyses and biomonitoring at Avedoere WWTP*
11:00	Selim AIT-AISSA (INERIS – France)	*Multiple endocrine disrupting activities in French river sediments as assessed by the combined use of in vitro bioassays and chemical analyses*
11:30	Shane SNYDER (Southern Nevada Water Authority – USA)	*Risk Assessment of Pharmaceuticals in US Drinking Waters*

12:00 Lunch and poster session (1st Floor)

14:00	Thierry BAUSSANT (IRIS – Norway)	*Monitoring methodologies for accidental spill of hazardous and noxious substances at sea: An integrated approach*
14:30	Steiner SANNI (IRIS – Norway)	*Biomarker response distributions as tool to validate environmental risk of hydrocarbon discharges and to monitor early effects of emerging pollutants in artic species*
15:00	Peter von der OHE (UFZ – Germany)	*The use of the SPEAR and toxic units indicators to link ecological status to chemical pollution*

15:30 coffee break (1st floor)

16:00 Discussion session and concluding remarks (Ground level)

17:30 End of the workshop

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<u>SESSION I</u>

Prioritisation of emerging substances for field monitoring and risk assessment

ACHIEVEMENT OF A GOOD CHEMICAL AND ECOLOGICAL STATUS : EXISTING CHALLENGES AND A WAY FORWARD TO IMPROVE THE RISK ASSESSMENT STRATEGIES

Eric VINDIMIAN

Head of research department - French Ministry for ecology, sustainable development and sustainable spatial planning

The existence of several regulations on chemicals in waters leads to a paradox.

One could see a great coherence in such a set of regulations. Those concerning chemicals and pesticides are based on ecotoxicological risk assessment strategies that avoid improper use of toxic substances in a way that could impair water bodies. On the other hand the water framework directive pushes a strong pressure on water quality on the basis of a list of priority substances using water quality standards derived from ecotoxicity tests. And, last but not least, the overall ecological quality should be the overall chapeau that assures that no anthropogenic activity creates any detectable impairment within water bodies, such an impairment being from habitat disturbance, exploitation of resources or chemical pollution.

The main question for a scientist is the following : is there a solution to this system of equations? In other terms : what happens if a chemical is authorized when its concentration in waters overpass quality standards? How can public authorities regulate such authorized substances? Can ecological status be good in such a situation? Alternatively, is toxic pollution detected at the community level using standard methods for ecological assessment? How joint effects of several chemicals, joint effects of chemicals and nutrients and joint effects of chemicals and habitat disturbance are assessed? Are there any risk assessment and risk management strategies that would solve such problems?

Ecotoxicological hazard assessment and ecological risk assessment are not exact sciences. They both use a lot of approximation strategies to be able to deliver results without understanding all the mechanisms behind the fate and effects of chemicals. For instance, the fate of a chemical is based on a generic environment that has nothing to compare with local reality. Another example is the fact that the toxicological profile of a chemical is based on a very simplified series of tests and large safety factors. Chemicals are not isolated substances, they interact between themselves or at the organism level, they also interact with complex water sediment interfaces leading to unexpected hazards.

The only virtuous cycle that can be used is to continuously move from prediction to monitoring and then to the revision of decisions based on field evidence. This implies that the right monitoring strategies are being implemented by member states on a sufficient set of substances. The loop between monitoring and regulation of substances must also be continuously closed, which is not the case for the moment.

Scientist can play a great role in such an improvement. They develop accurate cost-effective analytical tools able to deliver real time knowledge of the concentration of chemicals in the environment. They combine Earth observation systems to provide local data at the right scale in order to refine fate assessment. They develop biomarkers that integrate responses of several bioavailable chemicals with a common mechanism of action. They investigate relationships between biological traits and the presence of toxicants to be able to diagnose causes of ecological disturbances. They try to assess effects of mixture of substances. They develop economical tools that shift regulations from go-no go strategies to incentives for continuous improvement.

Policy makers might benefit from such progress in science under two conditions. First, they should be involved in the management of research programmes designed to support policy making and second, they should set-up multidisciplinary programmes aiming tot develop converging strategies for different disciplines.

For the moment, is seems that such strategies are not really at work. Hopefully, Norman could help to achieve such a goal.

IMPLEMENTATION OF A PRIORITIZATION METHOD FOR SELECTING HUMAN PHARMACEUTICALS TO SURVEY IN SURFACE WATERS IN FRANCE

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In the framework of the French National Environmental Health Plan, the river basin authorities have to implement a comprehensive survey of several organic compounds, including pharmaceuticals, in surface waters. Before implementing such a large survey, a list of priority molecules, based on their risk on the aquatic environment, must be established. Using recent data on the most consumed pharmaceuticals in France and based on the EMEA (2006) model used for human pharmaceuticals (ERA), we proposed a list of the priority pharmaceuticals to survey. In this aim, we reviewed and combined physico-chemical properties, fate in wastewater treatment plants (WWTP), pharmacological and ecotoxicological data available from the literature, and implemented a selection methodology. This prioritization approach allowed us to propose a sound selection of pharmaceuticals and metabolites to survey in WWTP effluent and in the aquatic ecosystem, which will be discussed. We also evaluated our results for specific pharmaceuticals using data available from literature on removal efficiency of WWTP for biological treatment and on occurrence in effluents and surface waters.

Keywords : pharmaceuticals, prioritization, aquatic ecosystems, risk evaluation

SCREENING OF PRIORITY SUBSTANCES IN FINLAND

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A nationwide screening project was designed to enhance the knowledge on the distribution and concentrations of the priority substances and other pollutants defined by the EU Water Framework Directive: chemicals from consumer and industrial use (project VESKA 1) and pesticides in agricultural use (project VESKA 2).

A survey extending to twelve densely populated and industrialized areas, concentrations were measured in sludge and waste water samples and surface water, sediment and fish samples. Groups of substances of importance include PAH-compounds, alkylphenols, phthalates, volatile organic compounds, brominated flame retardants (PBDE) and organic tin compounds. Sampling matrix for each substance group was chosen according to the compound properties (water solubility, affinity for adsorption and bioaccumulation) and the source of emission.
Organotin compounds were found in all matrices, often exceeding EQS or "benchmark" values, which will trigger more research, monitoring and management activities. Only few industrial chemicals (mainly nonylphenol ethoxylates) were found in surface waters at the EQS-level. For some substances it is difficult to assess the need for monitoring (PAH, phthalates, chlorobenzenes). It seems that natural conditions in Finland (population/water volume, lake-richness) would favour sediment and biota over water, in monitoring industrial and household chemicals.

A pesticide survey in 2005 covered 40 streams situated mainly in southern and western coast. Sites were chosen from watershed register using agricultural land-use percent as the main criterion: most sites from agricultural areas while 5 reference sites from non-agricultural areas. Multi-residue analyzing methods gave information of the concentrations of over 100 substances in water samples. In addition, 14 substances were analyzed from river sediment samples, taken from the same sites. The median of the number of detected pesticides per water sample was 3 in reference sites and 6 in agricultural sites (only summer-samples included). Phenoxy herbicides were most frequently detected compounds. Although the number of detected pesticides was rather high, the detected concentrations were mainly low compared to available reference values (EQS or Nordic target / limit values). Only traces of WFD priority substances were found in water samples. Nevertheless, notable concentrations of atrazine, prochloraz (a fungicide in the national list of harmful chemicals), endosulphan and its metabolite were found in sediment samples.

Simultaneous screening of several substance groups is suitable for pesticides but not for industrial and household chemicals with several, poorly known sources. By comparing the results with the Environmental Quality Standards (EQS), a decision-making model will be developed to evaluate the need for future monitoring. The measured environmental concentrations of harmful substances are needed in the risk assessment carried out in the EU for consumer and industrial chemicals. Information gained can also be utilized in national risk assessment work and emission source identification.

'EMERGING AND FUTURE ENVIRONMENTAL CONTAMINANTS: WHAT SHOULD WE BE TESTING AND MONITORING?'

Alistair B A Boxall

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In recent years there has been increasing concern over the so called 'emerging contaminants' such as degradates (formed in the environment and treatment processes), human pharmaceuticals, veterinary medicines, nanomaterials and personal care products. These substances have been shown to be released to the environment and in the monitoring studies that have looked for them, they have been detected in surface waters, groundwaters and drinking waters.

While limited information is available on the potential impacts of many of these substances, there is some evidence that selected substances may adversely affect human and environmental health. For example, many degradates of pesticides are potentially more toxic than the parent compound, selected adjuvants are suspected endocrine disruptors and there is concern that long-term exposure to antibacterial pharmaceuticals may be contributing to the development of resistant bacteria.

It would therefore be prudent to 1) develop a better understanding of which emerging contaminants are likely to contaminate the environment, 2) determine what are the potential risks of these to environmental and human health; 3) develop analytical methodologies for substances identified as high risk; and 4) expand current monitoring programmes to quantify those emerging contaminants that are likely to pose the greatest risk to environmental and human health.

This presentation will provide an overview of a range of approaches for identifying those emerging contaminants of most concern. Information sources that could be used to identify the inputs of emerging into environmental systems will be described. The application of predictive approaches (e.g. read across and i*n silico*) for estimating physico-chemical properties, environmental parameters, ecotoxicity and mammalian toxicity will be described. Prioritisation frameworks, that draws on this information and that can be applied to identify those compounds that pose the greatest risk will then be illustrated for veterinary medicines, engineered nanoparticles and pesticide transformation products. Finally, the implications of climate change on the chemical risks in the future will be described.

<u>SESSION II</u>

Effects–driven approaches for field monitoring and risk assessment of emerging pollutants

TOXICITY IDENTIFICATION IN CONTAMINATED SEDIMENTS

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Introduction

Sediments are valuable ecosystems with high biodiversity providing numerous very important goods and services. These include for example the decomposition of litter and waste, nutrient cycling, carbon storage and the provision of food and clean drinking water. Biodiversity as well as these goods and services are at risk due to many reasons among them the contamination with toxicants. It could be clearly shown that e.g. pesticides in aquatic ecosystems do not only affect sensitive species but also ecosystems services like leaf litter degradation (*1*);(*2*).

Sediments are often contaminated with a huge number of chemicals. Thus, *a priori* selected target compounds such as priority pollutants in general do only cover a tiny portion of possible but also of actually present contaminants. While more than 16 million compounds are known only 33 of them are part of the priority pollutant list according to the EU Water Framework Directive list that have to be monitored in European surface waters to define the chemical status. It is a general observation that measurable effects or ecological degradation in most cases cannot be explained by these priority pollutants. This situation is often hardly improved if other pre-selected target compounds are added.

Thus, in order to make sure that chemical analysis is actually relevant for effects of contaminated sediments it has to be directed to those compounds that cause the effects. This may be done with effect-directed analysis that wants to break down complex mixtures such as sediments and extracts thereof to individual toxicants that can be made responsible to cause effects.

EDA Approach

Effect-directed analysis is a stepwise approach comparable to Toxicity Identification Evaluation (TIE) (*3*);(*4*);(*5*). Three steps may be discriminated: Toxicity characterization, toxicity identification and toxicity confirmation. Toxicity characterization focuses on the assignment of toxicity to distinct groups of chemicals such as metals, organic toxicants and ammonia. While procedures for water samples are quite straight forward, for sediments the selective removal of a group of toxicants prior to biotesting is much more challenging. One example based on a sediment contact test with embryos of *Danio rerio* together with the application of selective sorbents will be presented.

Toxicity identification (Fig. 1) is based on the combination of biotesting, fractionation and chemical analysis that sequentially reduces the complexity of a mixture by removing non toxic compounds (*6*) till only few compounds are left in the mixture that may be subjected to chemical analysis.

Toxicity confirmation may be seen as a tiered approach to 1) make sure that the suggested chemical structures actually fit the real compounds, 2) confirm and quantify the contribution of identified toxicants to total effects and 3) confirm the relevance for hazards and risks on higher levels of biological organization.

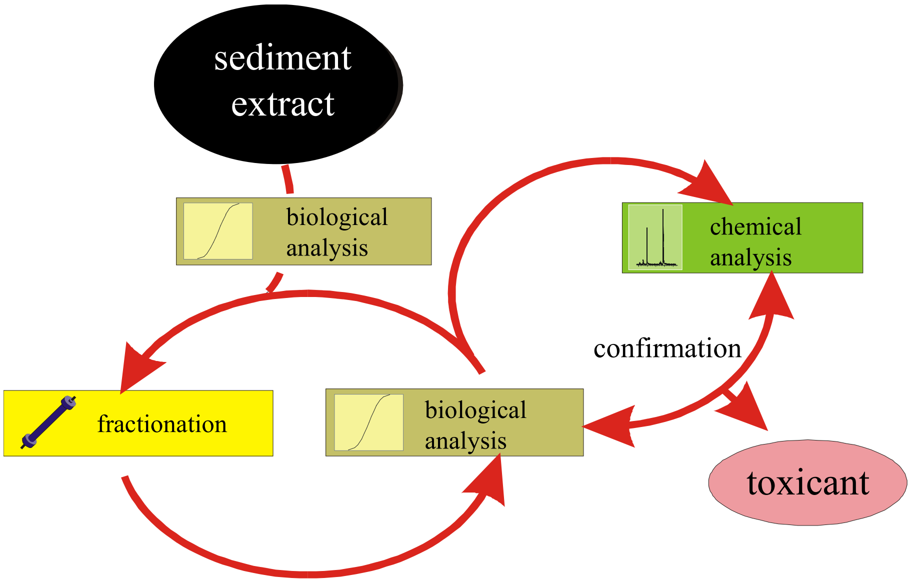

Fig. 1: Effect-directed analysis

Example Bitterfeld

At the example of contaminated sediments from the industrial area of Bitterfeld, Germany, an extensive EDA study was run using a broad array of toxicological endpoints together with multi-step fractionation procedure. Bitterfeld is a site of chemical industry since 1894. Starting with chlor-alkali electrolysis within the more than 100 year history thousands of compounds have been produced in Bitterfeld including pesticides, dyes, polymers, metals and others. Careless handling of wastes and by-products resulted in highly contaminated groundwaters, soils and sediments impacting on the River Elbe. Key toxicants identified in Bitterfeld sediments include N-phenyl-2-naphthylamine and prometryne as major toxicants to green algae (*7*), the insecticide methyl parathion affecting invertebrates, organotin compounds affecting bacteria, algae and daphnids and PCDFs and O- and S-heterocyclic polyaromatic compounds dominating the strong AhR-mediated effects (*8*);(*9*).

Evaluation of EDA procedure

As shown for Bitterfeld sediments EDA actually proved its strength allowing the isolation and identification of individual toxicants even in very complex mixtures and for many toxicological endpoints. No advance information on target analytes is required while EDA directs analysis to hazardous compounds non-regarding priority and monitoring lists.

The procedure itself however still faces problems and challenges that need to be addressed in order to make EDA more efficient and successful. Approaches to simulate realistic exposure conditions and to address bioavailability are required. Automated multidimensional time- and cost-efficient fractionation procedures would help to reduce costs and risks of artefacts due to extensive sample manipulation. Structure elucidation if no standard compounds are available as well as the identification of non-volatile compounds that are not analyzable with GC-MS are still very complex, laborious and often not successful. Confirmation of structures, toxicity and hazards is still a challenge (*10*). New approaches to meet these challenges are in development and will be briefly presented.

Bioavailability

We assume that the bioavailable mixture rather than the extractable mixture is relevant for hazards and risks. The composition of both may be quite different. However, since bioavailability is rather a general concept than a measurable process or property, for EDA we need an operationalisation. Two approaches are tested at present: 1) bioaccessibility-directed extraction procedures based on desorption kinetics (*11*) and 2) partition-based dosing techniques.

The use of "mild" extraction techniques such as e.g. TENAX extraction indicates desorption of lipophilic compounds from sediment particles in three phases with very different kinetics. While a rapidly desorbing pool that is regarded as bioaccessible desorbs in hours slowly and very slowly desorbing pools are released in a time frame of weeks to centuries and thus with kinetics that are not relevant for partitioning into mobile and short-lived benthic organisms.

Partition-based dosing techniques try to simulate partitioning processes of lipophilic compounds between sediment, pore water and organisms. Extracts or fractions are loaded on a solid phase such as PDMS. After introduction of the loaded solid phase into the test medium, concentrations in the water phase are established according to the partition coefficient of the compounds similar to partitioning processes in aquatic sediments.

Both concepts focus on different aspects of bioavailability (*12*) and may be combined.

Fractionation

Fractionation is an important key to successful toxicant identification. Optimized fractionation procedures provide a predictable separation with high resolution (low overlap of fractions), low risks of artefacts and a high degree of automation. For complex mixtures such as sediments often multi-dimensional separations according to different properties are required. In this paper, two recent developments will be presented: 1) an automated multi-step NP-HPLC procedure (*13*) (Fig. 2) and preparative capillary gas chromatography that adds volatility as a new dimension to fractionation procedures (*14*).

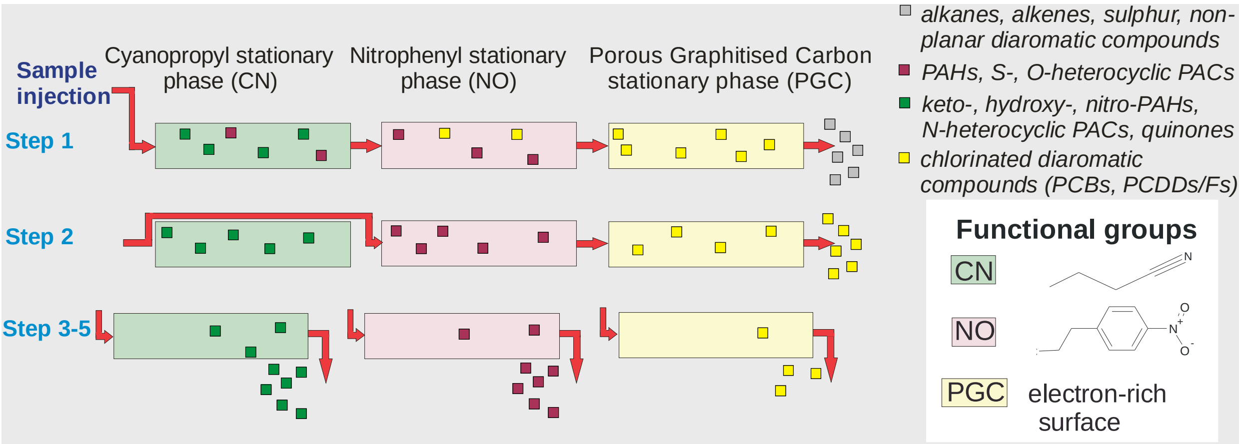

Fig. 2: Automated multistep NP-HPLC procedure

Structure elucidation

Compound identification based on GC-MS analysis together with mass spectra library search is a time- and cost-efficient and generally accepted approach. However, if no neat standards are available this approach leads to only tentatively identified compounds. One reason for that is the limited number of compounds available in spectral libraries such as NIST. This may be illustrated by an example. NIST lists 376 mass spectra for a molecular weight of 150, the Beilstein database lists 5,300 while structure generation results in 615,977,591 possible structures. That means that there are numerous components for which you do not find an appropriate suggestion in the library and even if you got a good match this is no guarantee that you have correctly identified your compound. An alternative approach presented here is based on the generation of all possible structures fitting to the molecular weight using software such as MOLGEN (*15*) together with stepwise reduction of the number of candidate structures using classifiers from fragmentation, chromatographic retention data and other spectra to end up with a (hopefully short) final list of candidate compounds. Ultimate confirmation can be based on these candidates that need to be synthesized and tested.

Confirmation

The different steps towards the confirmation of a compound as a relevant toxicant have been recently reviewed (*10*). In this paper only the question of hazard confirmation will be discussed. It focuses on the question whether compounds identified by EDA as key toxicants actually pose a hazard to *in situ* communities. The approach that will be presented here is based on pollution induced community tolerance (PICT) (*16*). This approach helped to confirm hazards of prometryne to biofilm communities in sediments from the area of Bitterfeld (*17*). Prometryne has been identified in a previous EDA study as a key toxicant to green algae in extracts of these sediments (*7*).

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EFFECTS-DIRECTED IDENTIFICATION OF EMERGING CONTAMINANTS IN AQUATIC AND OTHER SYSTEMS

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Identifying new environmental pollutants is one of the greatest challenges that face environmental scientists (1). Pollutants co-exist as complex environmental mixtures along with natural compounds, some of which are toxic and some of which are benign. Identifying which substances have the potential to cause harm is therefore a major and important challenge. Emerging environmental contaminants have been traditionally identified through assessing the volumes of chemicals used and calculating the volumes potentially discharged to the environment. This source specific approach uses instrumental chemical analysis to target the specific compounds of interest (i.e. perfluorinated substances, brominated flame retardants, pharmaceuticals) and record their occurrence in the environment. However, this is not the only available approach for identifying emerging substances and there is an alternative complementary approach that integrates both bio-analysis with instrumental chemical analysis; effects-directed chemical analysis (EDA). In order to truly protect the environment both approaches need to be used in a complementary manner. Targeted chemical analysis is the most appropriate approach when it is known which compounds should be looked for. However a source-based approach does not account for the substances that we know little or nothing about (i.e. metabolites, natural products, low volume/high toxicity or those substances that are known to us but may exert other previously unknown effects). Clear examples of such substances that are relevant to Scandinavia are naturally occurring brominated dibenzo-*p*-dioxins (2) and petrogenic naphthenic acids (3).

EDA combines effects based techniques (bioassays) with chemical analysis to identify the compounds present in the environment that are responsible for specific effects. This is achieved through isolating environmental samples with known ecotoxicological effects and using the response from mechanism specific assays to direct the isolation of the compounds responsible for the measured effects and then analyse them using advanced modern analytical chemistry. This presentation will provide examples of how EDA has been successfully used to identify the cause of endocrine disruption in estuaries (4,5), as were the occurrence of alkylphenols and naphthenic acids as endocrine disrupters and other toxicants in North Sea offshore produced water discharges (3,6). NIVA is currently involved in a number of key national and international projects which will provide important data on the types of emerging contaminants that contaminate our environment (i.e. EU funded Modelkey [www.modelkey.org] and NFR funded Eceda). As well as demonstrating the effectiveness of the EDA approach and its role in emerging contaminant research the presentation will also discuss current perspectives and future recommendations on how EDA can be incorporated to complement existing approaches to informing risk assessments.

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ADAPTATION OF MEASUREMENTS ON SMALL MODEL ORGANISMS TO SEMI-ROBOTISED READOUTS

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Any attempt to further our understanding of how environmental stressors impinge on environmental health must involve increasing our knowledge of the physiological effects of synthetic chemicals. However, it is almost impossible to make causal links between contaminant exposure and major patho-physiological damage.

In collaboration with Veolia research teams, we are addressing the problem of identifying endocrine chemicals in mixtures and complex liquid matrices as a function of the OECD criteria of endocrine disruption of the thyroid and estrogenic axis.

Small models organisms derived from fish and amphibians exploit the physiological pertinence of whole organism approaches with the advantages of accelerated testing in either micro-volumes (96 well plates) or flow through readings.

Many environmental biomarkers could be good candidates to mimic the potential effects of emerging contaminants on vertebrate physiology. For example, *in vivo* reporter gene assays can be particularly useful for studying nuclear receptor signaling, as numerous chemicals interfere with hormonal signaling pathways, which most often act by modulating transcription of target genes.

We have developed models to detect hormonal disruption of thyroid and estrogenic functions.

Fluorescent *X. laevis* tadpoles bearing genetic constructs integrating hormonal responsive elements are used for physiological-based screens for potential endocrine signaling disruptors.

The control of large volume samples (such as continuous monitoring of water) is best addressed using a flow through methodology. It has already been established that the principle of flow-through readings is compatible with detection of fluorescence in biosensor organisms. Preliminary results have confirmed both the sensitivity of the method and the possibility of obtaining a quantitative signal.

Furthermore, hormonal-dependent switches in gene transcription underlie establishment of different physiological functions and organs including maturation of the central nervous system, respiration and metabolism in a manner that closely parallels the neonatal period in mammals. All these similarities argue that amphibian development can serve as a model for mammalian development, with the distinct advantage that gene function and regulation can be analyzed in an early stage model.

The genetic and physiological proximity between humans and xenopus is particularly well established and recognized for endocrine regulation (OECD reference model). Other physiological and pathological fields can also benefit from small model organisms technology. For these, xenopus is again relevant in that it very rapidly develops a vascular system, a complete immune system and a complex central nervous system in the course of its growth.

A main outcome exploiting these aquatic models will be the possibility to adapt an *in situ* platform for semi-robotized monitoring of environmental health impacts directly on industrial sites. We will adapt existing measurement tools to perform our amphibian based tests in a continuous real time sampling of water. This platform can be adapted, after specific industrial design and modification, to any *in situ* localisation where evaluation of industrial waste or measurements of water quality is needed.

BIOMONITORING STRATEGY OF CHEMICAL POLLUTION ALONG THE IBERIAN MEDITERRANEAN COAST: DEVELOPMENT OF THE MED POL APPROACH

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V.M. León, J.A. Campillo, B. Fernández & J. Benedicto

Introduction

Barcelona Convention constitutes the legal framework of Mediterranean Action Plan (MAP) which is a cooperative effort involving 20 Mediterranean countries and the EU. The Programme for the Assessment and Control of Pollution in the Mediterranean Region (MED POL) is MAP´s first operational programme, which was adopted in 1975 and signed in the Barcelona Convention (1976), being the first UNEP's regional Sea Convention. As Spain is a contracting party to both Oslo Paris Commission (OSPAR) and the Barcelona Convention, it must contribute to both the Co-ordinated Environmental Monitoring Programme (CEMP) and MED POL monitoring programme. During the last decades, the research group of Marine Contamination and Biological Effects (MCBE) belonging to the Spanish Institute of Oceanography, IEO (www.ieo.es), has been performing monitoring activities in Mediterranean waters of Spain, following the recommendations of the MED POL programme related to marine chemical contamination, through research projects mainly funded by the IEO. Since then, the biomonitoring strategy followed by MCBE (IEO) is continuously evolving and updating to new necessities and recommendations, considering those proposed within the MED POL programme as well as those arising from expert groups of the International Commission for Exploration of the Mediterranean Sea (CIESM), the International Council for Exploration of the Sea (ICES) and different policy frameworks.

Biomonitoring strategy for chemical pollution

MED POL phase II was a long-term phase (1981-1995) and aimed to provide further information on possible reviews and implementation of existing protocols and on future ones as well to provide regular assessments on sea water quality. Along the Iberian Mediterranean coast, a network to assess the chemical contamination of coastal waters, using wild mussel (*Mytilus galloprovincialis)* as a target species, was initiated in 1991 by MCBE (IEO). During the first years, only trace metals content in mussel tissue were analysed (cadmium, mercury, zinc and lead) but analysis of more metals (arsenic, selenium, copper, etc.), and a wide variety of organochlorinated compounds (PCBs, DDTs, etc.) and polycyclic aromatic hydrocarbons (PAHs) were also included in subsequent years. So far, selection of chemical compounds to be monitored has been based and exclusively focused on a group of conventional and/or regulated contaminants prioritized within the MED POL programme. However, the number of such contaminants represents only a small part of the total number of potentially hazardous chemicals present in the marine environment. Furthermore, toxic effects in the field often occur as a complex mixture of both known and unknown contaminants.

Such circumstances motivated toxicologists to develop a wide variety of biomarkers of marine contamination during last decades and their potential as useful tools in monitoring programmes started to be validated. Basic principle to use biomarkers in monitoring programmes is they offer early-warning information, detecting sublethal effects of pollutants in the organisms and integrating the combined effects of mixed contaminants available in the marine environment. Detection of sublethal effects helps to identify areas of serious concern/hotspots and, through a more exhaustive chemical assessment, may elucidate their underlying causes. During the MED POL Phase III (1996-2005), biological effects monitoring using biomarkers in sentinel organisms for the Mediterranean Sea (*Mytilus sp. and Mullus sp.*) was recommended to be included in the national monitoring programmes as a pilot activity to test the methodology. Since 2001 and to fulfil this purpose, chemical data obtained from our mussel net work started to be complemented by measuring some biomarkers of stress (i.e.

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lysosomal membrane stability) and of exposure (i.e. metallothionein content) recommended by MED POL guidelines.

For the future implementation of the biological effects monitoring in MED POL Phase IV (2006-2013), the Contracting Parties to the Barcelona Convention adopted the strategy for the development of Mediterranean marine pollution indicators (MPIs) to be considered as the basis for the preparation of marine environment assessments in a manner which could facilitate the development of policy for the protection and conservation of the Mediterranean Sea and coastal areas and track its implementation (UNEP, 2003). At 2006, the biomonitoring programme was substantially extended and coastal sediments (chemical measurements) and a target demersal fish species *(Mullus barbatus)* (chemical and biological measurements) started to be monitored on regular basis, and new MPIs proposed started to be progressively incorporated.

Therefore, current biomonitoring programme conducted by MCBE comprises different partial monitoring programmes and the main objectives to achieve are:

(1) The determination of spatial distribution and temporal trends of selected contaminants in mollusc, fish and sediments in coastal, hot spots and reference areas;
(2) To seek evidence of detrimental biological effects in mollusc and fish and assess them over time.

With such aims, MCBE (IEO) is conducting two field samplings yearly along the Iberian Mediterranean coast. The first survey is conducted between April 15 and April 30 (pre-spawning period for *Mullus barbatus*) in order to collect in a coordinated way sediments and also fish samples for chemical and biological effects in selected areas of concern along the Iberian Mediterranean coast. The second field sampling is conducted between 15 May and 15 June (outside spawning period for *Mytilus galloprovincialis*) in order to collect wild mussel samples for chemical and biological effects along the Iberian Mediterranean coastline (Figure 1).

For chemical concentration and biomarkers in biota, the temporal monitoring programme comprises a number of locations that are sampled yearly, while the spatial monitoring programme comprises a larger number of locations that are sampled once every 5 years. For chemical concentration in sediments, the temporal monitoring programme will be conducted at least once every 5 years, once the appropriated areas have been identified. At present, a pilot study (2006-2010) is being conducted to identify suitable sediment sampling areas along the Spanish Mediterranean coast with the best characteristics (undisturbed bottoms by anthropogenic activities with high sedimentation rate, percentage of fine fraction and content of organic matter, etc.).

Integrated assessment and novel contaminants

The approach of an integrated assessment of the health status of the marine environment is being stressed and expert groups (ICES, MED POL) agree that a joint assessment of the water, sediment and biota performing chemical and biological measurements (biomarkers and bioassays) is preferred. In order to progress on it and optimize the funding resources available, fish sampling is being carried out in a coordinated way with sediment sampling and catching fish from main fish grounds in the regional vicinity of sediment sampling areas. MCBE (IEO) started at 2006 the integrated assessment of the chemical contamination in some selected sites/areas chemically well characterized as hot-spots. In such areas, biomarkers and secondary parameters are measured in fish and/or mussels, and selected contaminants (PAHs, OCPs and trace metals) are measured both in biota and superficial sediments. The final objective to achieve at 2010 is to identify the main areas of concern along the inner continental shelf (<70 m) to perform the chemical and biological temporal trend monitoring in fish underpinned with the data obtained from chemical trend monitoring in sediments.

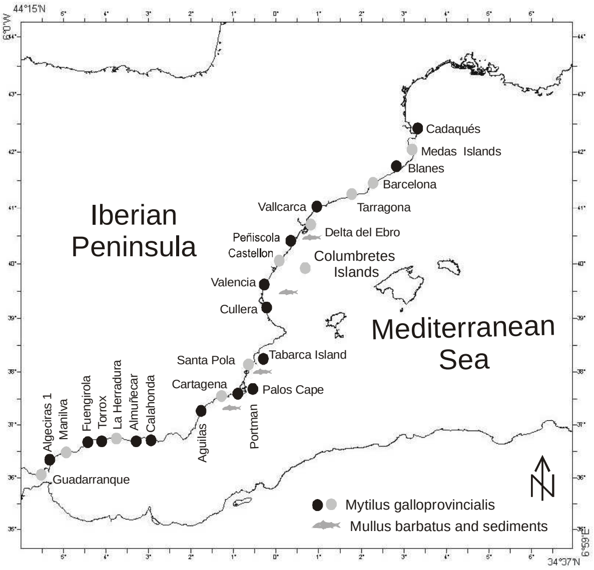

Fig. 1 Sampling sites corresponding to field samplings conducted in 2006 to study temporal trends of contaminant content (black and grey circles), biomarker responses in mussels (grey circles) and contaminant content and biomarkers in fish. Sediment samples were also collected in fish sampling areas.

On the other hand, after a review of the monitoring activities and the use of indicators within the framework of the MED POL programme (UNEP, 2007), the need was highlighted to establish national reference laboratories and provide analytical capacity for emerging contaminants. Consequently, the study concerning the presence of novel contaminants has been recently incorporated to our monitoring activities, using new methods now available in our laboratory with stir bar sorptive extraction (SBSE) coupled to gas chromatography - mass spectrophotometry (GC/MS) to determine simultaneously PAHs, PCBs, organochlorinated pesticidas, organophosphorates and triazins in sea water and interstitial waters by SBSE/GC/MS (Pérez-Carrera *et al.*, 2007).

At 2007, a number of core samples were also taken from a limited number of areas. These areas were selected on the basis of highest concentrations of regulated contaminants and possible emerging contaminants, in the vicinity of a major river mouth and industrial/urban centres where mixed contaminant inputs are expected. Interstitial water and sediments samples of these core samples will be screened using SBSE/GC/MS methodology to detect and identify the presence of regulated and also novel contaminants (i.e. pharmaceutical products, non-regulated pesticides, etc.). Once major emergent contaminants have been identified, the analytical methodologies will be developed or adopted, validated and included in our monitoring programme. Superficial sediments samples from the same box corer were also sampled to perform sea urchin embryotoxicity bioassays. The study of contaminants in interstitial waters will help to evaluate the biomarkers and bioassays results in a more comprehensive way.

Perspectives of the UNEP MAP MEDPOL programme: The caging strategy and the two-tier approach

The MED POL IV proposal is focused on the use of chemical data and associated biomarkers in marine monitoring. The programme aims to evaluate not only the organism's health status but also chemical compounds that induce the stress syndrome. During the last Workshop on the MED POL Biological effects programme (Alessandria, Italy, 2006) it was proposed to include in the MED POL Phase IV the application of a 2-tier approach with caged mussels in the national monitoring programmes. The two-tier approach is highlighted as an effective strategy for routine biomonitoring of marine chemical contamination. The proposal is based in the assessment of the level of pollution-induced stress syndrome in sentinel organisms. The 2-tier approach considers lysosomal membrane stability (LMS), stress on stress and mortality as core biomarkers than can be easily applied by any MED POL laboratory. During 1º tier, two end-points are screened (early-warning and mortality). If as a result of the 1º tier the effects on LMS are not detected, the site may be considered as a clean site and no other (biological or chemical) analysis would be required. If during the 1º tier an increase of mortality is observed then it should proceed directly to chemical analysis to identify contaminants that are inducing biological effects. In sites where critical alterations in LMS are observed, analysis should be supplemented (2º tier) by utilising a battery of biomarkers (proposed on the basis of the results from the BEEP project (EU VI programme) to be analysed by competent labs in the region.

So far, the application of the 2-tier approach is mainly addressing the use of caged mussels, though it can be extrapolated to some other sentinel organisms. The main reason to use caged organisms is the standardization of the methodology and comparability of results. For assessment in mussels, caging the organisms for 30 days allows physiological parameters not to be affected by differential changes in the reproductive cycle and variations occurring in the polluted sites allow comparison with chemical data obtained for organisms sampled in control/clean sites. To initiate and validate this new strategy, the MCBE (IEO) will conduct pilot field studies using caged mussels at selected locations in 2008 and 2009 along the SE Spanish coast.

The use of caged mussels for biomonitoring purposes is being proposed as a way to standardise and facilitate the comparability of results among different areas and to solve the scarcity of natural mussel stocks. It offers a chance to relate the caging strategy and EU Marine Strategy, being a relatively easy tool to make and assessment of the chemical water quality of the different body waters among regions. The use of models to validate and to normalise contaminant concentration in caged mussel makes the comparison between sites possible, regardless of their physicochemical and trophic characteristics (Andral *et al*., 2004). In fact, development of an interregional network of monitoring of the quality of coastal water by bio-integrators was initiated in 2004 in the western Mediterranean basin throughout the MYTILOS project (2004-2006) (<http://mytilos.tvt.fr>). This evaluation and monitoring network of the coastal contamination carried out an active biomonitoring through caged mussels immersed for a period of 3 months. This strategy was also considered by MAP/ MEDPOL and as a continuation, the MYTIMED project (2006-2007) was developed for the Aegean Sea and the North-East Mediterranean. Both projects are actively involved in the integration, standardisation and harmonisation of activities among EU and non-EU member states around the Mediterranean Sea.

However, the optimum length of the exposure period to assess the pollutant-induced stress syndrome in mussels (3-4 weeks) is not well fitted with the longer advisable period to identify changes over time in contaminant concentration and/or in biological responses (temporal monitoring). A longer exposure period lasting several months is usually required to cover the bioaccumulation phenomenon of most persistent organic contaminants and to reveal more subtle chronic effects on organisms. That happens mainly because the bioaccumulation phenomenon covers a period lasting several months (depending of the type of substances) and also because most of the persistent organic pollutants bioaccumulated on tissues produce chronic effects on organisms and affect their physiology in long term periods. Furthermore, the use of caged mussels for wide area biomonitoring programmes entails a higher-cost monitoring strategy than the use of wild ones because at least, two field sampling have to be performed, and moreover, the recovery of the samples is not always been guaranteed.

Therefore, future developments in the biomonitoring programme conducted by IEO will continue using wild mussels for study temporal trends on chemical concentration and the long-term effects on biological effects but also will use transplanting mussels to solve the problem of scarce natural mussel stocks in certain areas. Results of the pilot study to validate the two tier-approach should help to make a final decision if the spatial biomonitoring with wild mussels can be simplified using the two-tier approach.

Discussion on future developments

Due to the Spanish territorial organization, competences to implement the EU Water Framework Directive (WFD) are transferred to the Autonomic Communities (CCAA), which are developing their own monitoring programmes, being primarily focused on chemical concentrations in water and sediments. At present, CCAA monitoring programmes along the Mediterranean waters don't assess the biological effects of pollutants by using biomarkers but they are monitoring a substantial number of chemical compounds than are not incorporated in both OSPAR-CEMP and MED POL programmes. Therefore, to meet the WFD, but also the coming EU Marine Strategy monitoring requirements in coastal and open sea waters, new considerations concerning the biomonitoring strategy should integrate the information obtained through the MED POL Spanish biomonitoring programmes with those ones obtained by CCAA. That would allow a better insight into the quality of individual water bodies (because biological effects measurements would be considered) and it will reduce the costs caused by some overlapping monitoring activities and lastly obtaining a more realistic picture of the marine ecosystem health status. Responsibility of chemical and biological measurements could easily be shared among different institutions, organically dependent from autonomic or national governments, provided quality assurance of such measurements are being controlled: using reference material, participating in the intercomparison exercises organized by International Agency of Atomic Energy (IAEA), QUASIMEME, BEQUALM (www.bequalm.org) and/or those organised by MED POL (Prof. A. Viarengo, University of Piemonte Orientale, Italy). At present, a national biomonitoring programme for marine contamination has not been established yet, and it will be necessary to demonstrate to the policy makers and environmental managers the convenience and advantages of the continuous financial support for the future development of the Spanish biomonitoring programmes that are currently contributing to MED POL and OSPAR-CEMP programmes.

The main challenges arise to perform different standardised samplings surveys in a coordinated way to allow that all measurements can be comparables and to integrate data to give an objective global evaluation of the prevalence situation regarding marine chemical contamination. An important and essential step to integrate information from contaminants, biomarkers and biological data is the establishment of assessment criteria (AC) for all parameter measured, especially biomarkers, scoring them in a second step in order to obtain a generic risk assessment. But the selection of contaminants/biomarkers/biological measurements as well as the applied methodology in the different biomonitoring programmes is not always the same. Such circumstances entail that the establishment of ACs result in a long task, mainly due to the absence of the necessary number of data to validate them effectively. In this context, the use of a synthetic index can play a fundamental role when such differences were insurmountable because geographical/economical/political reasons, as happens when whole Mediterranean basin countries or different regional programmes (OSPAR-CEMP, MED POL, and HELCOM) are considered. Together with the MED POL proposal for implantation of the two-tier approach, the use of a recently developed Expert System (Dagnino *et al*., 2007) is being proposed for integrating biomarker data in a synthetic index. Classification of the Stress Syndrome Level is performed on a five threshold scale, as identified in the WFD, from A (no stress) to E (pathological stress), considering the alteration reached by each biomarker on the basis of its stress response profile and the level of biological organisation (cell, tissue or organism). In a similar same way, synthetic indices are being developed for different groups of data to evaluate the ecological risk. These include the AMBI index to assess the ecological quality of soft-bottom benthic communities and the POMI index, based on structural and functional attributes of the *Posidonia oceanica* ecosystem (endemic sea grass and a major primary producer in the Mediterranean Sea) to assess the ecological status of coastal waters following WFD requirements. Despite differences among national/regional programmes, combination of “particular” synthetic indices for contaminants, biological effects and biology into generic assessment frameworks are under development (ICES, 2008) and may be a feasible solution to improve comparisons of results among regions.

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THE ARCTIC AS SENTINEL FOR ENVIRONMENTAL PROCESSES AND EFFECTS

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AMAP was initiated in 1991 by ministers from the eight Arctic countries (Canada, Denmark, Finland, Iceland, Norway, Russia, Sweden and USA) with the task to monitor and assess the pollution of the Arctic, including effects on biota and humans. The initiative for the Arctic Environmental cooperation goes back to Gorbachov's speech in October 1987 where he called for international assistance to handle the pollution of the Northern Russia[1] and to the observations of high levels of PCB in Inuit women in Northern Canada[2]. In 1993 AMAP was asked to include assessment of climate change and UV/ozone. AMAP has established expert groups for each of the key issues of concern, e.g. persistent organic pollutants (POPs), heavy metals, radionuclides, human health and climate/UV. These expert groups are composed by scientists from the eight Arctic countries and non-Arctic countries with research activities in the Arctic. The programmes for monitoring, assessment, QA/QC, data handling, etc. has been developed and is available from www.amap.no .

Figure 1. AMAP`s geographical area

In 1997 the first comprehensive circumpolar AMAP assessment was presented[3, 4] and in 2002 an updated assessment[5] on the highest priority issues of concern such as POPs, heavy metals, radionuclides and human health and changing pathways of pollution were presented.

The AMAP assessments have mainly focused on the geographical area shown in Fig.1. and have documented that a variety of contaminants such as POPs, heavy metals, including mercury and lead, radionuclides and acidifying components such as sulphur and nitrogen are transported into the Arctic area and deposited in the environment. Some of these contaminants bioaccumulate in the food chain. POPs accumulate mostly in the marine food chain affecting high trophic level predators such as polar bears and killer whales, but also the indigenous populations living mostly on a marine mammals diet. The same goes for mercury while for radionuclides these have accumulated mostly in the terrestrial food chain and shown to affect mostly those indigenous peoples living of reindeer meet. Effects due to the exposure to POPs and methyl mercury have been documented in Arctic animals, e.g. reduced immune system and survival of cubs of polar bear and glaoucous gulls, and on humans[5]. The situation is a paradox – the people living in the Arctic have hardly used any of the products containing these contaminants, but are among the most highly exposed people on the globe. This has been called “the Arctic dilemma”, because the contaminants are found at high concentrations in those parts of the animals (blubber and meat) that also are important as food (energy and vitamins) for the indigenous peoples.

The assessment has also documented that while male mammals accumulate many of these contaminants over their lifespan, the females transfers POP and mercury to their babies during pregnancy and nursing, as illustrated for DDT in Bowhead whales in Fig. 2.

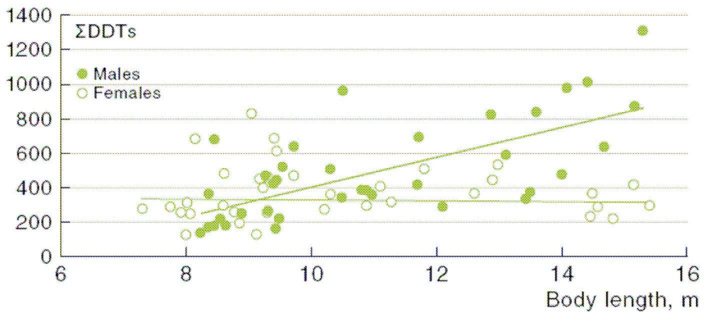

Figure 2. Body length versus OC concentrations in blubber samples from male and female bowhead whales.

For some organochlorines, such as HCH, DDT and PCB, concentrations have generally decreased in both biota and in abiotic matrices in the Arctic, and these declines have been documented in the recent AMAP POPs Assessment report[6]. However, new chemicals of concern such as brominated flame retardants and perfluorinated compounds show increasing temporal trends. PBDE concentrations have shown similar and rapid increases in Canadian ringed seals, beluga, and sea birds as well as in peregrine falcons from Greenland. Similar increasing trends are being seen for the perfluorinated compounds such as PFOS and some PFCAs in ringed seals from two sites on Greenland[7]. Archived polar bear liver tissues from the eastern and western Canadian Arctic showed exponential increases for PFOS and PFCAs[5, 7].

A recent review of brominated flame retardants in the Arctic[8] reports that not only lower brominated PBDEs, but also DecaBDE, hexabromocyclorodecane (HBCD), PBBs and tetrabromobisphenol A (TBBPA) are found in both abiotic and biotic samples. Spatial trends of PBDEs in marine mammals and seabirds show highest concentrations on East Greenland and Svalbard. Highest HBCD levels are also seen in polar bears from East Greenland and Svalbard. These spatial trends are similar to those seen for Organochlorine Compounds (OCs) indicating source regions in eastern North America and Western Europe. DeBDE has been found in polar bears and Arctic seabirds. Fig. 3 shows levels of PBDE and HBCD in ringed seal and Polar bear.

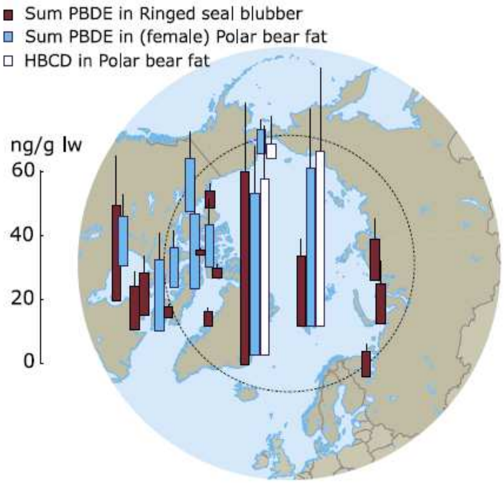

Figure 3. Sum PBDE concentrations in Ringed seals and Polar bears.

Various perfluorinated compounds (PFCs) have been found in a wide variety of animals including polar bear, ringed seal, whales, Arctic fox, seabirds and fish in Canada, Greenland, Faroe Islands and Norway[7]. A spatial study of polar bears covering Alaska, Canada, Greenland and Svalbard indicates highest PFA concentrations in East Greenland, Svalbard and Hudson Bay[8]. PFOS is the predominant PFC in most species studied and concentrations are as high as or higher than those of single PCB congeners in polar bears.

The sources and levels of “classical” contaminants in humans living in the Arctic have been thoroughly evaluated in the recent AMAP Human Health Report[9]. The most exposed people in the Arctic are the indigenous peoples living in Greenland, the North Eastern territories of Canada and, partly, the indigenous peoples of the Russian Arctic. There are certain differences in the contaminant patterns, with typical global exposure in Greenland and Canada, but more evidence for local use of insecticides in the Russian Arctic. Remnants from military activities, especially PCB contamination, are documented in Canada and the Russian Arctic. Examples of organic contaminants of concern are the PCBs, the DDT-group, Chlordane and the HCH-group. Geographical differences in maternal blood PCB levels are visualized in Fig. 4.

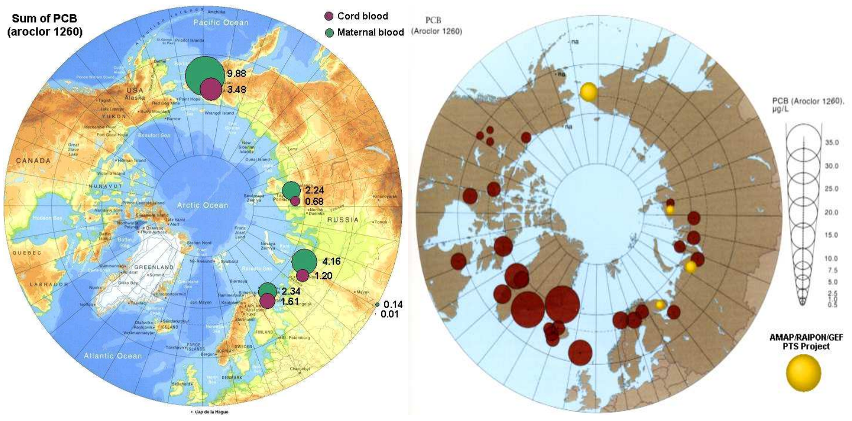

Figure 4. Circumpolar comparison of PCB levels in maternal blood, µg/L, median.

Specific biological effects from some Persistent Toxic Substances (PTS) have been discovered, e.g., direct toxic effects on cellular level; endocrine activity on cellular level effecting the hormonal balance of the body; effects on reproduction, fertilization, early pregnancy development, birth weight, early child development and brain development; carcinogenic properties, immune depression, respiratory diseases and cardiovascular diseases. New research has focused on combined effects of different substances as well as different biological properties of the substances and their metabolites. In the PTS Report of the Russian Arctic[10] a number of health effects of concern are discussed, e.g., many aspects of reproductive health, gestational age, birth weight, and a change in the sex ratio of the newborn babies in female direction. In this study an interesting observation was the change in sex ratio of the newborn babies associated with moderately elevated levels of maternal blood PCB, see Fig. 5.

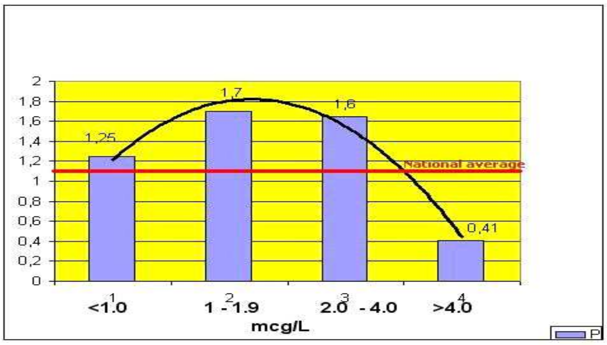

Figure 5. Sex ratio (male/female) of newborns by PCB concentrations in maternal plasma, median, µg/L.

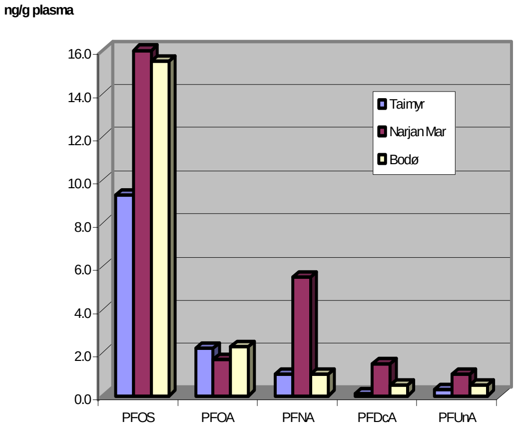

Figure 6. Distribution of some of the dominating PFAS in different populations (ng/g plasma, median levels).

In the group of toxic metals, mercury is the element of concern in the Arctic, with highest exposure in coastal populations of Faroe, Greenland and Canada, with a high intake of fish and marine mammals. Effects such as increased blood pressure and subtle neurotoxin effects have been documented in children. However, local action to reduce the exposure of women of child –bearing age has been succesfull[9]. As for the Russian study groups, they are more exposed to contaminants resulting from local activities. Recently "new" contaminants, like the brominated flame retardants (BFR) and the fluorinated substances (PFOS) have been documented in human blood in the Norwegian and Russian Arctic [11]. The pattern is different, with higher levels in blood from urban populations in Norway, pointing out items connected to modern civilization as the most important sources, wit diet as a less important factor. Geographical differences are visualized in Fig. 6.

In November 2004 the Arctic Climate Impact Assessment (ACIA)[12] was presented as a joint work between AMAP and CAFF (Conservation of Arctic Fauna and Flora) and IASC (International Arctic Science Committee). In addition to document changes in temperature and precipitation, the report document changes in permafrost, erosion, snow and ice conditions and effects on animals and human activities. Models predict an increased temperature that may have a strong effect on the ice and snow conditions in large part of the Arctic. It has also been documented a correlation between changes in climate, UV/ozone and mercury deposition/accumulation in Arctic areas (Fig. 7).

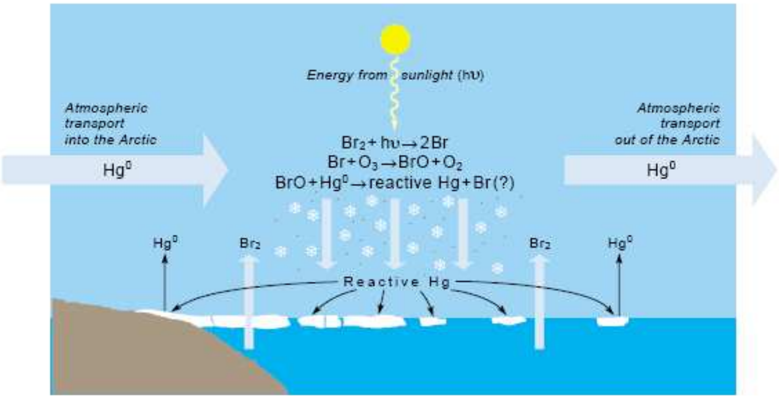

Figure 7. Reactions involving sunlight and bromine remove gaseous elemental mercury from the atmosphere (mercury depletion) and transfer it to the surface as reactive mercury. Part of the reactive mercury may reach the food web; part is re-emitted as Hg0.

At the international level, a main issue for AMAP has been to develop and integrate methodology that is easy to implement globally, standardized due to international QA/QC criteria. It is especially important for the human health activities, to integrate information extracted from research on breast milk and blood on a global basis. Efforts are now coming to develop a common strategy with UNEP and WHO on this issue. An example of correlations between blood and breast milk levels of organic, fat-soluble compounds.

At the Arctic Council ministerial meeting in Russia in October 2006, AMAP presented an assessment on Acidification and Arctic Haze in the Arctic[13]. The level of Sulphate has been decreasing, while Nitrogen oxides have increased in high Arctic. In January 2008 AMAP presented an updated assessment on the consequences of Oil and Gas activities in the Arctic[14] since the beginning of oil drilling in the 1920ties. The levels of petroleum hydrocarbons in the Arctic environment are generally low and the main source is natural seepage. The threat for serious effects on Arctic ecosystems and species due to an oil pills is however, very significant. In 2009 AMAP will present updated assessments on trends for POPs and mercury in the Arctic environment and on the exposure of Arctic peoples to contaminants. In the period of 2009 – 2012 several assessments related to the effect of climate change and combined effects with contaminants will be prepared. For further information, please visit: www.amap.no .

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THE GERMAN ENVIRONMENTAL SPECIMEN BANK PROGRAM AS A TOOL FOR THE RETROSPECTIVE MONITORING AND ASSESSMENT OF EMERGING CHEMICALS

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The German Environmental Specimen Bank (ESB) is part of the national environmental observation program. Every year since 1985 plant and animal samples are taken from representative marine, freshwater and terrestrial ecosystems. Specimens are stored in the gas phase above liquid nitrogen at temperatures below -150°C. The inert gas layer and the low temperature rule out, as far as possible, changes in state or the loss of chemical characteristics over a period of several decades. All tasks are performed according to standardized protocols to ensure the comparability of specimen properties in space and time (Umweltbundesamt 1996).

The greatest potential of the ESB is the possibility of retrospective monitoring. The archive permits specimens to be retrieved for analysis at a later date, should unexpected questions arise or more sensitive analytical methods be available. Thus it is possible to analyze compounds which were not known as pollutants at the time of sampling or which could not be analyzed due to insufficient analytical methods. The specimens from the past can now be examined for all kinds of compounds with the analytical methodology of today or even tomorrow.

Recent examples of the application of the German ESB in the field of emerging pollutants are studies on the occurrence of ingredients of commonly used personal care products like biocides and fragrances in biota samples. In one study (Boehmer et al. 2004) levels of the biocide triclosan, which is used in personal care products and textiles, and its transformation-product methyl-triclosan were investigated in muscles of fish (bream). In specimens from a reference site neither triclosan nor methyl-triclosan could be detected. In bream samples from German rivers up to 69 ng/g triclosan and up to 650 ng/g methyl-triclosan were detected (lipid-based data). For most sampling areas statistically significant increasing trends of the methyl-triclosan contents could be recognized for the period from 1993 to 2003. As consequence of this study methyl-triclosan could be identified as emerging chemical in German freshwater fish.

In a second study on personal care products the polycyclic musk compounds Galaxolide (HHCB) and Tonalide (AHTN) which are commonly used as fragrances were investigated. Highest levels were found in bream from the river Saar with up to 46 µg/g HHCB and 14 µg/g AHTN. The range of HHCB and AHTN concentrations in bream from the river Elbe were 0.5-6.4 µg/g and 0.05-2.1 µg/g, respectively (five sampling sites, period 1993-2003; lipid-based data). Levels of both compounds decreased in bream from most sampling sites within the period analyzed. It is assumed that HHCB and AHTN are substituted by other compounds. A further retrospective monitoring was initiated to investigate whether the fragrance OTNE (tradename 'Iso E Super') is of relevance as emerging compound (Bester et al. 2008).

Another example is the retrospective analysis of ESB samples for organotin compounds from marine and freshwater ecosystems (Ruedel et al. 2003, 2007). The major organotin contaminants in all matrices were tributyl tin (TBT) and triphenyl tin (TPT) compounds. While in marine samples the concentration of TPT was decreasing in the period from 1985 to 2006, levels of TBT remained relatively constant until 2001. Only afterwards concentrations decreased as analyses of marine samples from recent years revealed. This corresponds to the use pattern of TPT and TBT in antifouling paints. While TPT was phased out in 1985, the use of TBT was restricted only for small boats in 1989. A decrease of TBT in freshwater ecosystems was obvious as consequence of this restriction. However, the ban of the TBT application to small boats had no effect in the marine ecosystems where large vessels dominate. Only in 2001 an EU-Directive banned the organotin usage for all applications resulting in decreasing TBT residues in marine biota in recent years, too.

Beside the application of the ESB as an instrument for the exposure assessment also effect assessments can be performed. As a first step an indirect approach can be applied. From the tissue levels of a chemical in aquatic organisms the ambient water concentration to which the organisms had been exposed can be estimated. For this approach bioconcentration factors (BCF) reported in the literature or derived by QSAR may be applied. The water concentrations calculated by this means can be compared to effect concentrations derived from literature sources or estimated by QSAR. This approach was used for example in the assessment of the results of the retrospective monitoring studies for organotin compounds (Ruedel et al. 2003) or musk fragrances (Ruedel et al. 2006). Thus it could be shown that in the 1990s the estimated concentrations of TBT and TPT in marine waters which were derived from tissue concentrations in mussels were in the range where imposex effects in sensitive species may occur. For HHCB and AHTN extrapolations of the detected freshwater fish residues by means of published BCF indicated that the levels of exposure in hot spot regions (e.g. the river Saar) exceeded predicted no effect concentrations (PNEC) for aquatic organisms.

A more direct way for effect assessments are analyses of molecular biomarkers. To investigate the possible usage of biomarkers in cryogenically stored samples taken from the ESB archive an initial study was performed (Seidel et al. 2004). For this feasibility study freshwater fish (bream, *Abramis brama*) was used which is sampled for the ESB since 1992. By comparison of gene sequences of bream with zebrafish (*Danio rerio*) specific genes could be identified which are regulated in bream by different stressors. It was possible to detect genes which are expressed in an agent-specific or agent-unspecific way. HSP (heat shock protein) was identified as a marker to be regulated agent-unspecifically. HSP is over-expressed fast by the influence of different stressors and can therefore be used as a health indicator. Agent-specific genes like metallothionein and vitellogenin represent genes which are influenced by heavy metals or endocrine disrupters, respectively. The main result of this pilot study was that both RNA and DNA could be extracted from archived ESB tissue samples and analyzed with standard molecular-biological methods. If it is possible to identify appropriate molecular biomarkers also in other ESB sample organisms, an effective tool will be available which extends the retrospective monitoring from bioaccumulation markers to effect biomarkers including the detection of changes in the genome.

In some cases also direct effect studies are performed within the ESB program. An example is a project with bream which was initiated to investigate the possible linkage between bioaccumulation of alkylphenols and the effects on male fish (Klein et al. 2005). From a previous ESB monitoring study it was known that estrogenic disruptors like alkylphenols and their ethoxylates (AP, APEO) were present in German freshwater fish (Wenzel et al. 2004). Concentrations of AP and APEO in fish from the river Saar were much higher than those found at other sampling sites. Therefore this area was chosen to study the relationship between AP/APEO accumulation and effects. Although a direct linkage between tissue levels and effects of AP and APEO could not be established, the relationship between elevated vitellogenin concentrations and sewage treatment plant discharges was evident (Klein et al. 2005).

Furthermore, preliminary studies reveal the usefulness of concomitant histopathology of fish gonads to detect potential signs of reproductive impairment. Recent histological examinations of eelpout (*Zoarces viviparous*) collected from North Sea and Baltic Sea sampling sites showed moderate prevalence of intersexuality in male fish and surprisingly high prevalence of atresia in female fish (Gercken and Sundt 2007).

The German Environmental Specimen Bank is in the responsibility of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety and is coordinated by the Federal Environment Agency. More information on methodology and results is available via the internet site www.umweltprobenbank.de (English language pages are available).

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SESSION III

Integrated approaches within risk assessment strategies for monitoring risk of emerging pollutants at local and large scales

INTEGRATED CHEMICAL AND BIOLOGICAL MONITORING OF THE MARINE ENVIRONMENT

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Marine ecosystems worldwide are increasingly subject to stressors that originate from anthropogenic activity. Such activities include fisheries, transport, industry, energy production, aquaculture, agriculture and household waste production. Most of these will to some extent result in the input of toxic substances, contaminants, to the marine environment. Contaminants in marine ecosystems are relevant both because they may accumulate in food chains, thus affecting human use of marine resources, and because they may impact marine life, reducing biodiversity. The concentration of contaminants in marine matrices, be it abiotic compartments or organisms, can provide the answer to the first issue (if the quality of the monitoring programme is satisfactory), but there is a need for additional information about effects on organisms to be able to address the second question.

There has been a general agreement for more than a decade that there is a need to co-ordinate chemical and biological monitoring. Although this has resulted in monitoring guidelines, e.g. within OSPAR[1], integrated monitoring has been implemented to a limited extent and using different strategies in different countries.

Over the past few years there has been work within ICES[2]/OSPAR to develop a framework for such monitoring, bring forward appropriate protocols and decide on assessment criteria and strategies. This presentation will briefly review the strategies chosen in some countries and then expand on the framework developed within the above process (WKIMON). Prior to implementation, important components the framework will be tested out in an international workshop (ICON), due to take place in the North Sea in 2008 as part of the national obligations to OSPAR for some countries.

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[1] Oslo and Paris Commissions, http://www.ospar.org

[2] International Council for the Exploration of the Seas

FIELD MONITORING AND EFFECT ASSESSMENT OF EMERGING SUBSTANCES IN THE MARINE ENVIRONMENT: INTEGRATED APPROACHES AND FUTURE CHALLENGES

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Introduction

Despite increasing legislative activity to improve protection of the aquatic environment throughout Europe, new potentially hazardous substances are constantly emerging. These new substances have an actual or potential impact on the aquatic environment, though establishing appropriate indicators for their presence and effects has proven to be a challenge. It is generally recognized that monitoring programs for hazardous substances, including endocrine disruptors (e.g. organotins, estrogen-actives substances), should be based on an integrated approach using a combination of chemical and biological data. These typically include: confirmation of exposure through determination of body burden concentrations and biological responses in the same target species, joint application of chemical analyses (external and internal concentrations) and biological effects assessment techniques (using diagnostic biomarkers, mechanism-based bioassays and transplanted sentinels). An approach similar to that described above can be envisaged for other known classes of emerging substances, such as brominated flame retardants, poly- and perfluorinated compounds, pharmaceuticals, and nanoparticles.

We review the status of integrated chemical-biological effect methods for known emerging substances in the marine environment. We describe an integrated method for a generic preliminary hazard assessment of known and yet unknown emerging substances for aquatic environments. We then discuss some future challenges and opportunities in this field.

Biological effects monitoring programmes and integrated methods

The use of biological effect methods is well established in European programmes, including the Oslo-Paris Commisson (OSPAR) Joint Assessment Monitoring Programme and the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) (for details see http://www.ospar.org/). The JAMP (Agreement No 2003/22) specifies requirements for information on the biological effects of the substances on the OSPAR List of Chemicals for Priority Action and on any emerging problems related to the presence of hazardous substances in the marine environment. The specific focus is on determining whether there are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, as a result of exposure to hazardous substances. A well-known case is the development of imposex in gastropod molluscs due to extremely low concentrations (close or below to analytical detection limits) of tributyltin (TBT). As a consequence, studies on TBT-specific biological effects are now mandatory under the CEMP. This integrated package include a suite of parameters relevant to imposex/intersex in gastropods, TBT, DBT, MBT, TPhT, DPhT, MPhT in sediments (for offshore monitoring) and in biota where appropriate (JAMP, 1998b). More general biological effects measurements, including whole sediment bioassays; sediment pore-water and elutriate bioassays; water bioassays; lysosomal stability; macroscopically visible liver neoplasms>2mm; externally visible fish diseases; and reproductive success in fish and those associated with PAHs and metals (JAMP, 1998a,b), are now part of the CEMP (voluntary), but some are awaiting development of quality assurance procedures and assessment criteria.

Currently, OSPAR in association with the International Council for Exploration of the Sea (ICES) are developing an integrated chemical-biological effects monitoring strategy which addresses three ecosystem components: water, sediment and biota The latter includes fish, gastropods and mussel themes with tissue chemistry, whole organism responses, tissue responses and subcellular responses (for details see presentation by Ketil Hylland, this workshop). Fundamental aspects of the design of such an integrated programme include the selection of appropriate combinations of biological effects and chemical measurements and the design of sampling programmes to enable the chemical concentrations, the biological effects data and other supporting parameters to be combined for a generic assessment. Further, it requires robust assessment criteria and background responses for all the techniques and a generic risk assessment framework. This integrated approach to monitoring marks an important step towards effective monitoring of the impact of hazardous substances on ecosystem health and will likely become part of monitoring requirements under the EU Marine Strategy Directive (MSD).

Integrated methods for estrogenic and other emerging substances

In order to aid interpretation of biological effects measurements, an integrated assessment may require information on specific mode of action and data on related contaminants which would elicit a biological response. This approach is well illustrated by the example of estrogenic substances and their monitoring requirements in a recent proposal to OSPAR (OSPAR, 2008).

Estrogens and xenoestrogens act directly on estrogen receptors and mimic the effects of natural female sex hormones, such as estradiol. Since estrogen disruptors are biologically active at very low concentrations, and given the additive nature of their action, in assessing the presence and significance of estrogenic substances chemical analysis should be complemented by biological screening. To date, marine monitoring programmes of estrogen-specific effects should primarily focus on fish (appropiate biomarkers for invertebrates are not available). The following 3 endpoints in individual male fish should be measured: (1) in vitro bile estrogenic activity, using deconjugated extracts and application of estrogen-receptor based in vitro assays; (2) plasma vitellogenin (VTG) concentrations using immunoassays (3) prevalence and severity of gonadal intersex by standard histological procedures. These endpoints are collectively diagnostic of estrogenic contaminant exposure and effects and can provide information on the severity and duration of exposure, the stage of development at which the organism was exposed, as well as the severity of damage or effects on fish health. Recommended chemical determinants and necessary co-factors that should be recorded for this integrated method package include: alkylphenols and alkylphenol ethoxylates, natural (estrone, 17β-estradiol) and synthetic hormones (17α-ethynylestradiol), and phthalates in environmental and/or biotic samples, fish age, gonad somatic index and stage of sexual maturation. In addition, *in vitro* assays to determine bile estrogenicity can also be used for measuring the estrogenicity of environmental extracts and in relation to a bioassay-directed fractionation approach (e.g. Toxicity Identification and Evaluation/TIE) to identify those chemicals responsible for the observed estrogenicity of environmental and biotic samples (e.g. Houtman et al 2004). Quality assurance procedures and assessment criteria have been established for VTG, but not yet for estrogenicity of fish bile (as E2-equivalent concentrations), or prevalence/severity of intersex in male gonads. These techniques require further work before they can be implemented within monitoring programmes such as the CEMP.

Another group of emerging substances concern the dioxin-like PCBs and polychlorinated dibenzodioxins and furans. An integrated package of methods relevant to this group of compounds include the induction of CYP1A/EROD activity in fish liver and application of the dioxin receptor based *in vitro* test, DR-CALUX. Several other general biological effects measurements in fish and invertebrates, including lysosomal stability, may respond to exposure to these substances. DR-CALUX is considered the most useful *in vitro* bioassay technique although chronic *in vivo* bioassays may also be relevant (see also WKIMON, 2008).

Very few biological effects methods are currently available for other known emerging substances, such as brominated flame retardants, poly- and perfluorinated substances (e.g. PFOS), and pharmaceuticals. These chemically different substances, however, may have endocrine-disrupting effects and some ED-relevant endpoints may be appropriate, along with general biological effect measurements such as reproductive success. For brominated flame retardants, thyroid hormone receptor assays in fish blood are relevant but have not been tested in the field and currently remain at the laboratory stage. Recent studies on the toxicological properties of brominated flame retardants in fish suggest that there are limited overt effects that can be detected by existing general biological effect techniques, such as reproductive success and subchronic developmental tests (Kuiper, 2007).

In the case of nanoparticles, both chemical analyses and biological effects methodologies are still lacking. However, there are indications that oxidative stress measurements (Moore, 2006) and embryo-larval bioassay may be appropriate for these contaminants (Zhu et al., 2008). These techniques are either available or under development by ICES.

A variety of passive sampling devices offer the potential for temporally integrated sampling of several of the emerging substances including brominated flame retardants and perfluorated substances in water and assessment of their availability in sediments, and these should be employed where possible. OSPAR is currently considering some of these tools (e.g. silicone rubber) for application in its monitoring programmes.

From the above, it is clear that very few biological effects methods are currently available for measuring the effects of relatively new emerging substances. For these substances, "general" biological effects methods which are indicative of stress (e.g. lysosomal stability) or the health status of marine organisms (e.g. disease outcomes), or general toxicity of the sediments and water that are likely to respond to these contaminants can be used.

Quality Assurance procedures for biological effects methods are provided by BEQUALM (http://www.bequalm.org) and, for marine chemistry and in the case of imposex/intersex in gastropods, by QUASIMEME (http://www.quasimeme.org). Whilst chemical analysis methodologies are available for some emerging substances, suitable External Quality Assurance (EQA) programmes do not yet exist at this stage. This may change in the near future, as many of the contaminants of interest must be monitored under the EU Water Framework Directive (WFD).

Preliminary hazard assessment using integrated bioanalysis in WFD

An assessment of "biological effects of contaminants" carried out under the OSPAR CEMP is not explicitly required by the Water Framework Directive (WFD), however opportunities for biological effects measurements can generally be seen in WFD monitoring. A potential application of socalled integrated bioanalysis relating to emerging substances is discussed below.

A first indication of the presence and effects of known and (yet) unknown emerging substances can be achieved by performing a battery of short-term low-volume screening assays (*in vitro* and *in vivo*) on extracts. Screening assays, especially those based on chemical sum parameters, may yield broader coverage of ecologically relevant compounds than can be achieved with chemical analysis alone. Maas and Van den Heuvel-Greve (2005) have explored the opportunities for using this integrated approach in WFD chemical monitoring. They conducted a field study to investigate the link between toxic effects and concentrations of priority pollutants in the same samples. A combination of broad spectrum and mechanism-based assays (Microtox®, Daphnia IQ, Daphnia 24-48 hr, Algal growth, 72 hr, Algal PAM, Umu-C/Mutatox®, DR-CALUX, ER-CALUX) were applied to samples both of surface water and of suspended matter taken at a number of inland, transitional and coastal waters sites. Effects in bioanalysis were demonstrated in an artificial sample composed of the 33 WFD priority contaminants at their maximum permissible level. It turned out that the most important effects were caused by PAHs, herbicides and insecticides. Field samples containing priority contaminants in low concentrations showed the same or greater effects. These field effects, however, were mainly caused by contaminants other than those on the WFD priority list of chemicals. Thus, inclusion of integrated bioanalysis into chemical monitoring programmes would enable the effects of substances other than the selected priority chemicals to be monitored alone. This would include known and unknown contaminants and their combined effects. In addition to generally being less expensive than chemical analyses, the method is general and can be applied to marine, brackish and freshwater environments. New studies will explore the use of passive samplers and its advantages as a time-integrating tool to obtain extracts for applying bioanalysis.

Integrated bioanalysis should be deployed as a "battery of tests". Many tests (e.g. CALUX assays, Daphnia test, bivalve embryo test) are already standardised and validated and in regular use. However, the range of methods needs to be expanded to include other ecologically relevant mode of actions (e.g. immunoassays), trophic levels (e.g. microbes) and underrepresented taxa (e.g. echinodermata and sponges). This list might also be extended with relevant biosensors designed for measurement either of specific chemicals or of their biological effects. The composition of what the set minimally needs to comprise of requires further work. Finally, there is a major need for development and validation of appropriate protocols for extraction methods and subsequent *in vivo* and *in vitro* testing.

Future challenges and opportunities

Although the use of biological effects in integrated environmental risk assessment frameworks and in environmental compliance offers obvious advantages, their linkage to population and community-level effects often remains tenuous and their application is not, therefore, straightforward. Nevertheless, a large number of biological effects techniques are available for immediate deployment in environmental monitoring programmes. Several of these methods could be integrated with chemical determinants to monitor the effects of emerging substances (e.g. estrogen-active compounds). On the other hand, very few specific biological effects methods are currently available for measuring the effects of emerging substances, such as brominated flame retardants, PFOS and nanoparticles. Although it is possible to design a programme that addresses effects of specific (groups) of emerging contaminants, observed effects will rarely be caused by one agent or group of agents only. Methods that relate directly to specific contaminants (e.g. organotins, estrogen agonists) are exceptions. This underpins the need to integrate chemical and biological methods in environmental-contaminant monitoring and not to use methods in isolation but as part of a suite.

The integrated multi-level approach to monitoring with its different ecosystem components, as proposed under ICES and OSPAR (see above), is essential for ascertaining the extent and significance of the impact of hazardous and novel emerging substances on marine wildlife populations and ecosystem health. New techniques for certain and new emerging substances need to be developed, validated and internationally standardized, and existing monitoring programmes such as CEMP should be augmented. At the same time, there is a need for consistent pan-European screening programmes designed for preliminary assessment and identification of hazard of yet unknown emerging chemicals. These programmes could include, amongst other techniques, the application of integrated bioanalysis, extended with TIE procedures, and novel sensors and micro-arrays, when they become available, and instrumental methods to identify causal compounds. In this connection, there are advantages to be gained for combining the use of passive sampler extracts and bioanalyses as an important linkage between the WFD and MSD. Both methods are generic and can be applied to a wide variety of environments.

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PASSIVE SAMPLING COMBINED WITH BIOLOGICAL AND CHEMICAL ANALYSIS OF ESTROGENS AND PHOTOSYNTHESIS INHIBITORS

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Summary

Grab sampling is the common tool to assess the presence of chemicals in the aquatic environment. As concentrations of chemicals vary over time, grab sampling poses limitations for studies that aim to link exposure and effect. A large number of water samples may be needed to assess exposure adequately. Also, it can be difficult for regulators to define efficient and meaningful grab sampling protocols to evaluate if water quality criteria are being met. Observations on the occurrence of estrogens in Swiss midland rivers will be used to highlight these issues. In order to improve exposure assessments, and as monitoring tools for water quality criteria assessments, passive sampling is a promising tool. Several examples will be shown of ongoing work at Eawag. Sampling campaigns have been run with two samplers that target the more polar substances: POCIS and Chemcatcher. Besides field applications of passive sampling, we also perform semi-field experimental work. We address the interaction between hydrodynamics and the sampling properties of various sampler designs. For the analysis of the passive sampler extracts the main focus has been on two bioassays: the well known yeast estrogen screen, and a recently developed combined algal assay to analyse PSII inhibitors and baseline toxicity.

Bioassays as integrative assessors of environmental pollutants

Target analysis of environmental samples yields exact information on the presence and concentration of pollutants in the environment. Furthermore, using internal standards, and other quality assurance methods, chemical analysis yields robust data. However, there are some limitations with chemical analysis. One constraint is that only substances can be found that are being targeted by the analytical method. Another drawback is, that the method is costly, especially when numerous and diverse chemicals – and their metabolites – have to be measured. These are some of the reasons why biological assays are becoming increasingly important in monitoring programmes that aim to assess the load of pollutants in the environment, or that aim to assess the treatment efficiency of the sewage treatment process. Besides, biological assays can be combined with chemical analysis to assist with the identification of unknown toxicants. However it is not possible to resolve the responsible chemical(s) with bioassays. Therefore, the information of chemical and effect analysis is always complementary and one cannot replace the other.

A large number of cell-based assays have been developed. Some of these assays, particularly receptor based assays, cover a clearly defined class of chemicals, e.g. estrogens; other assays cover more non-specific modes of toxic action, such as baseline toxicity. In this presentation some data will be shown from a well established yeast based assay for estrogenic activity, the yeast estrogen screen (YES[1]), and a relatively novel high through-put assay for photosynthesis II inhibitors[2] and algal toxicity, the combined algal test[3]. For both assays we compare biological with chemical analysis of substances that are known to cause the observed biological effects.

Fluctuating concentrations of estrogens

Concentrations of aquatic pollutants vary. It is very obvious, that chemicals that enter rivers during storm water run-off events show a very dynamic concentration range. This situation has been relatively well documented for biocides (e.g.[4,5]). However, when we started characterising the estrogenic activity in Swiss rivers and effluents, we expected a variable but still relatively constant exposure situation. Estrogens are mainly reaching rivers via treated domestic sewage effluent. Setting industrial and agricultural sources aside, the main load of estrogenic activity in influent to sewage treatment plants is coming directly from humans. Thus, the release of these estrogenic substances to the influent is fairly constant over time[6,7], they are pseudo persistent[8].

In two studies some 400 grab samples were collected and analysed for estrogenic activity. Even with this fairly large data set it was difficult to link estrogenic activity with river and/or sewage treatment works parameters. It was also not possible to confidently tie the measurements of estrogenic activity with effects in fish[9]. Therefore we started investigating passive sampling as an alternative and more integrative sampling strategy[10]. Another reason to explore passive sampling was our interest in sampling chemicals from the water in a more biomimetic fashion (e.g.[11]).

Some background to passive sampling

Passive sampling of lipophilic substances from water became well established some 15-20 years ago with the development of the semipermeable membrane device (SPMD[12]). An SPMD was designed to mimic bioconcentration. It consists of 1 g of lipid contained in a long and flat polyethylene membrane – ensuring a large surface to volume ratio. Chemicals diffuse from the bulk water through the aqueous boundary layer to the sampler surface, and then partition between the sampler and the water. Initially the sampler will mainly take up a chemical, but as more of it has been sampled, also the elimination, i.e., partitioning of chemical from the sampler back to the water, starts to play a role. In the initial linear uptake phase the amount of chemicals in the passive sampler may be extrapolated to average concentrations over the sampling period – time weighted average (TWA) concentrations – provided sampling rates have been established. This situation is typical for SPMDs and chemicals with a $\log K_{OW}$ larger than 6. However, samplers can also be configured to quickly reach equilibrium between the uptake and elimination processes, this is applied in the area of SPME – solid phase microextraction[13]. Also for hydrophilic compounds with low $\log K_{OW}$, SPMDs quickly reach equilibrium.

As passive sampling is partly a diffusion process, it is affected by environmental conditions that affect diffusion. These include, for example, temperature, the thickness of the aqueous boundary layer and the fouling of the sampler during its deployment[14,15,16]. This means, that sampling rates of a passive sampler will vary across deployment sites, as the environmental conditions are always different. With SPMDs, an elegant solution is available, where an SPMD can be spiked with a substance that is lost from the SPMD during deployment (see also[16]). The degree of loss of this performance reference compound (PRC) can be used to calculate a sampling rate in the field for each individual SPMD, these can then be used to calculate fairly robust TWA concentrations[17]. Passive samplers have also been developed for metals, e.g. the DGT (diffusive gradients in thin film[18]). As DGTs are relatively unaffected by environmental conditions such as flow rate, also these samplers can be used to calculate TWA concentrations (e.g.[19]).

In this presentation the focus will be on samplers for more polar substances. Currently there are two basic types of polar organic samplers for which several studies have been published (for an overview of more sampler types see[20,21]). One sampler is called POCIS (polar organic chemical integrative sampler[22]): it consists of a polymer powder that is contained between two membranes. The other sampler type was recently named Chemcatcher[23]. The Chemcatcher is also a polymer-based sampler, but the polymer is attached to a teflon matrix (Empore disk; www.3m.com) and therefore can be applied either with or without a membrane. Both samplers have been used to sample chemicals with a $\log K_{OW}$ between 0 and 6 (for reviews see: [20,21]). Although sampling rates have been calculated for these samplers, the effects of environmental conditions on these lab-based sampling rates are not very well described yet. Also, a PRC based system has yet to be developed for these samplers; thus, it is not yet possible to assess environmental effects on the sampling rates. Finally, it is unclear to which extent the passive sampler models that have been developed for the SPMD can be applied to both polymer based samplers[24].

Overview of some passive sampler studies

At Eawag we have applied both types of polar organic samplers in the field – in rivers and treated sewage effluents – and under more controlled conditions. In a first field study with the POCIS that focussed on estrogenic substances, we could show that: 1) the amount of estrogenic activity in POCIS, assessed with the YES, correlated well with the amount of steroidal estrogens measured with chemical analysis; 2) POCIS data correlated well with average concentrations assessed with repeated grab samples; and, 3) the amount of estrogenic activity collected in the POCIS correlated with the amount of estrogenic activity accumulated in the bile of male fish[25]. Based on these promising results, we conducted a larger POCIS study where we screened many different effluents[10]. Some preliminary results of this field work will be shown.

Parallel to the work with POCIS, we also performed several studies with the Chemcatcher. In a study that targeted herbicides that inhibit photosystem II, we deployed unprotected Empore disks and analysed the samples with an algae based bioassay and chemical analysis for two herbicides: diuron and simazine[26]. Using published sampling rates for the Empore disks[27], we calculated TWA concentrations; these matched the concentrations in a single grab sample rather well. In addition, there was a very good association between the data from the bioassay and the chemical analysis.

Semi-field experiments with passive samplers

As highlighted above, polar organic samplers can already generate useful data with respect to relative loads of chemicals (e.g.[28,29,30]). Also, when some calibration data are available, representative results (i.e. TWA concentrations) can already be obtained when sampling conditions are close to calibration conditions[26]. However, there is still a lack of understanding how environmental conditions, such as the water flow rate, affect the sampling process. Several studies looked at the effect of turbulence (by either stirring the water[22,31]; or by rotating the passive sampler[16,27]). However, it is not straightforward to translate the obtained sampling rates to field studies. For this reason, we constructed two channel systems where passive samplers can be subjected to river-like flow conditions[32]. In addition, the channels are being run with environmentally relevant matrices such as river water and treated sewage effluent. In contrast common calibration studies have worked with simple matrices, i.e. nanopure water with spiked chemicals.

Typically, the amounts of chemicals sampled by Empore disks from the channels increase 5-fold when the flow rate is increased 15-fold (from 2.5 to 37 cm/s). However, even at slow flow rates, some substances, particularly ones with a lowK_{OW}, rapidly reach equilibrium. Consequently, it is unclear if Empore disks are suited as integrative samplers for very polar compounds. Recent work demonstrated that the Chemcatcher housing has a pronounced effect on the sampling rate of the Empore disks[33]. In a flat Chemcatcher housing, the sampling rate of the Empore disk appears to be double that of the rate obtained with the original housing, where the Empore disk is placed at the bottom of a shallow cylinder [23] [27]. For our studies we mainly employed the flat housing. Thus, one way to increase the integrative sampling period of Empore disks is by modifying the sampler housing. Examples of recent channel studies will be shown, where we applied biological analysis and chemical analysis for a suite of different chemicals, including those that partially explain the biological responses.

Required developments for polar organic samplers

Passive sampling of polar organic compounds, linked with biological and chemical analysis, already yields very useful and biologically relevant data. However, environmental conditions and sampler housing (placement) significantly affect the results obtained in the field. If the technique is to develop further – for example as part of programmes to monitor compliance with water quality criteria – it is essential that a performance reference system for polar organic samplers can be implemented. This development should be matched by a widening of the data base of sampling rates. Sampling rates should be shown to be robust, i.e. repeatable in different laboratories and there should be a good comparability between lab-based and field-based sampling rates.

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COMBINED CHEMICAL ANALYSES AND BIOMONITORING AT AVEDOERE WWTP

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A strategy for characterising influent and effluent from a 350,000 PE_{B60} urban, publicly owned, tertiary wastewater treatment plant (WWTP) was implemented in 2002 and in 2005. Repeated study is scheduled for 2008 synchronised with the national monitoring programme NOVANA.

The strategy includes chemical analyses of sets of 4 flow-proportional weekly samples per year, algal toxicity tests on the same samples and exposure of freshwater mussels in the effluent for a 6-week period. The analytical programme for the effluent samples included heavy metals, pesticides, PAHs, phenolic compounds, PCBs, phthalates, chlorophenols, P-triesters, aromatic hydrocarbons, halogenated aromatic hydrocarbons, halogenated aliphatic hydrocarbons, organotin chemicals and a few sum parameters. The total number of compounds/parameters analysed were 125 and 58 in 2002 and 2005, respectively.

The toxicity level has remained at a constant level since 1999 (Table1). For all the effluent samples investigated, the effluent toxicity has been below detection limit of 2 – 3 TU (toxic units). The effluent has an initial dilution factor of more than 6 and the acute toxicity of the effluent can not be considered a problem for the aquatic recipient. Calculations on "explainability" for expected versus observed toxicity show reasonable agreement and a very limited influence from the organic compounds compared to heavy metals.

Table 1 Development in algal toxicity for the freshwater algae *Pseudokirchneriella subcapitata* expressed as average toxic units (TU) for EC_{10} (72 h)

Year	n^1	Average (TU)	
		Influent^2	Effluent
1996	3	6^3	< 3
1999	4	21	< 3.3
2002	4	28	< 2
2005	4 and 2^4	21	< 2

1: Number of 1-week flow proportional samples; 2: Including internal load from reject water
3: Extrapolated to 72 h values from 48 h mini test; 4: 4 influent samples and 2 effluent samples

Chemical analyses of sampled mussels exposed directly in the effluent in 2002 and 2005 showed bioaccumulation of diethyl phthalate, LAS and Cr. The investigation in 2005 included brominated flame retardants (BDE) and organotins. It showed bioaccumulation of tetra and penta BDE (0.2 to 0.6 µg/ kg wet weight), few PAHs (e.g. phenanthrene from 1.3 to 2.1 µg/ kg wet weight) and two organotins as well. During the 6 weeks of exposure in 2005 the concentrations of Cu, Pb and carcinogenic PAHs in the mussels decreased. Using the mussels for screening of bioaccumulation it was possible to detect compounds which were undetectable by the chemical analyses of effluent samples. From these results we can conclude that the method is a promising tool for screening of bioaccumulative compounds in effluent.

Keywords: emission control, mixture toxicity, wastewater management, occurrence, hazardous substances, bioaccumulation

MULTIPLE ENDOCRINE DISRUPTING ACTIVITIES IN FRENCH RIVER SEDIMENTS AS ASSESSED BY THE COMBINED USE OF *IN VITRO* BIOASSAYS AND CHEMICAL ANALYSIS

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We report the combined use of both *in vitro* bioassays, based on several reporter cell lines, and chemical analyses to assess endocrine disrupting chemicals (EDCs) in environmental samples. Sediments from three French river sites located at stations where endocrine disrupting effects in wild fish (*i.e.* alteration of estrogenicity and androgenicity biomarkers) have been previously evidenced, were analysed. In the most impacted site, multiple *in vitro* endocrine activities, including estrogen (ER), androgen (AR), dioxin (AhR) and pregnane X receptor (PXR) ligands were detected in organic (hexane/acetone) extracts of sediments. Targeted chemical analyses performed on the samples demonstrated the presence of several well known EDCs (E2, E1, octylphenol, BPA, 16 PAHs), but at levels that only partly explained, on a concentration additive model basis, the toxic-equivalents given by the ER and AhR bioassays. In order to further characterise and identify the compounds responsible for the *in vitro* activities, bioassay-directed fractionation approaches coupled to chemical analyses are developed (ongoing work). Firstly, the use of both recombinant hER and recombinant hPXR immobilised on sepharose columns allowed to purify the different *in vitro* endocrine activities. ER and AhR activities are separated, suggesting that multiple endocrine activities are due to different chemicals. ER and PXR activities are only partly separated indicating that some compounds bind to and activate both receptors. Secondly, in an attempt to identify the nature of active chemicals, the application of an established bioassay-directed identification procedure using HPLC fractionation and subsequent chemical identification is under progress. The overall approach and preliminary results will be presented.

RISK ASSESSMENENT OF PHARMACEUTICALS IN US DRINKING WATERS

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Pharmaceuticals, suspected endocrine disruptors, and other emerging contaminants have been detected ubiquitously in wastewater effluents globally. Many drinking water facilities utilize water supplies that are impacted by treated wastewater outfalls. The Awwa Research Foundation (AwwaRF) has conducted several studies to evaluate the occurrence, treatability, and relevance of trace emerging contaminants in North American drinking waters. This presentation will provide information on a recent human health risk assessment conducted for pharmaceuticals in drinking water. Through an iterative process of literature review and consultation with water experts, a suite of 16 pharmaceuticals and 4 pharmaceutical metabolites were chosen for evaluation based on magnitude/severity of toxicological effects, representation of major pharmaceutical classes, and analytical considerations. A robust analytical method utilizing solid-phase extraction followed by isotope-dilution liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) was developed and utilized. An MCF-7 human breast carcinoma cellular bioassay also was used to detect and quantify total *in vitro* estrogenicity. More than 15 US water utilities from various geographical locations were evaluated. At a minimum, each utility provided raw, finished, and distribution system water samples. Several water reuse systems also were evaluated. In order to place *in vitro* bioassay results into a context readily perceivable by the public, several beverages were also analyzed (i.e., juices, beer, coffee, etc.). Of the pharmaceuticals evaluated, meprobamate and dilatin exhibited the most widespread occurrence with detectable concentrations in 73% and 60% of the finished water samples tested, respectively. Median concentrations of meprobamate and dilatin were 6.2 and 5.8 ng/L, respectively. Using US Environmental Protection Agency (EPA) risk assessment paradigm, including the most conservative safety factors, the maximum detected concentrations of selected pharmaceuticals in US drinking waters were several orders of magnitude lower than the calculated acceptable daily intakes (ADIs). Estrogenicity, measured as estrogen equivalents (EEQs), was detected in only one of the finished drinking waters evaluated, and at a level just about the minimum reporting limit (MRL) of 0.07 ng/L. In contrast, beverages such as soy milk and certain fruit juices exhibited EEQs of greater than 4000 ng/L. From this study it is clear that: a) pharmaceuticals can and do occur at ng/L levels in US drinking waters, b) the concentrations selected pharmaceuticals in US drinking waters occur at levels far below any meaningful toxicological significance, and c) the estrogenicity of US drinking waters is trivial compared to the estrogenic potential of several common dietary items.

MONITORING METHODOLOGIES FOR ACCIDENTAL SPILL OF HAZARDOUS AND NOXIOUS SUBSTANCES AT SEA: AN INTEGRATED APPROACH

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During the last twenty years, there has been a considerable development in the transport and handling of chemical substances by ships along the European maritime waters. There is a huge range of compounds trafficking and new products are regularly launched in the market. A number of them are poorly described and may pose hazard and threat to the marine environmental in case of accidental discharges. The MARPOL 73/78 international convention aims at defining hazard profiles of substances transported by ships. It is enforced by the international maritime organization (IMO) for the prevention of marine pollution. Since 1995, the hazard criteria reviewed by the GESAMP working group that evaluates and lists hazardous substances, are very much harmonised with those used by OECD. In the new revised MARPOL classification, there is goal to categorize the substance according to bio-accumulation; biodegradation; acute toxicity; chronic toxicity; long-term health effects; and effects on marine wildlife and on benthic habitats. However, in many instances, there is no sufficient or available data for these criteria. Hence, the monitoring methods to evaluate the status of the environment following a chemical spill are often based on the measurement of concentration found in the water or in biota, and on the basis of acute toxicity. In some instances, the experimental conditions in which these parameters are established do not reflect the actual field conditions. For example, the behaviour and fate of chemicals in seawater are often poorly described. Laboratory tests are carried out in freshwater environments and the toxicity is based on freshwater species. In most cases, the biological impacts at short- and longer term remain poorly described. Consequently there is a gap of knowledge and further improvements of the methods describing the environmental effects and the recovery after accidents should be addressed.

Within the Community framework for cooperation in the field of accidental or deliberate marine pollution (DG Environment), two recent projects have been launched to select, propose and implement in environmental programmes methodologies for the monitoring of hazardous and noxious substances following accidental spill. The questions addressed in these projects are: "Can we propose additional methodologies based on biological and ecotoxicological markers and what is their responsiveness towards chemicals?"; "Which methods can we recommend and can we use these methods *in situ* for the biomonitoring following chemical spill events?"; "How can we use these methods to assess recovery post-spill and help decision-making by EU regulators and stackeholders?"

BIOMARKER RESPONSE DISTRIBUTIONS AS TOOL TO VALIDATE ENVIRONMENTAL RISK OF HYDROCARBON DISCHARGES AND TO MONITOR EARLY EFFECTS OF EMERGING POLLUTANTS IN ARCTIC SPECIES

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Strict environmental regulation is set forth to oil and gas activities in Arctic waters offshore. Oil and gas activity discharges may contribute to combined effects of long transported pollutants, and potentially impact vulnerable ecosystems and important fisheries. An environmental management plan has been made recently to set standards for the Norwegian part of the Southern Barents Sea.

The environmental management of this maritime area will be risk based, and the state of the environment will be controlled through monitoring of environmental indicators. Adequate environmental indicators will have to be defined, and there is a need for coherent assessment schemes, i.e. that information from risk assessment and field monitoring can be evaluated in relation to the same established set of environmental standards and discharge requirements.

Preventive environmental management will require the capacity to make early diagnosis of subtle anthropogenic effects as well as to detect possible changes in populations of ecological indicator species and eco-fisheries parameters. The regional environmental management of the southern Barents Sea must clearly take into account specific regional characteristics. Therefore, assessments and monitoring must be based on species and conditions of the region, rather than on generic eco-toxicological model species.

We think that assessment of environmental sensitivity in the region can partly be accomplished through a set of representative ecological indicator species for which sensitivity distributions can be established in relation to relevant stressors. This concept unifies risk assessment of discharges and bio-monitoring and it can be applied both to the end-points of fitness as well as to early diagnosis parameters at low levels of biological organization in the selected ecological indicator species. Further, this integrated approach helps to set a common scale for predicted risk of chemical hydrocarbon discharges as well as for actual bio-monitoring of the same discharges possibly augmented by effects of other emerging pollutants.

This presentation will focus on the generic principles for establishment of sensitivity distributions related to the early diagnosis parameters (Biomarker response distributions), their state of development, how it can be used as an integrated approach to risk assessment and bio-monitoring of oily discharges combined with emerging pollutants, and inclusion of Arctic species in order to make the system operative for Arctic assessment and monitoring.

THE USE OF THE SPEAR AND TOXIC UNITS INDICATORS TO LINK ECOLOGICAL STATUS TO CHEMICAL POLLUTION

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The European Water Framework Directive (WFD) requires the assessment of ecological status using biological indicators to evaluate water quality. Hence, these indicators acquire legal status and their interpretations (i.e. ecological status) become a binding factor in the management of water resources. In this way, the selection of biological indicators may also affect the design of the programmes of measures and the probability of achieving the environmental objectives of the WFD. As aquatic ecosystems are subject to various pressures, such as eutrophication, toxic substances or hydromorphological changes, biological indicators are needed that are able to detect insufficient ecological status. The European Commission suggests the use of multimetric approaches, which respond to a gradient of diverse pressures. However, the use of such approaches somehow obscures the interpretation of the results and makes it difficult to opt for appropriate restoration measures. At the same time, many European countries still employ only the locally developed water quality metrics that mainly assess the impact of organic pollution. These indices do not adequately notify the effects of other stressors, e.g. organic toxicants or elevated levels of nutrients. Hence, a set of specific indicators is needed that discriminates between different stressors and identifies the ones that are most likely responsible for insufficient ecological status. One example for such an indicator is the SPEcies At Risk (SPEAR) index that will be presented. It was developed to detect the effects of organic toxicants on the community structure of invertebrate assemblages. For this purpose, species are classified to be at risk or not at risk of being effected by organic toxicants. SPEAR is based on the physiological sensitivity of invertebrate species towards organic compounds as well as additional life history information, i.e. the recovery potential of species. It is computed as a ratio of the total abundance of sensitive species (species at risk) and the total abundance of all species. Hence, the SPEAR index met the requirements of an indicator of the WFD concerning both the composition and abundance of the biotic assemblages.

Concerning the physico-chemical conditions that affect water quality, it is generally agreed that improvement of hydromorphological conditions and control over eutrophication are crucial to achieve good ecological status in Europe. However, stream conservation and restoration measures in the past often failed to rehabilitate stream biodiversity. At the same time, elevated levels of numerous chemicals are frequently detected in European surface waters and hence, chemical stress may be one of the driving forces for an insufficient ecological status. The often poor correlation between ecological and chemical status does not indicate that toxic pollution is an insignificant pressure for European river basins. It rather suggests that chemical status, as defined by compliance with Environmental Quality Standards (EQS) of currently 33 priority pollutants (PP), may not be a good indicator for toxic stress. On the one hand, the short list of PP hardly reflects the often much higher number of chemicals in environmental samples that all may have potential toxic effects. On the other hand, the exceedance of protective effect levels for the most sensitive organism (i.e. EQS) is unlikely to correspond to biological indicator values based on only one of the Biological Quality Elements (BQEs). Hence, rather a BQE specific indicator of toxicity is needed to link an insufficient ecological status to chemical pollution. For this purpose, the Toxic Unit (TU) approach, based on acute toxicity test data on *Daphnia magna*, was used to derive a measure of the expected mixture toxicity of the analysed organic chemicals.

The aim of the present study was to link Ecological Status (expressed as SPEAR) to an integrated measure of chemical pollution (expressed as TU). Furthermore, the mean numbers of species per site (i.e. biodiversity) were compared for sites with three levels of modelled agricultural impact and with recovery potential present or absent. The study also includes a comparison of SPEAR with presently used biotic indices of organic pollution. The results showed that SPEAR was highly correlated with the gradient of computed TU. A significant decrease in the relative abundance of species classified "at risk" was observed at sites with medium and high TU by up to 60%. This corresponds to a much lower fraction of sensitive species at the higher impacted site and reduced species richness (mean number of species) at sites with high agricultural intensity by up to 30% compared to the reference sites (Figure 1). This would correspond to reduced species richness (i.e. biodiversity) in subbasins with high agricultural intensity. The biodiversity loss could be explained by a reduced number of SPEAR at those sites, whereas the number of $\mathrm{SPE_{not}AR}$ seemed to be unaffected. At the same time, the adverse effect of the organic chemicals (i.e. pesticides) could be partially compensated when uncontaminated stream sections were present upstream that allow for recovery of the impacted communities downstream. Based on these results, both the SPEAR index and the TU indicator are recommended for their use in the WFD.

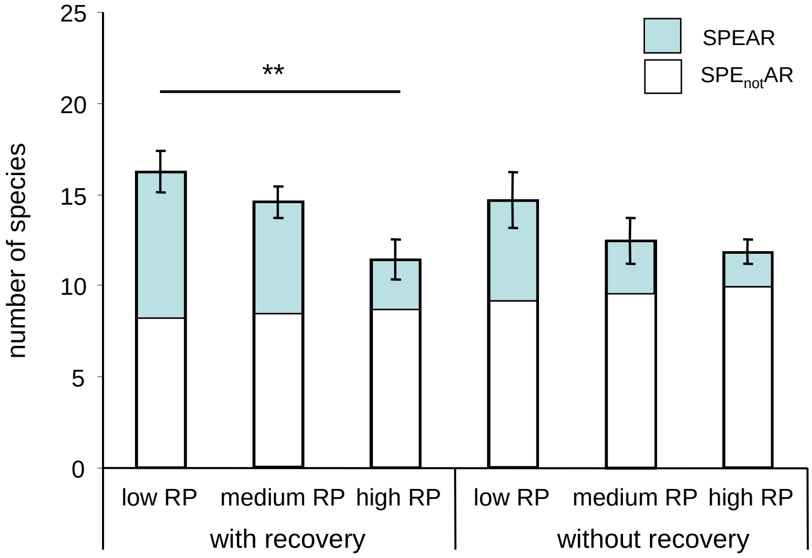

Figure 1. Differences in the mean numbers of species per site (i.e. biodiversity) were compared for sites with three levels of modelled agricultural impact and with recovery potential present or absent. The numbers of species are classified as either SPEAR or SPEnotAR.

POSTERS

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ANALYTICAL STRATEGIES FOR STEROID ANALYSIS IN WATER AT SUB PPT LEVELS

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The occurrence of steroids in aquatic environment and their effects on normal endocrine function in aquatic organisms have been subjects of current concern. Several studies have shown that also birds, reptiles and mammals in polluted areas undergo alterations of the endocrine-reproductive system. At present, a multitude of chemicals have shown to be endocrine disrupters. Among these, natural and synthetic estrogens are already effective at the lower ng/L. Their efficient control in environmental waters is made possible nowadays thanks to numerous analytical approaches available in the literature. This is the case for estradiol (E2), estrone (E1), estriol (E3) and ethinylestradiol (EE2) measurement. Estrone-3-sulfate (E1S) has also to be considered because of its stability in the environment. But less papers are concerned with androgens, gestagens and their phase I and phase II metabolites (in particular E1S).

Two analytical methodologies dealing with 32 steroids at low ppt levels, based on isotopic dilution are presented. The first one is dedicated to free steroids using two SPE preparation steps and a GC-MS/MS detection. The acquisition is carried out in SRM mode on a Quattro Micro instrument (Waters, two transitions followed per compound). In this way, DHEA, 4-androstenedione, testosterone and their main metabolites (androstanediols, etiocholanolone, androsterone), E1, E2, E3, EE2, progesterone and various contraceptive gestagens are monitored. The second method is focused on estrone-3-sulfate for which a specific combination between a SPE preparation and a LC-MS/MS detection (Gemini column, Phenomenex, Agilent 6410 LC-MS/MS system) has been developed. Identification relies upon 2002/657/EC decision to confirm unambiguously the steroid presence even at ultra-trace levels (<1 ng.L^{-1}).

ELECTROCHEMICAL IMMUNOSENSORS BASED ON ELECTROPOLYMERIZED FILMS FOR THE DETECTION OF ANTIBIOTICS, PESTICIDES.

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For four decades, biosensors and DNA and protein sensors have been the subject of increasing research effort and now constitute a major component of mainstream analytical chemistry. Among the various non-manual immobilization methods of biomolecules on transducer surface, the immobilization of biomolecules in or on electrogenerated polymer films has aroused a considerable attention because of its electrochemical addressing property. In particular, functionalized polypyrrole films offer very interesting features such as entrapment, affinity and grafting properties with biological macromolecules and their redox properties can be exploited for the reagentless transduction of hybridization and immunoreaction events [1].

Methods of detection of pesticides as the atrazine and antibiotics as the fluoroquinolone will be presented. With the aim to detect these pollutants rapidly and easily, models of these pollutants functionalized by pyrrole have been synthesized. The electrochemical behavior of these pyrrole derivatives and their electropolymerization properties have been investigated in organic media. Then the impedancemetric methods used for the detection of these pollutants will be presented.

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TOLERANCE AND EFFECT OF HEAVY METALS TO THE DUCKWEED, *LEMNA MINOR.*

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Lemna minor, an aquatic plant, was exposed to different concentrations of Cd, Cu, Ni and Zn in a modified quarter coïc solution at pH = 6.1 (± 0.1) and under a daily regime of 16 h light (101 $\mu mol.m^{-2}.s^{-1}$). Copper at concentration 0.3 mg/L and nickel at 0.5 mg/L promoted *Lemna* growth. The EC_{50} were 0.45 mg Cu/L and 1.9 mg Ni/L. Cadmium and zinc decreased by 50% the fronds growth when the medium contained respectively 0.64 and 5.5 mg/L (EC_{50}). Duckweed tolerated Cd, Cu, Ni and Zn at concentrations of 0.4, 0.4, 3 and 15 mg/L respectively without showing any morphological signs of toxicity (chlorosis, frond dislocation and necrosis). Biomass and growth rate reduction was observed at elevated metal concentrations. The toxicity metal in *Lemna* tissues was in decreasing order of damage: Cu, Cd > Ni > Zn.

Keywords: Cadmium; Copper; Nickel; Zinc; *Lemna minor*; Tolerance.

ESTONIAN BIOLOGICAL AND CHEMICAL MONITORING OF PRIORITY AND SEA/RIVER BASIC-SPECIFIC SUBSTANCES

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Introduction

The Estonian Environmental Research Centre (EERC) is specialised in chemical analyses in the field of environment protection. EERC also provide a comprehensive range of analyses for drinking water, foodstaff, fuel etc. The predecessor of the EERC was the Estonian Environmental Laboratory, which was founded in 1991 to serve the purposes of the Ministry of the Environment, Ministry of the Agriculture and the Tartu University, Estonian Marine Institute EERC facilities are well-equipped, enabling precise determination of environmen-tally dangerous substances in different sample types.

The Center is accredited by the German accreditation bureau Deutsches Akkreditierunssystem Prüfwesen GmbH (DAP) (Reg no DAP-PL-3131.99) and the Estonian Accreditation Centre (EAK) (Reg no L008). We are also licensed by the Estonian Ministry of Environment to perform environmental impact assessments.

Method

Objective of monitoring of priority hazardous substances in Estonian surface water bodies is to observe long-term changes of hazardous substances in Estonian water bodies is to observe long-term changes of hazardous substances in Estonian surface water bodies and aquatic ecosystem, and provide assessment of contamination of surface water bodies and aquatic ecosystem, which will be the basis for planning of further measures for the achievement of good condition of surface water bodies.

Discussion

As a result, the Estonian National Environmental Monitoring Programme (NEMP) was initiated in 1994 (Roots, Saare, 1996). Presently there are altogether around 1800 monitoring stations in the monitoring set of 68 sub-programmes of 11 monitoring themes, number of parameters reaching 250 (Roose, Roots, 2005).

Objective of the directive 2000/60/EC of the European Parlament and the Council of the European Union “Water Framework Directive” is to establish framework for the protection of inland surface water, transitional waters, coastal waters and groundwater.In order to fulfil the requirements proceeding from the Directive 2000/60/EC of the European Parlament and Council and the Council Directive 76/464/EEC all member states shall specify priority hazardous substances for surface water bodies on national level. The implemented Estonian national environmental monitoring programme of hazardous substances, which follows European Union and Helsinki Commission recommendations, covers all major problem areas, sites, and aspects on a national scale. Operational monitoring by companies, required by the environmental permit system, complements the national network and gives the opportunity for detailed assessment of trends in waterbodies (Roose, Roots, 2005).

Hazardous substances are divided into two lists on the basis of hazardousness. List 1 includes substances, which release of disposal into water shall be prevented and List 2 substances, which release or disposal into water shall be limited. Compared to the old member states of the European Union few attention has been paid on the monitoring of hazardous substances in Estonia, but it is still possible to draw the first conclusions for priority substances. List of priority hazardous substances for Estonian surface water bodies should include five hazardous priority compounds (Determination of priority hazardous subs-tances for Estonian surface water bodies and formation of monitoring network (Comp. O. Roots). 2005. Estonian Environmental Research Centre (Agreement no. 2005/K-11-1-2005/52), Report, 84p +Annexes). The mentioned list is not final, because upon reception of new information hazardous substances should be added into the list, or, if no hazard exists, removed from the list.

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ENVIRONMENTAL EXPOSURE OF PHARMACEUTICALS IN THE SOMES VALLEY WATERSHED IN ROMANIA

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The occurrence of pharmaceutical and personal care products in the aquatic environment has been recognized as one of emerging issues in environmental chemistry[1]. The widespread presence of pharmaceuticals in the aquatic environment is due to their extensive use in medical practices and incomplete removal in wastewater treatment plants (WWTP)[2,3].

The absence of data on the environmental exposure in Eastern Europe is significant, since use patterns and volumes differ from country to country.

In this part of Europe, the majority of wastewater, from highly-populated cities and industrial complex zones, is still discharged into surface waters without proper treatment or after inefficient treatment. In respect to this, it is important to determine the environmental occurrence and fate of PPCPs in wastewaters and surface waters.

The aim of the present study is to obtain systematic and extensive analytically measured data on the occurrence and environmental behaviour of pharmaceutical and personal care products in environmental samples of the aquatic ecosystem from Somes Valley watershed, Romania.

The objective were to increase our knowledge on the fate and behaviour of selected PPCPs in Rumanian WWTPs. The efficiency of the removal of selected PPCPs in conventional wastewater treatment was studied.

The occurrence of selected PPCPs was monitored in the aqueous phase in order to determine whether PPCPs occurring in the dissolved fraction are eliminated to any extend. Subsequently the occurrence of adsorbed PPCPs was determined.

Based on obtained results the mass balance was calculated by absolute loads in kg/d and mass flows of PPCPs through primary treatment (mechanical treatment) and secondary treatment (biological treatment).

The occurrence of PPCPs was monitored in the dissolved fraction of the wastewater effluents of different WWTPs discharging into the Somes River. This allowed calculating an average removal efficiency of different WWTPs along the Somes River and quantifying the PPCPs inputs into the receiving river. This information also allowed assessing the efficiency of different Rumanian WWTPs.

Analysing the main final effluents in the Somes Valley Watershed in Transylvania will account for the significant inputs of PPCPs into the river. The final effluents from 6 WWTPs was considered.

In order to investigate to which extend PPCPs are removed along the river, PPCPs was sampled at different locations. Sampling at the WWTPs were synchronized with the river sampling in order to account for different consumption patterns and water flow.

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FISH EROD ACTIVITY IS WIDELY USED TO ASSESS THE EXPOSURE OF ORGANISMS TO CYP P450 INDUCER IN MARINE AND FRESH WATER AND SEDIMENT

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Recently we developed a non « destructive » method (1) based on the biotransformation activity of the ethoxyresorufin substrate in the pro-larvae of the fish *Danio rerio.* EROD activity induction is detected by measuring the fluorescence increase in the test medium due to the excretion of the resorufin by the fish, after pre-exposure to chemicals or environmental samples.

Therefore, the proposed methodology allows a simple, sensitive and reproducible micro biotest to be performed for the detection of sublethal concentrations of AhR chemical inducers in environmental samples, such as natural waters and organic extracts of sediments. Here we present the results obtained with several water and sediment sampled from a French river upstream and downstream an urban area. *D. rerio* embryos and prolarvae were either exposed to water samples or to organic extracts of sediment.

Low but significant inductions were obtained with water samples downstream the city of Lyon. More severe inductions until 5 fold the basal activity were obtained with sediment extracts (closed to induction measured in positive control: Benzo-a-Pyrene 30 µg/l) .

The first results obtained are encouraging as this microbiotest allows to discriminate between low or moderate and contaminated sites downstream urban area sites. Moreover, the inter assays comparison have revealed a low variability of the negatives and positives controls, which allows reliable comparisons between sites and time of sampling.

This microbiotest, can be a valuable screening approach to quickly assess the presence of Cyp450 inducers in environmental samples such as water, effluent and sediment.

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XENOBIOTIC CONCENTRATIONS IN RIVER WATERS OF LUXEMBOURG

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The first studies which deal with the impact of xenobiotic compounds on human health and the environment were published in the early 1990's. These studies pointed out the endocrine disrupting effect of some xenobiotics on biological functions in animals.
This work deals with the study of some xenobiotics and their occurrence in the surface waters of Luxembourg. Different sample points were chosen along the Alzette river, including a waste water treatment plant. Furthermore, flood events were investigated in a tributary of the Alzette, the Mess river.

The twelve molecules analyzed belong to three families : the estrogens, the antibiotics (sulfonamides and tetracyclines) and the pain killers. With the goal to extract all these compounds with very different physico-chemical properties, a single step performing extraction method by solid phase extraction was developed on copolymer cartridges. After this concentration step, xenobiotics were quantified by High Performance Liquid Chromatography coupled with a tandem mass spectrometer with triple quadrupole. Among the twelve xenobiotics monitored, the pain killers are the most abundant in the surface waters. During flood events of the Mess river ibuprofen was measured at a concentration between 10 and 2300 ng/L; in the wastewater treatment plant, these concentrations reached 4000 ng/L. Compared to ibuprofen, sulfonamides reached concentrations of approximately 20 ng/L in the Alzette and 50 ng/L for the wastewater treatment plant. Because of their role in bacterial resistance these concentrations of antibiotics are of concern. Alarming are also the estrogen concentrations (1-240 ng/L) which may be responsible for disturbing the endocrine system in animals.

Keywords : surface water quality, antibiotics, estrogens, pain killers, HPLC/MS-MS.

FACTORS INFLUENCING THE METAL ACCUMULATION IN MOSSES

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In the late 1980’s, the International Cooperative Programme (ICP) on the effects of air pollution on natural vegetation and crops (*ICP Vegetation)* was established to quantify damage to plants by ozone and other pollutants. One part of the ICP Vegetation addresses the assessment of possible effects of atmospheric metal depositions to terrestrial ecosystems. Here, ectohydric mosses are used to monitor the atmospheric metal bioaccumulation eventually causing retarded growth of trees and crops and increased levels of toxic metals in the food chain leading to man. The monitoring is performed in terms of national moss surveys in more than 20 European since 1990 every five years. Each of the participating countries, including Germany, follow a UNECE monitoring manual, giving recommendations on the sampling procedures and on critical distances to keep from emission sources and tree crowns.

Several studies aimed at proving the influence of certain boundary factors on the bioaccumulation in the mosses (summarised in Zechmeister et al. 2003). Nevertheless, the discussion is rather controversial and therefore the interpretation of the spatial variability of the metal bioaccumulation remains difficult. Hence, this study uses the monitoring data from the German moss survey 2005 and other available geoinformation to examine the hypothesis that local and regional factors overlay the deposition effects on the bioaccumulation.

The monitoring data used for the testing of the hypothesis mentioned above consists of measurement data on up to 12 metal elements and multi-metal indices (MMI) that were computed by percentile statistics ranging from 1 for low to 10 for high metal accumulation (Schröder and Pesch 2004). The MMI aggregates the element loads of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), nickel (Ni), lead (Pb), antimony (Sb), vanadium (V), titanium (Ti) and zinc (Zn) which were analysed in moss samples collected across the entire German territory. Next to the measurement data sampling sites describing metadata is also included in the statistical analyses. Such provides information on the emission situation around the monitoring sites in terms of distances to roads, motorways, human settlements or industries. Further, the sampling sites are characterised in terms of growth type and frequency of occurrence of the sampled moss specie, topographical aspects and the potential influence of the filter effect of trees and bushes.

In addition to the site-specific data surface data were included in the hypothesis testing. Precipitation sums for the 2 to 3 year accumulation period were computed geostatistically from respected measurement data made available by the German Meteorological Service. To better account for the atmospheric emission situation around the monitoring site aerial percentages of selected land use categories within defined radiuses were derived from the Corine land cover map 2000. Maps on background values for metal loads in the upper soil were used to account for adsorbed soil particles. At last, a distance raster depicting the distance of each of 1 * 1 km² centers to the North and the Baltic Sea was calculated with help of a coastline GIS-layer to account for the sea-spray-effect.

For the assessment and ranking of boundary factors of the metal bioaccumulation two decision tree algorithms were applied: Classification and Regression Trees (CART) and Chi-Square-Automatic-Detection (CHAID). CHAID and CART produce easy to understand explanation schemes for a target variable, here the element loads in the mosses and the MMI, from the chosen set of meaningful predicting variables, here the above described metadata and intersected surface information. With both procedures a given dataset (= root node) is subdivided into subclasses (= subnodes) via a series of multiple splits. Each split is performed by the features of that predicting variable that enables an optimal subdivision in terms of a defined statistical goal: CART chooses those predicting variables that optimise the homogeneity with regard to the features of the target variable. Provided the latter is metric, CART aims at minimising the within node variance. An essential feature of CHAID is the use of inference statistics to decide which predicting variables are of maximum importance. Such is done by means of chi-square tests when the target variable is categorical or ANOVA F-tests when the target variable is continuous.

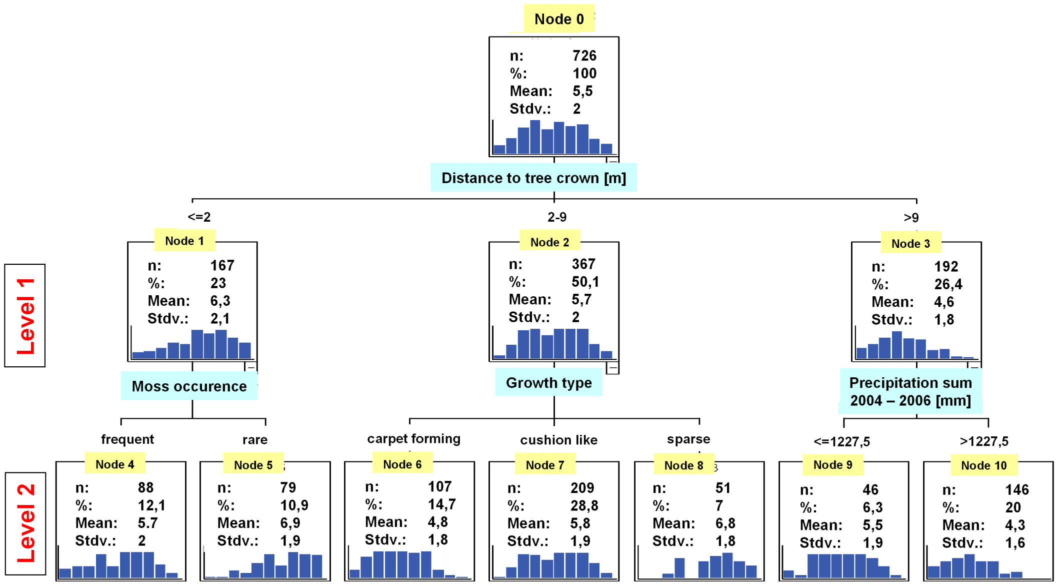

Fig. 1. CHAID-dendrogram for the MMI 2005 (Levels 1 and 2)

The results of the study are exemplified in terms of the calculated CHAID-tree for the MMI (first two levels). The entire data set, consisting of 726 monitoring sites, is at first subdivided into three subnodes with the help of the distance of the monitoring site to the nearest tree crown: one subnode containing sites sampled less than 2 m, one with sites sampled between 2 and 9 m and one with sites sampled more than 9 m afar from the nearest tree crown. The respective mean values of the MMI clearly indicate the effect of canopy drip: The nearer the mosses grow to trees the higher the metal bioaccumulation is by average. Further splitting is done with help of the predictors 'Moss occurrence' and 'Growth type'. In both cases higher MMI values occur in classes with sites that exhibit rare moss occurrence and sparse growth, possibly relating to toxic effects of the metal accumulation. The class with sites sampled the most afar from trees are subdivided by the precipitation sum for the accumulation period. The node with the higher precipitation sum by average thereby shows less metal bioaccumulation than the one with less precipitation. This tendency may be related to the wash-off-effect of soil particles and dust that are adsorbed by the mosses.

The presented tree is only one of 26 decision trees that were calculated to test the hypothesis mentioned above. Next to the ones calculated for the MMI, CART and CHAID-trees were also derived for each of twelve metal elements. The resulting trees are quite different, referring to metal specific bioaccumulation of the mosses. In summary, predictors related to canopy drip, precipitation, the sea-spray-effect, the collected mosses (species, growth type, moss occurrence) and factors related to the metal emission situation around the monitoring sites were chosen most often. In order to substantiate the findings the presented approach should be applied on data from other countries participating in the European moss surveys.

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VITELLOGENIN-LIKE PROTEINS IN CLAMS AND NONYLPHENOL LEVELS IN WATER, SEDIMENTS AND BIOTA: THE LAGOON OF VENICE AS A CASE STUDY

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Nonylphenol (NP) is commonly used in the production of nonylphenol ethoxylates (NPEs), NP phosphites and insecticides. As a consequence of their widespread use, particularly of NPEs as nonionic surfactants in household and industrial detergents, large quantities of these compounds are discharged into aquatic environments, either directly from untreated effluent or indirectly from sewage treatment plants (STPs). In aquatic ecosystems, NPEs biodegrade to de-ethoxylated intermediates, whose final product is NP. It is known that NP can have estrogenic effects in aquatic organisms, being able to mimic the action of endogenous estrogens by binding to estrogen receptors. As a consequence, it can induce vitellogenin (Vg) synthesis, the major precursor of the egg-yolk proteins of oviparous organisms.

In this study, Vg induction was evaluated in the clam *Tapes philippinarum* seasonally collected (from October 2003 to June 2004) in 3 sites of the Lagoon of Venice: Marghera (MA), characterised by high industrial contamination levels, Campalto (CA), nearby a STP, and Poveglia (PO) close to the lagoon inlet, which was considered as a reference site. Vg-like proteins were determined in the haemolymph and digestive gland of sexually differentiated males and females when it was possible to distinguish them by microscopic observation of a smear of gonadal tissue. In the haemolymph Ca^{2+} levels were also measured, this parameter being strictly related to the presence of Vg-like proteins. During clam samplings, water and sediments were also collected to determine the concentrations of NP, which was chosen as a marker of the presence of anthropic sources of estrogenic compounds.

Both male and female clams collected at CA and MA generally showed significantly higher Vg-like proteins in haemolymph with respect to specimens from Poveglia throughout the study period. Significant differences in Vg-like proteins were also detected in digestive gland of clams from the 3 sampling sites. In particular, sexually undifferentiated clams collected at CA showed the highest protein levels in January 2004, suggesting that endocrine alterations mostly occurred far from the reproductive period of animals. Ca^{2+} levels were generally higher in haemolymph of both female and males clams from CA and MA. Ca^{2+} levels showed also a clear seasonal trend, with higher values in spring-summer period than in autumn-winter.

In the same campaign NP levels were determined in sediment, water and Tapes philippinarum samples. MA and CA sampling sites were found to be more contaminated than PO. The highest concentrations in the range of 64-246 ng NP/L d.w., were always measured in MA sediments while the lowest concentrations in the range of 27-99 ng NP/L d.w. were measured in sediments from PO.

Nonylphenol was found to be the most abundant compound in all sediment samples in every sampling season. Sampling sites showed a difference in concentrations but none in typology of pollution that seems to be mostly related to industrial origin. Indeed NP/NPE ratios that indicate difference in degradation processes, resulted always homogeneous (always > 1) remarking the same typology and origin of the sediment pollution in the Venice lagoon.

The NP concentration in water samples collected from different sites in different seasons was as often as not close to the detection limit(50 ng/L). The concentrations of NP in the range of 60-180 ng/g d.w. measured in clams, were instead of the same order of magnitude in the three sampling sites, the highest values were found in January and April in two sites (PO and MA) whereas the lowest value was measured in June in samples collected at CA. The concentrations of NPE1 and NPE2 were always below the limit of detection, thus reflecting on one hand the different lipophilic power of the compounds, on the other hand their water concentrations.

The analyses on *T. philippinarum* showed a seasonal trend characterised by a raising in the NP concentration measured in soft tissues of clams collected from October to April, followed by a prompt decrease in June, when the sexual maturity and spawning phase are reached.

This study demonstrates that Vg-like protein levels significantly increased in both hemolymph and digestive gland from sexually differentiated and undifferentiated clams from the most NP contaminated sites. Considering that endocrine disruption due to exposure to estrogenic compounds, such as NP, may cause fertility reduction, alteration in sex ratio, and a decrease in reproductive rate, a condition of potential risk for clam populations in estuarine areas is highlighted.

APPLICATION OF ANTIBIOTIC SENSITIVE BIOASSAYS TO ASSESS WATER AND WASTE WATER QUALITY

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Bioassays are powerful tools for the assessment of environmental quality, and therefore the potential toxicity of the surface waters in the Netherlands is being monitored with a battery of bioassays. This test battery is responding well to most compounds causing a narcotic effect, but nowadays more and more attention is paid to compounds with a specific mode of action, like endocrine disrupters and pharmaceuticals.

To detect specific effects of antibiotics we tested a 96 well application of the Poultry Meat Scan© from RIKILT (multiplate) and a 96 well application of a test measuring photosynthesis efficiency of cyanobacteria (cyanotox). The obtained results were compared with those from two tests used in the traditional test battery (photosynthesis efficiency of green algae with PAM fluorescence and inhibition of luminescence with acute Microtox®).

To compare the specific sensitivities of these four test systems, we used 6 antibiotics from 5 different classes (sulfamethoxazole, trimethoprim, flumequine, tylosin, streptomycin and oxytetracycline).
The multiplate test and the cyanotox test yielded lower EC_{50} values than the two traditional tests for most of the antibiotics, because the prokaryotic organisms used in these tests are more sensitive to antibiotics. Consequently these tests can be used to measure effects at lower antibiotics concentrations. The multiplate test has the advantage that it gives information on the effects on five different bacteria genera, increasing the chance of identifying the responsible group of antibiotics.

We used the multiplate test in a pilot study to test for the effect of antibiotics in effluent from waste water treatment plants. Since inhibition of growth in bacteria sensitive to antibiotics from the macrolides/ ß-lactam group and the sulfonamides group was found, attention should be given to these antibiotic groups.

Current research focuses on optimization of the 96 well application of the multiplate test and on determining the specificity of this test by measuring the effect of other compounds than antibiotics (other pharmaceuticals, disinfectants, pesticides etc).

ORGANOHALOGEN COMPOUNDS IN THE EGGS OF PEREGRINE FALCONS AND OTHER WILD BIRD SPECIES IN BADEN-WUERTTEMBERG – PRESENT STATE AND TIME TREND

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The concentrations of some organohalogen (OH) compounds have been measured in eggs of Peregrine falcons, owls, jackdaws and tits from the German state of Baden-Wuerttemberg. Data from failed falcon eggs are available for dichlorodiphenyl-trichloroethane (DDT), hexachloro-cyclohexane (HCH), hexachlorobenzene (HCB), heptachloro-epoxide (HCEP) and polychlorinated biphenyls (PCB) from 1970 to 2007.

During the past 40 years the organochlorine (OC) pollution of the eggs of wild birds shows a general decrease which is paralleled by an encouraging increase of the number of breeding pairs of the Peregrine falcon in the state. Of 88 analytes searched for, only 51 could be detected in last year's eggs. PCBs and total DDT, which consists of the DDT-metabolite DDE to >99%, were found as the two main pollutants in the Peregrine eggs. Attempts were made to establish factors that influence the regional distribution of PCBs and DDE.

Despite the decline observed in the past, the OC pollution of eggs of the Peregrine falcon is still alarming at present. DDE exceeds 50fold the German limit value for the human consumption of chicken eggs and PCBs exceed 9fold this limit. Seventeen falcon eggs were analysed for polychlorinated dibenzodioxins and –furans (PCDD/F) in the years between 2000 and 2003. Dioxins and furans contributed only 15% of the dioxin equivalents (WHO-TEQ), whereas 85% may be attributed to the PCBs. The contamination peaked at >900 pg/g (dry matter). This value exceeds the EU limit value for chicken eggs by a factor of 600. The dioxin equivalents have reached the no observed adverse effect level (NOAEL) for birds of prey (200 pg/g dm) in the least polluted eggs, and exceed this level 5fold in the most polluted ones.

Eighteen eggs were selected from the years 2004 to 2007 and analyzed via the dioxin responsive chemically activated luciferase expression bioassay (DR CALUX®). The bioassay results (ranging from 44 to 2163 with a mean of 364 pg dioxin TEQ/g dm) confirm the earlier chemical analyses. Similar bioassays were performed with estrogen and androgen responsive cells, respectively (ER CALUX® and AR CALUX®). The results will be reported as 17β-estradiol and flutamide equivalents in the egg samples.

Polybrominated diphenylethers (PBDEs) have been measured in Peregrine eggs since 2003. After an initial sharp rise by a factor of 14 their levels seem to be declining in the years after 2005. Further research is necessary to develop adequate criteria for a reliable assessment of the human health and ecological risk caused by environmental OH pollutants and their metabolites. Despite the decline in OH concentrations in the past decades the Peregrine population of Baden-Württemberg is still at risk through OH compounds in their environment. Therefore, the egg monitoring should continue, and measures to reduce the contamination should be considered where appropriate.

A GIS-BASED CHEMICAL RISK ASSESSMENT FOR INFORMING MONITORING STRATEGIES AT THE CATCHMENT SCALE

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Contamination of surface waters by micro-organic compounds is a matter of concern for specialist scientists and the public at large. This concern arises from possible impacts on the use of the water for recreational activities, protection of ecosystems and the loss of drinking water resources. Environmental damage has been shown to have occurred, examples include intersex in fish and water snails that were related to endocrine disrupting chemicals (1,2). Other (so called emerging) contaminants at trace levels are also raising concerns for their potential effects on wildlife. These include some brominated flame retardants, pharmaceuticals and nanoparticles (3-5). Scientists and regulators need to examine and understand how wildlife may be harmed by the chemicals, but also to assess the extent of the problem. In assessing the overall risk to the aquatic environment the concentration of the chemical becomes the key question, specifically:

a) Is the chemical present in the aquatic environment?
b) Where and when will the concentration of the chemical be such that it might harm aquatic organisms?

Clearly, only reliable measurements can unequivocally answer question (a). However, guidance from a hydrologist on when and where to measure would increase the chances of detecting the chemical, as would knowledge of the elimination efficiency of different sewage treatment plants in the case of point sources. To answer (b) requires either reliable measured environmental concentration (MEC) or plausible predicted environmental concentrations (PEC) following a modelling exercise. These concentrations can then be combined with ecotoxicological data to make a risk assessment for the chemicals of interest.

This poster describes a modelling approach to estimating concentrations of chemicals whose primary source is sewage treatment plants (STPs) and whose polluting loads are linked to the populations served by those STPs. The modelling system is GIS based and can therefore predict the spatial variation of a contaminant throughout a catchment.

The application of the model to predict concentrations of three steroid estrogens, estradiol (E2), estrone (E1) and ethinylestradiol (EE2) in river water is presented as an example of its use in the context of identifying concentration "hot spots" and also in risk assessment. These chemicals are of concern because they have been shown to cause feminisation of male fish in rivers receiving STP effluents: the effect is widespread in the UK (1).

The model has been setup to cover the inland waters of England and Wales, including 357 catchments, covering 122,000 km^2, incorporating over 2,000 STPs serving over 29 million people. The model predicts the mean (plus standard deviation) and 90th percentile concentrations of E2, E1 and EE2 for 10,313 individual river reaches (21,452 km). The results are output as an ArcGis shapefile, which allows the user to easily assimilate the data in map form.

The model computes how much of each of the three oestrogens would be discharged into the receiving waters from the flow of the STPs, the type of treatment and the populations they serve. The in-stream concentrations are then calculated from dilutions down through the catchment river network including a first order natural attenuation rate. It also accounts for the initial conversion of oestradiol into its first degradation product, oestrone. The model inputs and some model parameters are described by distributions. Therefore outputs are in terms of predicted distributions of concentrations for each river reach.

For the risk assessment these concentrations were compared to predicted no effect concentrations (PNECs) established for the three steroid oestrogens individually and in combination to define river reaches that would be at risk of endocrine disruption. PNECs of 0.1 ng/L, 1 ng/L and 3 ng/l were established for EE2, E2 and E1 respectively. The combined effect was made in terms of the E2 equivalent concentrations which divides each steroid concentration by its respective PNEC to introduce a measure of relative potency before the values are summed (the effects of steroids have been shown to be additive). Thus the [E2 equivalent] = [EE2]/0.1 + [E2]/1 + [E1]/3 (where the square brackets denote concentrations).

The mean concentrations of the steroids were used in the risk assessment. Overall, 61% of river reaches in England and Wales were predicted not to be at risk from endocrine disruption (< 1 ng/L E2 eqv), 38% were predicted to be at risk (>1 ng/L E2 eqv) and 1% at high risk (>10 ng/L E2 eqv) based on river length. However, while the overall extent of the risk is of interest to regulators, the individual detail of the maps is of more interest when it comes to the targeting of chemical or biological monitoring programmes (selected, detailed maps are presented in the poster). For example this risk assessment indentified the locations of 186 STPs whose discharges would be likely to lead to a "high risk" (> 10 ng/L E2 eqv) of intersex in fish living in the receiving water downstream of these plants. If it was not already known that the effects of these chemicals were widespread, it is clear that these locations would be ones that could be considered for futher investigation. It is apparent, therefore, that the method presented here has a wide potential application for assessing known and emerging contaminants whose source can be attributed to STP discharges.

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DEVELOPMENT OF AN ANALYTICAL METHODOLOGY TO ASSESS CONTAMINTION OF INVERTEBRATES BY ENDOCRINE DISRUPTORS.

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Endocrine disruptors (EDs) are a structurally diverse group of compounds that may adversely affect the health of wildlife and fisheries, by interaction with the endocrine system. Both vertebrates and invertebrates are susceptible to the action of EDs. However, the issue of endocrine disruption has been studied mostly in vertebrates, due to the lack of knowledge on endocrine system and mechanisms of action of EDs in invertebrates. Studies on endocrine disruption in marine invertebrates are important because they account for 95 % of the known species of animals on earth and provide some of the best documented examples for deleterious effects of EDs.

In this context, two common freshwater gastropod molluscs, representative of freshwater ecosystems, *Potamopyrgus antipodarum* and *Valvata piscinalis*, have been chosen as sentinel species for the determination of endocrine disruptor effects. As there was no data available on endogeneous levels of steroids in these gastropods, the first step of the study was to further investigate the presence and levels of sexual steroids (progesterone, testosterone and estradiol) in the freshwater molluscs.

An Analytical method based on liquid chromatography – tandem mass spectrometry was developed because the technique combines high sensitivity and specificity. Preliminary steps of sample preparation including sampling, liquid-liquid extraction and purification by solid-phase extraction were optimized. The described analytical procedure was sufficiently sensitive and selective to detect the target analytes in the ng/g range. Both free and esterified steroid levels were evaluated.

MULTI-RESIDUE ANALYTICAL METHODOLOGY BASED ON LC-MS/MS FOR TRACE LEVEL DETERMINATION OF PHARMACEUTICALS IN SURFACE AND GROUNDWATERS

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Large amounts of pharmaceutical compounds from a wide spectrum of medicinal classes are prescribed in human medical care and are used in veterinary medicine worldwide. So far, therapeutically active compounds have not generally been seen as potentially toxic substances, therefore, unlike many other anthropogenic pollutants, pharmaceuticals have not been subjected to detailed research regarding their possible adverse environmental effects. So, pharmaceutical residues are environmental contaminants of recent concern and the requirements for analytical methods are mainly dictated by low concentrations found in aqueous and solid environmental samples.

Today, the technique of choice for analysis of these groups of substances is liquid chromatography coupled to mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS-MS). In the last decades, this technique has made impressive progress that has made possible the analysis of many environmental pollutants in a faster, more convenient, and more sensitive way. In some cases the analysis of compounds that could not be determined before has become possible.

In this context, we decided to develop a new multi-residue analytical methodology based on LC-MS/MS for trace level determination of 29 pharmaceuticals belonging to different therapeutical classes: antibiotics, beta-blockers, psychiatric drugs, non-steroidal anti-inflammatories, analgesics and lipid regulating agents cholesterol lowering agents. The method allows simultaneous extraction of the pharmaceuticals compounds by solid-phase extraction (SPE) using the Waters Oasis HLB. The analytes were then identified and quantitatively determined by liquid chromatography–tandem mass spectrometry (LC–MS–MS) using multiple reaction monitoring (MRM).

The developed analytical method was successfully applied to the analysis of pharmaceutical residues in surface and groundwaters. For both, the most ubiquitous compounds belonged to the group of anti-inflammatories and analgesics, antibiotics, psychiatric drugs being acetaminophen, salicylic acid, ibuprofen, ketoprofen, naproxen, diclofenac, trimethoprim, sulfamethoxazole, roxithromycin, carbamazepine and oxazepam the most frequently detected compounds.

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