Abstracts

Oral presentations

Research update on Priority and Emerging pollutants in the environment: occurrence and early effects detection

1. Identification of mutagens in the European Rivers. **Bougeard C., Gallampois C., Kormos J.L., Simon E., Brack W., Lamoree M. and Ternes T.A.**
2. Aquatic organisms as bio-indicator on multiple levels. **Agbo S., Möller A., Shinn C. and Slootweg T.**
3. Priority and Emerging pollutants in the environment: recommendations concerning sampling strategies and analytical procedures. **Barceló D. and López de Alda M.**
4. Search for key toxicants: Effect Directed Analysis and their applicability for monitoring. **Brack W., Balaam J., Bandow N., Barceló D., Brix R., Lamoree M., Leonards P., Lübcke-von Varel U., Machala M., Thomas K. and Weiss J.**
5. Priority and Emerging pollutants: the role of WWTP for sanitation and the significance of transformation products. **Kormos J.L. and Ternes T.A.**
6. Applicability and limitations of biomarkers. **Segner H. and Möller A.M.**

Research update on Priority and Emerging pollutants: ecosystem effects and recommendation for future

7. The use of biofilm communities to assess ecological risks of pollutants in aquatic ecosystems. **Bonnineau C., Sans-Piché F., Proia L., Lubarsky H., Geiszinger A., Romaní A.M., Schmitt-Jansen M., Gerbersdorf S. and Guasch H.**
8. Improving data sampling for better ecosystem diagnostic. **Faggiano L., Guénard G., Gevrey M. and Lek S.**
9. *Plenary session.* Ecotoxicology in fluvial ecosystems: from standardized single species tests to ecosystem manipulation. **Clements W.**
10. Current approaches used to evaluate ecological integrity: biotic indices versus community ecotoxicology studies. **Sabater S. and Muñoz I.**
11. Recommendations in the Design of Water Monitoring Programmes based on newly developed Complementary Methods. **Carafa R., Real M., Munné A., Ginebreda A. and Guasch H.**

Management Plans overview

12. Establishment of the good chemical status of fresh surface water in France. Application of the directive 2008/105/CE. **Rebillard J.P., Allonier-Fernandes A.S., Riou C., Halkett C., Pelte T., Verlhac A. and Demouliere R.**
13. Establishment of the good chemical status of water in Spain. Application of the directive 2008/105/CE. **Puig A.**
14. Methods of analysis of priority and emerging pollutants: problems and establishment of quality standards. **Caixach J.**
15. Monitoring of priority and related substances in the rivers of Catalonia, under the application of the WFD: overview of results and risk assessment. **Ginebreda A., Munné A., Carafa R. and Tirapu Ll.**
16. Control of hazardous substances in the Ebro River Basin. **Cortés S.**

Abstracts

Poster presentations

1. Linking SPATIOtemporal variations of diuron contamination to biofilm induced tolerance in a river. **Pesce S., Margoum C. and Montuelle B.**

2. Multi-biomarker responses of benthic macroinvertebrate species to diagnose the ecological status of polluted rivers. **Damasio J., Puértolas L., Prat N., Rieradevall M., Soares A.M.V.M. and Barata C.**

3. Patterns in diatom community structure in rivers submitted to heavy metal inputs in different countries and consequences for biomonitoring. **Morin S., Cordonier A., Duong T.T., Lavoie I., Tornés E., Bonet B., Corcoll N., Faggiano L. , Guasch H. , Sabater S. and Coste M.**

4. Pesticide gradients in rivers: fish-based investigative field monitoring. **Shinn C., Grenouillet G. and Lek S.**

5. Effect Directed Analysis of a benthic food chain using genotoxicity screening. **Simon E., Lamoree M., Leonards P., Hamers T., Reifferscheid G., Spira D., Rudoll R. and Boer J.**

6. Effect Directed Analysis performed on European river sediment with emphasis on the identification of androgen disrupting compounds. **Weiss J., Hamers T., van der Linden S., Leonards P. and Lamoree M.**

7. Identification of androgen disrupting compounds in effect directed analysed river sediment with an LTQ-Orbitrap. **Weiss J., Leonards P., Stroomberg G., de Boer R. and Lamoree M.**

8. Biodegradation of Pharmaceuticals during Wastewater Treatment and the Evaluation of Proteomics for Metabolic Pathway Elucidation. **Collado N., Osuna B., Comas J., Rodríguez-Roda I. and Sipma J.**

9. Uptake and effects of 1,2,3,5,7-pentachloronaphthalene in an aquatic food chain: from sediment via benthic organisms (*Lumbriculus variegatus*) to rainbow trout (*Oncorhynchus mykiss*). **Slootweg T., Dömötörová M., Fabišiková A., Igumnova E., Mayer P., Möller A., Nikiforov V., Schmidt J., Smith K. and Liebig M.**

10. The use of a new set of fluvial biofilms biomarkers to assess the effects of metals: contribution to the water framework directive application. **Bonet B., Corcoll N., Morin S. and Guasch H.**

11. Predicting the effects of toxic substances upon functional diversity of North-American fish species. **Faggiano L., de Zwar D., Dyer S., Lek S. and Gevrey M.**

12. Investigating the Environmental Fate of Emerging and Priority Contaminants - Identification of Transformation Products. **Kormos J.L., Schulz M. and Ternes T. A.**

13. Water toxicity assessment in Catalan rivers (NE Spain) using Species Sensitivity Distribution and Artificial Neural Networks. **Carafa R., Faggiano L., Real M., Munné A., Ginebreda A., Guasch H., Flo M. and Tirapu Ll.**

14. Are biotic indices sensitive to river micropollutants? A comparison of metrics based on diatoms and macroinvertebrates. **Blanco S. and Bécares E.**

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index | I

Identification of mutagens in the European rivers

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Potentially the most effective means of understanding cause-effect relationship of chemical pollutants on European river ecosystems is to identify key toxicants, their bioavailability, fate, transport and biological effects. Within KEYBIOEFFECTS, several approaches have been incorporated to identify the key environmental pollutants. However, the complexity of environmental contamination tends to limit the identification of unknown toxicants. It is a challenge to relate the presence of a compound or a complex mixture of compounds to the biological impact or outcome observed in the natural environment. To identify toxic compounds in complex environmental mixtures, effect-directed analysis (EDA), combining biotesting, fractionation and chemical analysis, was undertaken.

The focus of the EDA study presented here was on the identification of mutagens, which are mainly polyaromatic compounds with at least three fused rings. The EDA method relies on a specific passive sampler, blue rayon (BR) able to adsorb those planar aromatic chemicals, a three step fractionation method and a LC/MS/MS high resolution method. The fractionation and analytical methods were developed with 50 standards chosen regarding their planarity, mutagenicity, lipophilicity, polarity, etc. The first step, the separation of the compounds present in the sample was based on their behaviour in water; they act as neutral, basic or acid. The BR extract was fractionated using ion exchange cartridges into acids, bases and neutral compounds. The two next fractionation steps rely on HPLC separation, using 2 different static phases (Phenyl-hexyl and polymeric C18). The analysis of the mutagenic fractions was performed by a LC/MS/MS high resolution Orbitrap (Thermofisher) equipped with an electrospray ionisation source for the most polar compounds and an atmospheric pressure chemical ionisation source for less polar compounds. To determine the mutagenicity, the Ames fluctuation test was undertaken using the *Salmonella* TA98.

Although, EDA has been applied successfully to identify toxicants, the test of mutagenicity procedure can lead to a misinterpretation of the results. Hydrophobic organic compounds are thought to be lost by sorption to the plastic cell culture plates and to the bacteria, resulting in an over-estimation of the concentration that actually exerts mutagenicity. To overcome such problem, here, we applied passive dosing based on partitioning of the mutagens from silicone O-rings, hence, providing a defined and constant freely dissolved concentration. Previous experiments using a new SPME set-up on the 24-well plates combining with GC-MS showed similar compound diffusion with and without bacteria. Therefore, parallel tests were made in which the first batch of experiments was aimed to determine the mutagenicity from toxicants loaded on silicone O-rings and the second batch of experiments to quantify the freely dissolved concentration available in the mutagenic test by injecting into LC/MS the whole well containing the silicone O-rings and bidest water.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index |

Aquatic organisms as bio-indicator on multiple levels

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Introduction

In order to maintain and restore the ecological quality in a river system, it is essential to have a good understanding of the functioning of this system. If adverse effects that are observed in the field can be linked to the responsible stressors in the ecosystem, it will be possible to take effective measures. Considering the complexity of the ecosystem, it is a real challenge to establish those cause-effect relationships in the field, due to the many factors that could influence the ecosystem, like for example physical and chemical parameters, changes in habitat, and the presence of pollutants. In the Keybioeffects project, different types of research are conducted which investigate the cause-effect relationships of pollutants on multiple levels. In this presentation, we would like to focus on fish and aquatic invertebrates as bio-indicators of toxic stress. In the Keybioeffects subprojects, effects on fish as well as on the aquatic invertebrates were studied at different scales:

- Population scale: by investigative field monitoring and observing different fish traits
- Individual scale:
    • by studying bioaccumulation and effects of organic substances in aquatic invertebrates in relation to abiotic conditions
    • by studying uptake (bioavailability) and effects (linked to specific pollutants) on fish in laboratory systems
- Molecular scale: studying immunotoxic mechanism of pollutants in fish

Mechanistic research on effects of pollutants on fish is important since it contributes to our knowledge on the way pollutants interact with fish in the environment and their potential mode of actions. This knowledge does not only enable the establishment of a cause-effect relationship, but also informs us about measures that can be taken to minimize effects in the field.

Our objective is to demonstrate firstly the contribution of the overall outcome of these studies, and secondly how combining the results of the studies has additive value in ecological quality management of river systems.

Linking multiple levels

In figure 1 a scheme is given which demonstrates the links between the different levels.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

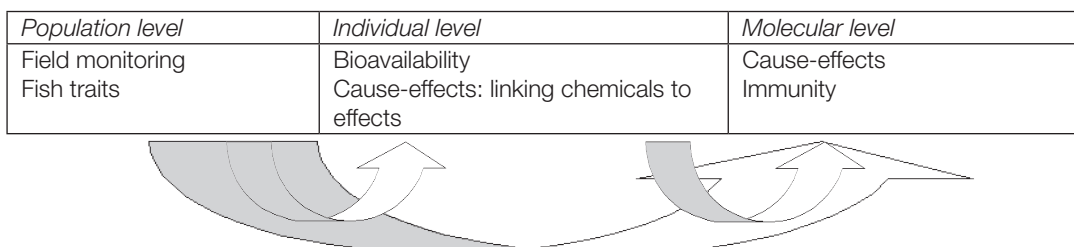| Population level	Individual level	Molecular level
Field monitoring Fish traits	Bioavailability Cause-effects: linking chemicals to effects	Cause-effects Immunity

Figure 1. Linking multiple levels of research on fish

Since it is not possible to check for each chemical what specific effects they cause, one option is to follow a downscaling pathway (figure 2), which starts with the monitoring of the chemical and biological status of a river system. When adverse effects on fish populations are observed, the focus shifts to the individual level, leading to investigations on the bioavailability of single chemicals and their effects on invertebrates and fish. To further explore the specific mode of action of the chemical, the study zooms in further to the molecular level and look at the toxicity mechanism of chemicals. By downscaling from effects on the population level to effects on molecular scale, it becomes possible to more specifically link effects to certain causes. The gained knowledge will help to make the right decisions on measures that have to be taken in the river system.

One example of the downscaling pathway is that during field monitoring parasites are observed in fish, which could be a sign of supressed immunity. At the molecular level the toxicity mechanism of chemicals which are detected in the river are investigated to correlate effects on immunity to adverse effects on the population level. Here, fish cells are used to investigate the tissue-specific response to the toxin.
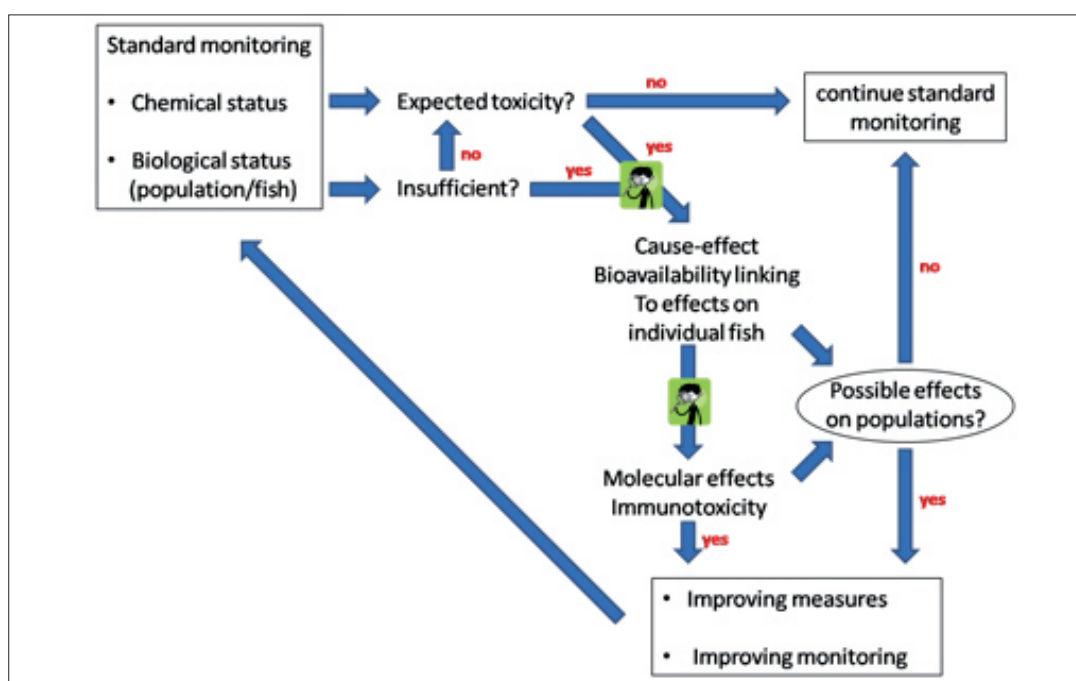

Figure 2. Decision tree for water management

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Another example is that toxic effects on fish can be linked to bioaccumulation of toxicants in aquatic invertebrates. Considering the abundance, feeding pattern and the potential of aquatic invertebrates to bioaccumulate toxicants, their position in food chain, predisposes fish and other species to chemical risks that could result from feeding. The end result could be the accumulation of potentially harmful chemicals in fish tissues.

Conclusion
- Research on multiple levels contributes to knowledge on cause-effect relationships of chemicals in the environment
- For assessment, improvement and management of the ecological status of a river system it is possible to perform downscaling from observed effects on fish/population scale to the molecular scale to find out specific mode of actions of a chemical
- The sensitivity of smaller aquatic invertebrate species could be harnessed in determining the potential risks of chemicals.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Priority and emerging pollutants in the environment: recommendations concerning sampling strategies and analytical procedures

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Investigation of the presence of priority and emerging pollutants in the environment is mandatory in the first case (in some environmental compartments) and necessary in the second in order to prevent and control pollution and protect the environment and human health from adverse effects caused by exposure to these compounds. Their analysis requires in general the use of highly sensitive and selective methods for their reliable determination at the very low concentrations at which they are found in the environment and/or at the levels that have to be measured according with the established environmental quality standards.

In the last decades the analytical instrumentation has experienced an impressive progress that has translated in more efficient and reliable methods and improved analytical performance. This presentation addresses with examples the last trends in the application of this instrumentation to the sampling, extraction, and analysis of priority and emerging pollutants in the environment.

Sampling is the first and often the most important step in the analysis of environmental contaminants; however, most frequently, it does not receive the necessary consideration. Most current works rely on the use of discrete, and to a lesser extent, integrated samples. In the last decades, other more advanced sampling strategies such as, for instance, passive sampling, which combines sampling, analyte isolation, and pre-concentration into a single step, are being developed and implemented in an effort to both save money and gain in information.

Extraction techniques have improved considerably through the years. Examples of advanced extraction techniques applied to the environmental analysis of priority and emerging contaminants are pressurized liquid extraction (PLE), applied to solid matrices, and on-line solid-phase extraction for water samples. They offer important advantages with respect to traditional techniques, especially in terms of time and cost of analysis, and also of volume of solvents and reagents used.

For analysis, gas chromatography (GC) and liquid chromatography (LC), both coupled to mass spectrometry (MS) or tandem mass spectrometry (M/MS) detection, are the most used. GC-MS is the technique of choice for analysis of volatile, thermolabile compounds, and the most extensively used in the analysis of priority pollutants, such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenylethers (PBDEs) , polychlorinated biphenyls (PCBs) or dioxins.

Most emerging pollutants, however, are polar compounds whose analysis is better suited by means of LC-MS techniques. Today, the various available LC-MS/MS techniques, with triple cuadrupole (QqQ), cuadrupole-linear ion trap (Q-LIT), and cuadrupole-time of flight (Q-TOF) analysers, make it possible to measure very low levels of pollutants, even at the pg/L

or pg/g range, in complex matrices, such as wastewaters, with great selectivity. However, the main drawback of such techniques is the presence of matrix ionization effects that interfere in the analyte signal and lead to inaccurate results. There are various means to try to overcome this problem; one of the most suitable is the use of isotopically labelled compounds as internal standards for quantification. These various LC-MS/MS techniques, recommendations about the operating conditions to be used to ensure unequivocal identification of the analytes, their capabilities in the identification of known compounds, and the use of isotopic dilution to compensate potential matrix effects, are illustrated with examples of the investigation of emerging contaminants, such as, pharmaceuticals, drugs of abuse, etc. in environmental samples.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index |

Search for key toxicants: Effect Directed Analysis and their applicability for monitoring

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The assessment of European water bodies according to the EU Water Framework Directive is based on the ecological status considering the biological quality elements (BQEs) fish, macro-invertebrates, phytobenthos, phytoplankton and macrophytes while the chemical status focuses on the concentration of presently 33(+8) priority pollutants in water and sediments. MODELKEY provides strong evidence that these priority pollutants explain only minor portions of measurable effects in European water bodies. Many other key toxicants have the potential to affect the ecological status in rivers and lakes.

Since the European commission is aware of that problem the list of priority substances is under review following two approaches: a monitoring-based approach and an approach based on modelling of exposure using production volumes and use patterns. Although this revision will provide significant progress, non-monitored compounds and limited data available as well as the great number of by- and transformation products may significantly hamper these approaches. Thus, to actually fulfill the requirements of the WFD and to protect European water ressources we suggest to add another approach based on field-evidences downstream of known sources of potential basin-wide relevance (big cities, industrial complexes, areas of intensive agriculture), at integrative sinks (reservoirs, estuaries, harbours) and at sites of specific concern (e.g. drinking water abstraction, valuable ecosystems, dredging areas). The most promising approach to derive water body specific key toxicants based on field evidences is effect-directed analysis (EDA). This approach uses water, sediment or biota samples from sites of interest. These samples or extracts thereof are subjected to effect assessment ideally applying a broad range of toxicological endpoints that are relevant for ecosystems and for human health. Subsequent fractionation of toxic samples is used to reduce complexity of the environmental mixtures. Biotesting of the derived fractions is applied to identify those with greatest hazard potential and thus highest priority. Hazardous fractions are subjected to chemical target and non-target analysis. Structure elucidation of unknowns using GC-MS and LC-MSn techniques together with modern computer tools is one of the greatest challenges. Finally, tentatively identified compounds need to be confirmed as compounds that significantly contributeg to the measured effect. In MODELKEY the EDA approach has been significantly advanced and applied to several sites of interest in three river basins. Together with classical in vivo tests representing members of WFD BQEs several in vitro test systems were applied covering a broad array of endocrine endpoints including estrogenicity, androgenicity, Ah-receptor mediated effects, thyroid hormone disruption but also mutagenicity and antibiotic

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

activity. A specific focus of EDA was given to sediments because of their potential to accumulate many toxicants. While classical non-polar persistent organic pollutants still are of relevance in the water bodies assessed by MODELKEY, EDA clearly indicated a high potential of polar fractions to affect most endpoints. This tendency was further enhanced when bioavailability was considered in EDA studies. Compounds stemming from personal care products such as the biocide triclosan and different musk compounds were identified as key toxicants in sediments. Other examples include steroid compounds, the flame retardant tris(2-chloroisopropyl)phosphate and different nitro-PAHs. A first comparison of toxicants identified with the EDA-based approach with prioritisation attempts based on monitoring and modelling indicates that our approach based on field evidences adds new potential key toxicants, which should be considered in monitoring and prioritisation. In addition to its value for prioritisation, the EDA approach is a powerful tool for investigative monitoring within the WFD at sites where there are indications for toxicants (e.g. by a low SPecies At Risk (SPEAR) index) as the cause of the insufficient ecological status, while target analytes can not explain measured effects.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Priority and Emerging Pollutants: the Role of WWTPs for Sanitation and the Significance of Transformation Products

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The presence of emerging pollutants (i.e. pharmaceuticals, personal care products, perfluorinated compounds, brominated flame retardants) in the environment has been well described in the literature. These contaminants have been detected in various environmental matrices up to low µg/L range in Europe as well as North America and Asia. It has been concluded that the primary source of these emerging contaminants entering the aquatic environment is through wastewater treatment plant (WWTP) discharges. Additional sources include industrial discharges, illegal disposal, leaching from landfill sites and hospital wastewater discharges.

In recent years, research has focused on trying to optimize and/or develop wastewater treatment technologies to effectively remove these emerging compounds and prevent further contamination of the aquatic environment.

The application of membrane technologies (i.e. nanofiltration membranes), sorption filters (i.e. GAC, PAC), chemical oxidation (i.e. ozone, UV/H2O2), operational parameters (i.e. sludge age, hydraulic retention time) as well of biological treatment (i.e. bioreactors, nitrifying bacteria) has been investigated to limit or prevent these compounds from entering surface waters and groundwater. However, limited research has focused on the impacts these treatments have on the composition of the effluent being discharged into aquatic ecosystems.

The formation and identification of transformation products (TPs) of emerging pollutants is a relatively new area of research which attempts to gain a better understanding of the implications of the applied treatment processes. In most cases, the removal of a compound does not necessarily indicate that mineralization has taken place. It is likely that the parent compound has been transformed to some extent, with possible changes to the functionality and toxicity of the compound. Research has investigated the biotransformation of selected pharmaceuticals (i.e. iodinated X-ray contrast media and opiates) as well as the chemical oxidation of selected micropollutants. The results clearly illustrate that transformation products of selected pharmaceuticals are formed in bench-scale batch systems, and the same TPs are detected in WWTPs and the aquatic environment.

This presentation will focus on the application of various wastewater treatments, the removal efficiency of certain polar micropollutants (i.e. pharmaceuticals), as well as the formation of TPs after the application of certain treatment processes.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index |

Applicability and limitations of biomarkers

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A biomarker is defined as a biological change in response to exposure to and/or effects of environmental chemicals. In principal, any response ranging from molecular up to ecological changes can serve as biomarker. In practice, however, usually the term "biomarker" is restricted to molecular, biochemical, cellular and physiological responses of organisms, whereas higher level response may be designated as "bioindicators" or "ecological indicators". Examples of biomarkers used in monitoring of aquatic organisms include biotransformation enzymes and products, stress proteins, metallothioneins, immunological parameters, endocrine parameters, genotoxic parameters and histopathological alterations. With the advent of toxicogenomic technologies, new biomarkers may become available.

Since biomarkers signal whether organisms are exposed to toxic chemicals and whether this is associated with adverse health impacts, they have the promise to link chemical and ecological status of aquatic habitats, as it is required, for instance, in the Water Framework Directive. The strength of biomarkers is the use as diagnostic tools in supporting the establishment of cause-effect relationships. However, the prognostic value of biomarkers, i.e. their ability to predict ecological change, is debated.

Biomarkers represent integrative responses of biological organisms. This has the advantage that biomarkers are indicative of the cumulative impact of all chemicals the organisms is exposed to. The disadvantage is that the biomarker response is influenced not only by chemical exposure, but also by a range of other chemical, biological and physical factors, e.g., repoductive status of the organism or environmental temperature. This multiple regulation of biomarker responses needs to be adequately considered in the design of monitoring programs in order to discriminate between the influence of chemical stressors and of other factors.

The presentation will discuss scopes and limitations of biomarker applications for the purposes of the monitoring and status assessment of aquatic habitats.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   l

The use of biofilm communities to assess ecological risks of pollutants in aquatic ecosystems

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Within the European Water Framework directive, a good ecological status has to be reached within all water bodies by 2015. Biofilm is integrated in the WFD as biological quality element to be monitored for quality assessment of surface waters. Biofilms are attached living communities growing on sediments (phytobenthos) or on other surfaces (periphyton). Algae, bacteria, protozoa, and fungi compose these communities, all embedded in an extracellular matrix of microbial secreted polymers. Guidance for monitoring biofilms focuses on structural elements of the autotrophic community using well-established tools: determination of taxa composition, abundance and related indices. However, it is also important to focus on the functional response of these communities as they provide crucial ecosystem functions. Thus, Keybioeffects project concerned the development of different tools to assess these functional responses of biofilm communities.

1. How to investigate biofilms complexity ?

Biofilm communities can be found in a variety of compartments within the aquatic ecosystem, and represent an interface between flowing water and stream bed/sediments. Due to their omnipresence and important role as primary producers in nutrient fluxes and trophic cascades, biofilms are good indicators of an integrated ecosystem health. Furthermore, biofilms are characterized by a high species richness with a reliable amount of sensitive and discriminating species, this feature combined with a short generation time make them pertinent early warning systems of disturbances within the ecosystem [1, 2]. The different levels of complexity have been studied in our groups on appropriate stages of biofilm colonization: from monocultures to mixed monocultures, up to natural biofilms in microcosms, mesocosms and artificial channels or in streams and rivers. To investigate the biological complexity of biofilms, we developed tools targeting different levels of biological organization: from gene responses to ecosystem function (Fig. 1). We focus on functional and integrated responses rather than on structural responses.
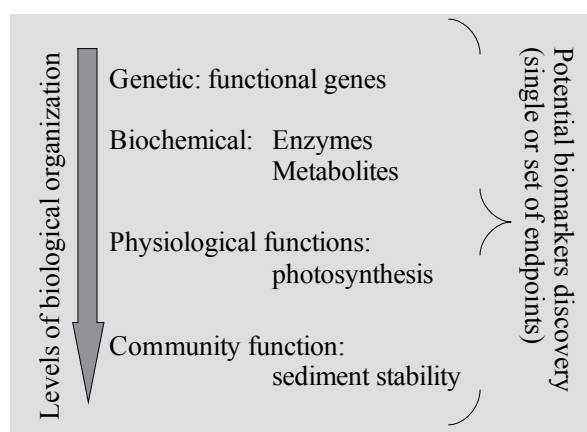

Levels of biological organization →

- Genetic: functional genes
- Biochemical: Enzymes / Metabolites
- Physiological functions: photosynthesis
- Community function: sediment stability

Potential biomarkers discovery (single or set of endpoints)

Figure 1. The different levels of biological organization integrated in tool development. At each level an example of endpoint used is indicated

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

2. Some examples of specific investigation:

Our groups are working on the development, optimisation and validation of these tools (Fig. 1). Responses to abiotic changes and toxicant pressure are being tested in order to investigate the potential of these tools in assessing risk of chemicals in the environment.

2.1 Interactions between environmental factors and pollutants

Biofilms were grown in mesocosms under different light intensities and were then exposed to different herbicides for 6h. In general, light history was found to affect biofilms responses to the herbicides. The analyses of antioxidant enzymes responses showed that depending on their light history, biofilms would use different enzymes to cope with oxidative stress due to these herbicides.

2.2 Emerging contaminants: β-blockers toxicity assessment on fluvial biofilms [3]

A biomarker approach, encompassing different levels of biological organization, was used to assess toxicity of 3 β-blockers, found in the ng/L range in the Llobregat river (Spain). After 24h of exposure in microcosms, the response of various biomarkers were tested. Propranolol was found to be the most toxic β-blocker, affecting strongly photosynthetic efficiency of biofilms. While the 3 β-blockers are expected to act similarly in target organisms, our approach showed that they affected differently the compartments of the biofilms and may have adverse effects when found in mixture.

2.3 Pollution pulses of triclosan and diuron

Fluvial biofilm was used to assess ecological risk associated with entrance of pulses of the bactericide triclosan (TCS) and the herbicide diuron (DIU). TCS directly inhibited phosphate uptake, increased bacteria mortality and indirectly affected diatoms. Phosphate uptake inhibition might determine a reduction of the self-depuration capacity in the river ecosystem. DIU mainly affected algal compartment of biofilm: photosynthetic efficiency decreased and diatoms mortality increased after exposure. These results confirm different modes of action for these 2 products at the biofilm community level and highlight the risk represented by them at river ecosystem level.

2.4 Metabolomics to understand prometryn toxicity on biofilms

An investigation has been conducted to assess the suitability of metabolomics technology to study periphyton incubated in microcosms. During the incubation, some microcosms were exposed to the herbicide prometryn. The metabolic profiles of periphyton could be successfully extracted using an optimized "metabolomics protocol". Though changes in the metabolic profiles of the community were observed after exposure to prometryn, the reduction of unwanted variability is still a challenging task.

2.5 Keycomponents for biostabilization of sediments

Triclosan, a common and persistent anthropogenic pollutant in aquatic habitats, affected strongly the directly exposed bacterial assemblage within a concentration range of 2 – 100 µg /l. The stabilisation potential of the bacteria, an important ecosystem function within the aquatic habitat [4,5], was strongly inhibited by TCS, both variables were negatively correlated. TCS exposure had a clear impact on bacterial cell numbers and bacterial growth that in turn affected the EPS secretion to influence sediment stability.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index | I

3. **Perspectives for use of biofilms in toxicity assessment and investigative monitoring**

3.1 Toxicity assessment

Biofilms, as complex communities, integrate over long periods both direct and indirect effects of toxicants and thus are very interesting for toxicity assessment. To develop laboratory bioassays, some standardization of colonization and toxicity assessment procedures is needed. The type of substrata, the colonization and exposure time, the set of reliable and pertinent biomarkers to use may be determined depending on the habitat and the community studied (small streams, lakes, sediments...). This uniformisation would be helpful to compare ecotoxicological parameters obtained for different communities, in different laboratories.

3.2 Investigative Monitoring: biofilm as a sentinel indicator of habitat health

Investigative monitoring may focus on the development of early warning systems. Since functional responses may occur before a structural effect is visible, the tools developed within Keybioeffects would be pertinent for an investigative monitoring. Before routine usage, these tools need to be adapted and optimized to field situations, therefore a field-lab approach is suggested.

Tools developed in laboratory to screen new parameters (e.g. genes, enzymes, metabolites) and community' functions (e.g. primary producer through photosynthesis or sediment stability) may be applied to specific field situations. Results of field sampling may then be used for further optimization in the lab and for validation. Finally combination of relevant tools should provide a biomarkers toolbox that can be used in investigative monitoring, for instance when pollution sources are unknown.

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Improving data sampling for better ecosystem diagnostic

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Freshwater ecosystems are strongly impacted by land-use, pollution and climate change. The EU Water Framework Directive (WFD) has committed all EU states to reach good ecological condition of surface water by the year 2015. The WFD was meant to promote the development of tools to assess the chemical and ecological status of water bodies reliably and create common standards for surface and groundwater management. In this way pollution should be stemmed and the condition of the ecosystem improved in order to protect the water resources in the long-term. In spite of the efforts made by the scientific community, it remains difficult to correctly define what bad water quality is and to reliably identify the causes of the impairment. In most instances, bad water quality is the consequence of multiple factors acting in synergy or hindering one another (e.g., chemical pollution, habitat alteration or climate changes) rather than the outcome of a single stressor. Furthermore, these multiple factors are themselves interwoven into spatial and temporal processes occurring on different scales within the landscapes. In order to help reach or surpass the goals of the WFD, obtain geographically-oriented assessments of biological impacts, and allow dependable identification of stressors, spatially- and temporally-explicit information is needed.

The European national water agencies, which are in charge of the implementation of the WFD, have proposed several monitoring programs, hinging for most instance on biological inventories or the measurement of chemical pollution such as the detection of pesticides and priority substances in freshwater ecosystems. However, in the absence of prior inter-agencies agreements regarding sampling sites, these monitoring efforts are generally done on different sets of locations. For example, in the Adour-Garonne basin (south-western France), pesticides data (collected by the French Water Agency at 130 sampling sites) and fish data (collected by the French Fishery agency at 140 sampling sites) were too far away from one other (median=10.5 km) to allow reasonable matching. As a consequence, datasets obtained by merging information from different agencies most of the time gather a weak amount of usable data to further investigate possible cause-effect relationships between chemical factors and biotic community responses.

Spatial database evaluations, as part of screening-level diagnostic assessment, aim at establishing scientific understanding of quantitative spatial associations between environmental (explanatory) and biological (response) variables. Such efforts are increasingly in demand as their results provide guidance for targeted follow-on studies and prioritization of regional and local watershed management goals [1]. Linking biological condition, habitat characteristics including mixture risks, and species abundance allowed us to separate natural from anthropogenic causes of ecosystem impairment. Methods may vary from simple correlation analyses to complex multivariate techniques [2-5]. For example, the methodology called Effect and Probable-Cause pie chart (EPC pie diagrams) allow mapping the relative probable contributions of different stressors to the loss of species with pie sizes corresponding to magnitude of local impairment (see figure 1a from Kapo et al., 2008). Moreover, the GIS-

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Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Based Weights of Evidence/Weighted Logistic Regression [6-7] uses relationships between a set of known training points and the map patterns of two or more variables to both predict the occurrence of undiscovered points of interest and determine the relative influence of individual variables (see figure 1b from Kapo et al., 2008) .
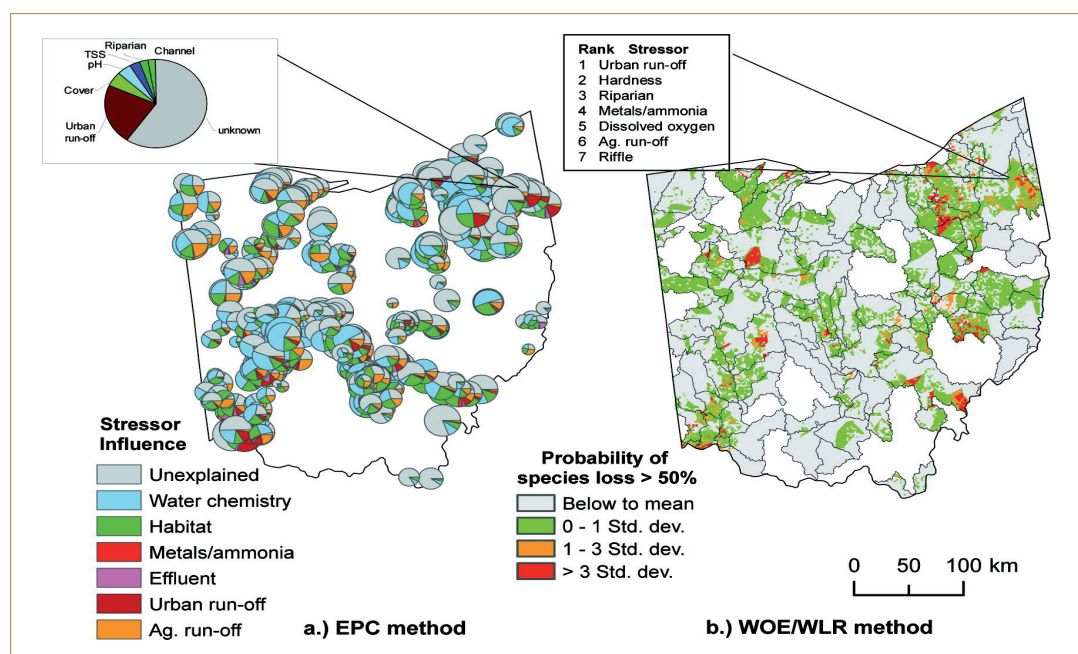

Figure 1. GIS mapping results for Ohio: (a) Pie and slice map from the Effect and Probable Cause (EPC) model. (b) Interactive raster map from the GIS-based Weights of Evidence/Weighted Logistic Regression (WOE/WLR) model. A model query example of stressor influence is shown for a site on the Mahoning River on each model map (from Kapo et al., 2008).

For now on, complete spatial database allowing this kind of analysis (including chemical, biological and habitat variables) remains scarce in Europe. In order to improve ecosystem diagnostic and to comply with the WFD, stakeholder should seek solutions to improve inter-agencies communication. In that regard, a possible solution may be the creation of inter-agency comities. For example, a geo-logistic comity shared by agencies responsible for biological inventories and chemical monitoring may have the responsibility of ensuring that both kinds of work are done within a standard set of sites. This kind of collaboration has started in the Adour-Garonne basin and a great improvement can be hoped in the next few years.

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Ecotoxicology in fluvial ecosystems:
From standardized single-species tests to ecosystem manipulations

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Biological monitoring of aquatic communities is routinely employed to assess impacts of physical and chemical stressors on ecological integrity. An important assumption of these assessments is that patterns of community composition observed at polluted sites reflect the presence of specific stressors. Because differences between reference and polluted sites result from numerous factors in addition to physical and chemical stressors, demonstrating causation in descriptive studies is challenging. For example, descriptive studies can show that two sites differ in community composition; however, these differences often cannot be attributed to a specific cause. Although approaches developed in human epidemiology (Hill 1965) have been proposed to strengthen causal relationships in stream bioassessments (Suter 1993), some researchers argue there is no substitute for experimental manipulation (Lubchenco and Real 1991).

The relationship between descriptive and experimental approaches in ecotoxicology can be depicted as continua along two axes that reflect the degree of experimental control and replication along one axis and the ecological relevance of a test system along the other axis. Traditional experimental approaches employed in aquatic toxicology, such as single-species laboratory bioassays, are the workhorse of environmental regulators (Cairns 1986). These experiments, which are routinely employed to demonstrate chemical effects and to establish chemical criteria, provide rigorous control over confounding variables and are easily replicated. However, laboratory toxicity tests lack ecological realism and fail to account for indirect effects that occur in natural communities. In contrast, purely descriptive studies (e.g., routine biomonitoring) lack true replication and random assignment of treatments to experimental units. Consequently, differences between reference and impacted sites cannot be directly attributed to a specific stressor.

More sophisticated approaches, such as microcosm and mesocosm experiments, have been developed to assess responses at higher levels of biological organization (e.g., communities and ecosystems), but these approaches are rarely used in a regulatory context. I believe the historical focus on reductionist approaches such as single species toxicity tests has significantly impeded implementation of these ecologically realistic experimental approaches. Conducting experiments at ecologically relevant spatiotemporal scales is difficult at higher levels of biological organization (populations, communities, ecosystems), leading some researchers to question the validity of small scale studies (Carpenter 1996). For experimental approaches to play a more prominent role in ecotoxicology, researchers must address concerns about spatiotemporal scale and design more ecologically realistic studies.

In addition to assessing effects at the appropriate spatiotemporal scale, ecotoxicologists have become increasingly aware of the need to measure a diverse suite of ecologically

significant endpoints. Responses at lower levels of organization are often specific to a particular contaminant (e.g., metallothionein induction and heavy metal exposure) and generally have a well understood mechanistic basis. However, the ecological consequences for populations and communities of most biochemical, physiological, and individual responses have not been characterized. Although responses at higher levels of biological organization are more ecologically relevant, they are less specific and lack a mechanistic basis. Consequently, some researchers advocate integrating measures across levels of biological organization when assessing stressor effects (Clements 2000). Because microcosm and mesocosm experiments typically involve exposure of complex systems, they provide important opportunities to investigate responses to stressors across levels of biological organization.

In summary, the transition from purely descriptive to experimental approaches and the ability to test hypotheses with controlled experiments are generally regarded as evidence of scientific maturation (Popper 1972). Although this transition is currently underway in the field of ecotoxicology, serious questions remain regarding appropriate spatiotemporal scales and levels of biological organization. In this presentation I will describe the strengths and weaknesses of experimental approaches across a range of spatial and temporal scales in ecotoxicology. Using data collected during a 20 year natural experiment conducted in a metal-polluted stream, I will describe how results of descriptive and experimental approaches can be integrated to demonstrate cause-and-effect relationships between stressors and ecological responses. Results of a large-scale field manipulation conducted in 12 separate streams will be used to demonstrate that effects of stressors must be investigated within the context of global change. The goal of the presentation is to demonstrate how results of descriptive and experimental studies can be used to establish causal relationships and to identify safe concentrations of priority and emerging contaminants in aquatic ecosystems.

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Current approaches used to evaluate ecological integrity: biotic indices versus community ecotoxicology studies

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Complying the WFD implies that member states and the scientific community consider the protection and management of entire freshwater systems. To determine the ecological status of water bodies, the WFD considers not only the impact of single pressures on individual biotic groups but the deviation of the community from undisturbed conditions.

It is often difficult to establish relationships that imply causality between the chemical characteristics of river water and biological communities. Moreover, other stressors can be responsible in the impairment of ecological status. Common metrics based on community structure (e.g. biotic indexes) do not account for the combined stressor effects. On the other hand effects differ between biological levels. Assessing the relevance of multiple stressors requires that analysis include multiple parameters and the use of multivariate statistics, as tools to confirm the role of each stressor and stressor interactions.

Within the MODELKEY project a range of experimental, observational and modelling approaches has been deployed to evaluate the potential and limitations of multiple stressor assessment. Tools have allowed to analyse data on multiple levels, including community, chemicals and toxicity descriptors, in order to asses the impact of key pollutants on community structure and biodiversity. The Llobregat river is one of the basins examined by the project. The Llobregat River is characterized by high discharge fluctuations, which reflect the Mediterranean climate. The headwaters of this river are characterized by agricultural activities, and industry and urban agglomeration impact on the middle and lower reaches. More than 5 million people live in this basin and chemical pollution is accompanied by salinity, nutrient enrichment, habitat deterioration and water abstraction. In several sites of the lower part of the river the chemical analyses revealed the presence of priority and emerging contaminants, like pesticides (Ricart et al. 2009), pharmaceuticals (Muñoz et al. 2009) and alkylphenolic compounds (Petrovic et al., 2002).

Simultaneous chemical and biological sampling was carried out along the lower reach of the Llobregat River during spring and autumn of 2005 and 2006. Multivariate statistical techniques were applied to determine the best match between the patterns among-sample of an assemblage and that from environmental variables associated with those samples. Results obtained show that different factors affect the different communities studied.

- Physical and chemical variables, mainly temperature and sulphate concentration explained a high percentage in the variability of the exoenzymatic activities of the bacterial biofilm compartment.

Pesticide occurrence and concentration, as well as nutrient availability, were related with changes in biomass, community composition and photosynthetic efficiency of algae in the biofilm.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

The abundance and biomass of macroinvertebrate community were related with the concentration of some pharmaceutical agents (mainly anti-inflammatory).

Though these results were not determinant to find evidences on cause-effect responses, laboratory ecotoxicological experiments partially support results found in the field. The combination of the two approaches may be essential to elucidate which are the factors causing changes in the biological communities. This approach might be useful to find spatial and temporal correlation of stressor and effects along gradients, while community experiments in the laboratory are required to examine hypotheses generated from field studies. Knowledge of field patterns and combined laboratory efforts might help to focus on the subsequent monitoring efforts, as well as on identifying key taxa for their use as ecological indicators of specific (or predominant) stress factors in multistress conditions. This could be a desirable way to go on in future risk management decisions.

Recommendations

Some measures and needs are proposed to improve the assessment and management of river basins:

Simultaneous sampling in time and space including physical, chemical and biological parameters.

General screenings in river basins to detect new compounds and to determine their potential toxicity on community in order to select those to be included in the routine monitoring.

Modelization to estimate impacts of multiple stressor on biological communities, at site and basin scales.

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Recommendations in the Design of Water Monitoring Programmes based on newly developed Complementary Methods

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Introduction

According to the requirements of the EU Water Framework Directive, crucial aspects for the evaluation of chemical status of European water bodies are: the identification of key toxicants, the quantification of the influence of environmental conditions on their bioavailability, and the assessment of their effects in mixture at different ecosystem levels.

Regional Water Authorities need to complement the traditional monitoring procedures with more sustainable tools in terms of lower cost and lower ecosystem impact, greater robustness and greater efficiency in the identification of pollution sources. These emerging monitoring tools (as biomonitoring, biomarkers, etc.) can provide a better insight on the overall ecosystem status, as they provide a link between chemical and ecological status.

The integration of exposure and effects monitoring will facilitate more cost effective monitoring programmes as well as forming the basis of a risk based pollution control strategy.

The present study is part of the European Project Keybioeffects, a Marie Curie Training Network, aiming to provide a better understanding of problems related to biodiversity conservation and water pollution in European rivers. The knowledge achieved will be transferred to different stakeholders. A case-oriented guidance document is being developed to support the activities of Water Agencies in the evaluation of chemical and ecological status in relation to priority and emerging pollutants.

Prioritization and decision support tools

A justification of a reduction in sampling frequency can be considered using sensors as screening tools e.g., Early Warning Systems. Sampling for chemical analysis can be subordinate to the response of a sensor above a certain threshold.

Good examples are methods that use fluvial biofilms or fish biomarkers as bioindicators of environmental perturbations in the aquatic ecosystem. These real-time continuous systems provide rapid evaluation and detection of temporal variation in water quality. These methods are applicable to detect early stage biological imbalance, and potential toxicity, in addition are able to assess the joint action of several stressors (such as environmental factors and toxicant concentration), recognizing interactions and unexpected effects.

The identification of problem as well as non-problem areas e.g., in the grouping of water bodies for operational monitoring can be achieved using advanced modelling techniques.

Predictive and diagnostic models applied to existing monitoring data and emission data can be used to predict the impact of emerging key contaminants on freshwater ecosystems biodiversity.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

These models can predict the stress factors that have contributed to the impairment of biota communities as well as provide a predictive analysis of the more probable effects of individual or mixture of toxicants on biodiversity.

In particular a risk assessment procedure based on Species Sensitivity Distribution SSD and multi Artificial Neural Networks ANNs is proposed for the analysis of large datasets and the identification of patterns of spatial diversity of chemical toxicity.

Monitoring tools

Concerning water monitoring programs, a big effort should be done on harmonisation of methods at European level, especially for international river basins. Spatial coverage and regular sampling time are crucial factors to obtain a regular map of pollution evolution across time and space. This will allow the application of model tools resulting in a better identification of critical sites that need most attention to reduce contamination.

Passive sampling devices are able to give a representative assessment of water quality in a river basin with rapid fluctuations in pollutant concentrations and integrate contaminant concentration over the sampling time. In addition are very useful to concentrate compounds that show low environmental levels and can be used also for short sampling time (few hours) or simply for spot water sampling. The sampling, extraction and cleaning techniques are simple as well as the transportation and storage of samples.

The European Commission in the directive highlights the importance of sediment and biota in the process of distribution and bioaccumulation of toxic compounds and Member States are required to set monitoring program, including sediment and biota.

New methods for cleaning and extraction of toxicants in sediment and fish are proposed.

It appears important also to consider bioavailability and reliable water exposure concentrations of toxicants: partitioning extraction systems (e.g. SPME) or bioavailability models may contribute to estimate partitioning of toxicants in water, and associated toxic risk

The routine use of these techniques allows assessing long term trends in toxicant loads.

Investigation tools

In case of biota impairment and when the reason of the impairment is unknown (good chemical status based on priority substances) monitoring methods can be coupled with in vitro bioassays, fractionation and chemical isolation methods. New sampling, fractionation and analysis methods are developed for the screening and identification of unknown, emerging contaminants in a complex mixture from complex environmental samples and also to identify the pollutants causing adverse effects to biota, including mutagenicity, carcinogenicity, endocrine disruption and developmental effects (e.g. Brack, 2003).

A step forward in toxic stress identification, when bioassay test response is ambiguous or inadequate, can be performed with metabolomics techniques especially designed to understand and compare physiological processes under natural or stressed conditions.

Specific investigation tools are recommended for solving problems related to bioaccumulation, degradation pathways and sediment binding capacity and stability properties.

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ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Establishment of the good chemical status of fresh surface water in France.
Application of the directive 2008/105/CE.

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In France, water chemical status monitoring involved to water agencies.

In order to evaluate the water body chemical status, 41 priority or dangerous priority substances are followed (appendix X, Water Framework Directive).

A european directive named "priority substances" defines Environmental Quality Standards (EQS) for these molecules (maximal acceptable concentration and annual average concentration).

The qualification rule consists in comparing these EQS with the averages and the maxima observed.

In 2007, there were two sample's frequencies for these 41 substances:
- one per month for the river
- four per year for the lake

Every monitoring control network's water stations have been sampled.

That means only ¼ of French territory has got measured data (approximately 2400 stations for about 9300 water bodies).

In reference to the decree (March 17, 2006) which explains the content of SDAGE (tool of planification for water management), the chemical status of fresh surface water bodies has been made on these 2400 sites. The French Department of Environment, Energy, Sustained Development and Sea (MEDDM) decided to limit the number of water body with out chemical status. That why, each water agency assigns chemical status with own methods which can all be summed up in a two methods I) pressure-impact assessment and II) the same quality for the main water body and its tributaries.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Establishment of the good chemical status of water in Spain. Application of the directive 2008/105/CE.

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The approval of the Water Framework Directive in 2000 was a milestone in the development of water policies and, in particular, in the protection of water against hazardous substances. The new Directive includes the obligations set forth in the previous legislation, but adds new requirements. The strategy to fight against the contamination of water with hazardous substances is mainly developed in Article 16. *Strategies to fight against the contamination of water*, and it must be implemented in accordance with the requirements set forth in Article 10. *Combined approach to address the punctual and diffuse contamination sources.*

Article 16 establishes that the protection of water against priority substances must address two main areas. On the one hand, it must act on the contamination sources, establishing measures aimed at reducing or eliminating the discharge of these substances to the aquatic environment. On the other hand, action is required in the recipient environment to make sure that the environmental quality standard is observed at all times. This standard establishes a concentration threshold for the environment, under which the presence of a substance will not generate a negative impact to the aquatic environment.

The strategy is articulated on three main aims. Firstly, the relevant substances must be selected, i.e., the presence of dangerous substances that represent a risk to the environment or, through the natural environment, to the health of persons. Next, the contamination reduction measures must be implemented for each substance selected and, finally, the environmental quality standard must be calculated for the aquatic environment.

The development of the strategy described in Article 16 defines the approval of the two legislation acts derived from such article. The first one is Decision No. 2455/2001/EC, November 20, 2001, for the approval of the list of priority substances in the water policy scope, constituting Appendix X of the Water Framework Directive. The second one, after years of intense negotiations, is Directive 2008/105/EC, December 16, 2008, related to environmental quality standards (hereinafter EQS) in the water policy scope, modifying and derogating Directives 82/176/EC, 83/513/EC, 84/156/EC, 84/491/EC and 86/280/EC of the Council.

The latter establishes an EQS for each substance in continental, coast and transition waters. In addition, there is an EQS in biota for mercury, hexachlorobenzene and hexachlorobutadiene. The EQS is expressed as an annual mean and as the maximum admissible concentration. In the case of organic compounds, the values of the EQS range from 0.2 ng/L for tributyl-stannane or 0.5 ng/L for bromilated diphenylether, to 2.5 µg/L for trichloromethane. In the case of metals, the values range from 0.05 µg/L for mercury, to 20 µg/L for nickel. The Member States will draw up their own definition of the EQSs for sediments and biota in certain water mass categories. The values approved for these matrices must offer the same degree of protection to those defined in waters. This is important in the case of strong water-repellent substances, such as chloroalkanes or pentabromodyphenil ethers. It is also interesting in the

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index | I

control of coast waters, where mussels or sediments are the matrices that are usually captured to measure the effects of contamination.

In parallel, the Directive requires the observance of the trends of content of contaminating substances in sediments or biota. In particular, the substances that tend to accumulate in these matrices must be controlled, such as metals, polycyclic aromatic hydrocarbons or chloro-organic substances. In this case, the concentration of these substances must be monitored to make sure that they do not increase in time.

We must highlight that the Commission is working to modify the ratio of priority substances with the aim of adding more substances. It is currently working with an initial list of 42 substances obtained after a new prioritisation process, including the contaminating substances of Appendix III of the EQS Directive, with historical substances, such as PCBs or Dioxins, or other less common substances in control networks, such as EDTA, PFOs, Glyphosate, AMPA and Bisphenol A. New substances have been added, in some cases, emerging substances, such as Trichlorfon, Dichlorvos, Ibuprofen, Irgarol or Carbamazepin, among others.

The analytical controls must comply with the requirements set forth in Directive 2009/90/EC of the Commission, July 31, 2009, which establishes the technical specifications of the chemical analysis and the control of the state of waters, sediments and organisms, as well as the regulations aimed at demonstrating the quality of analytical results.

Such a complex and vast legislation framework establishes the Directives that are developing the strategy for priority substances in Spain. In addition, the same principles are applied to protect waters from other types of substances, i.e., preferential substances prioritised at the State level and the relevant contaminating substances of each River Basin Authority. Therefore, for example, the good chemical condition of surface water is achieved when complying with the EQSs of Directive 2008/105/EC. In parallel, there is a good ecological condition - in relation to contaminating substances - when complying with the EQS of preferential substances established in Royal Decree 995/2000, June 2, for continental waters and Sections B and C of Appendix I and Appendix II of Royal Decree 258/1989, March 10, on the discharge of hazardous substances from land to sea. Likewise, other contaminating substances must comply with the EQS, calculated in accordance with the provisions of Appendix V of the Water Framework Directive, approved in each Hydrological Plan.

The Spanish Ministry of the Natural, Rural and Marine Environment is working with the General Coast and Sea Sustainability Department to prepare the text required to transpose the Directive. Likewise, it is assessing the possibility of establishing reference EQSs for sediments and the biota.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

Methods of Analysis of Priority and Emerging Pollutants: Problems and Establishment of Quality Standards

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The Water Framework Directive (WFD) (2000/60/EC) shall provide regulation for the contamination of European water bodies through chemical pollutants. This is achieved via the Priority Substance List Decision (2455/2001/EC) and establishing of Environmental Quality Standards on European level through the Daughter Directive 2008/105/EC. For river basin specific pollutants the Water Framework Directive provisions include obligations for identification of relevant pollutants at smaller spatial scales and the derivation of appropriate limit values on national level. The Groundwater Directive (2006/118/EC) ensures the protection of groundwater against pollution and deterioration. Therefore, Member States should set-up water monitoring programs covering a wide range of possible contaminants in order to identify risks, priority issues and needs for action.

The WFD daughter Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy is regulating the pollution with chemical substances in European waters. The performance criteria are proposed in a draft Commission Directive on Analytical Quality Control.

JRC IES has been accompanying the preparation of the upcoming WFD Daughter Directive COM(2006)398 on Environmental Quality Standards EQS through chairing the workgroup on Analysis and Monitoring of Priority Substances AMPS (2003-2004), co-chairing the drafting of the CMA guidance document for surface waters within the Chemical Monitoring Activity CMA (2005-2006) and is currently co-chairing the Chemical Monitoring Activity in 2007-2009.

The assessment of available methods for WFD compliance checking is among the prime objectives of the chemical monitoring activity. It is important that methodologies fulfil the requirements of the WFD chemical monitoring, e.g.by delivering concentration data of sufficient quality in order to assess compliance with the WFD Directive. Guidance on general WFD monitoring provision is available through the Guidance Document No. 7 "Monitoring under the Water Framework Directive" and for implementation of the ground water through the WFD CIS Guidance Document No. 15 "Guidance on Groundwater Monitoring" and CIS guidance document No 19 on "Chemical Monitoring of Surface Waters".

The method performance criteria for analytical measurements in chemical monitoring have been proposed in the "Draft Commission Directive adopting technical specifications for chemical monitoring and quality of analytical results in accordance with Directive 2000/60/EC of the European Parliament and of the Council". In that draft document a LoQ $\leq$ 30 % of EQS is required for WFD compliance checking. (Dir 2009/90/CE) .

In general, the methodologies for the analysis of organic pollutants in water are well established and are based on GC/MS (or GC/HRMS) and LC-MS (or LC-MS/MS). However, the values of some proposed EQS are very low (For example: Endosulfan ($\sum$: 5-0.5 ng/L),

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Oral presentations

Index | I

PBDEs ($\sum$: 0.5-0.2 ng/L) and some PAHs ($\sum$: 2 ng/L)). These values need LoQs in the level of ng/L (ppt) or lower.

Also the low concentration levels of pollutants included in Interlaboratory exercises, in the frame of the Chemical Monitoring Analysis (CMA), require the use of sophisticated instrumentation (GC-HRMS, GC-MS/MS or LC-MS/MS), which is not available at present for routine laboratories.

On the other hand, the analysis of called "emerging" organic pollutants (ex: PFOS, drugs, pharmaceuticals, NDMA , ...) need as well the sophisticated instrumentation (MS/MS & HRMS). The methods have not been yet fully verified and /or do not exist reference materials, and there are not many interlaboratory exercises available. As a result, a rigorous intercomparativity is nowadays not possible.

In this context, the establishment of reliable and robust methodologies which attain the QA/QC standards is essential.

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ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index I

Monitoring of priority and related substances in the rivers of Catalonia, under the application of the WFD: overview of results and risk assessment

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Summary

Data obtained along the monitoring campaigns of priority, dangerous priority and other related substances in the rivers of Catalonia, carried out by the Catalan Water Agency during the years 2008 -2009 according to the application of the WFD are presented and interpreted from a double point of view:

a) Regulatory

The results of priority and dangerous priority substances have been evaluated in terms of Directive 2008/105/CE. For those compounds that are not covered by the said directive, they have been examined, when possible, under the light of other applicable local legislation, such as RD 995/2000 and RD 140/2003.

Furthermore, available data of emission inventories, obtained within the application of the E-PRTR regulation to WWTPs of more than 100.000 eq. inhab., have been reviewed and tentatively related to river values.

b) Risk Assessment

An ecotoxicological risk assessment study has been attempted using sequential advanced modelling techniques on a sub-set of the whole dataset of chemical monitoring data (232 sampling stations and 60 pollutants).

Data on concentration of contaminants in water were pre-treated in order to calculate the bioavailable fraction, depending on substance properties and local environmental conditions. The resulting values were used to predict the potential impact on aquatic biota of toxic substances in complex mixtures and to identify hot spots. Exposure assessment with Species Sensitivity Distribution (SSD) and mixture toxicity rules were used to compute the multi-substances Potentially Affected Fraction (msPAF).

In order to understand and visualize the spatial distribution of the toxic risk, several representation techniques, such as Self Organising Maps (SOM) derived from Artificial neural Networks, Cluster Analysis or Principal Component Analysis (PCA), have been explored.

Hot spots and contamination patterns have been identified, thus providing useful information to water managers in order to assess chemical risk at basin scale.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts

Index |

Control of hazardous substances in the Ebro River Basin

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The Water Framework Directive 2000/60/EC (WFD) and directives included in its Appendix IX, as well as Directive 2006/11/EC (coded version of Directive 76/464/EC) force Member States to establish control stations to monitor the contamination in aquatic environments (water, sediments and biota) caused by hazardous substances downstream of their source points.

The so-called hazardous substances can have an industrial (punctual) and/or agricultural (diffuse) origin and, therefore, it is not possible to apply the same criteria when designing the control network for all substances, i.e., pesticides must be classified separately. Therefore, the Ebro River Basin Authority has defined two networks with different control points, sampling frequencies, measurement parameters and analysis matrices:

Pesticide Control Network (PCN), aimed at controlling the contamination with an agricultural/diffuse origin

Hazardous Substance Control Network (HSCN), aimed at controlling the contamination with mainly industrial/punctual origin.

The purpose of the Pesticide Control Network is to monitor the contamination caused by the pesticides in List I, List II - Preferential and the List of Priority Substances, downstream of the main agricultural areas and, in particular, making sure that they comply with the Environmental Quality Standards (EQS) established in the current legislation.

The Hazardous Substance Control Network has been implemented in the Ebro River Basin Authority (ERBA) since 1992. The purpose of this network is to control the concentration of hazardous substances (Priority substances, substances in List I and substances in List II Preferential) downstream of their main sources. The control procedures require the intake of water, sediment and fish samples. In the case of water, the Environmental Quality Standards have been established (Directive 2008/105/EC and Royal Decree 995/2000) and, in the case of sediments and fish, the aim is to make sure that the concentrations in these matrices do not increase greatly over time (basic principle of improvement or standstill).

Table 1 shows the current number of stations in these control networks, as well as the matrices analysed and sampling frequency.

Table 1. Characteristics of the HSCN and PCN.

	No. of Stations	**Matrices analysed**	**Sampling frequency**
HSCN	18	Water	12/year
		Sediments	1/year
		Fish	1/year
PCN	22	Water	5/year

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Oral presentations

Index   I

37 different substances are controlled in the Pesticide Network and a total of 47 contaminating substances are controlled in the Hazardous Substance Network.

The results obtained in the Hazardous Substance Control Network during the 2008 campaign indicate that the points with a highest contamination with industrial origin are the stations of Gállego in Jabarrella, Ebro in Ascó, Cinca in Monzón, Zadorra in Vitoria-Trespuentes, Ebro in Tortosa and Huerva in Zaragoza-Fuente de la Junquera.

The most important concentrations of polluting substances in some of the analyses carried out during the year 2008 in each one of the matrices analysed from the Network of Hazardous Substances are as follows:

- **Water:** selenium, nickel, mercury and dichloromethane.
- **Sediment:** nickel, chromium, zinc, cadmium, DDTs, benzo(b)fluoranthene, benzo(a) pyrene, benzo(g,h,i)perylene and benzo(k)fluoranthene.
- **Fish:** DDTs, zinc, hexachlorobenzene and mercury.

The 2008 analyses of the Pesticide Control Network show that only 9 of the 37 pesticides analysed (atrazine, desethylatrazine, chlorpyrifos, isoproturon, metholachlor, molinate, simazine, terbutilazine and 3.4-dichloraniline) have exceeded 0.1 µg/l. In addition, during the year 2008, only terbutilazine and 3.4-dichloraniline had a concentration over 1 µg/l.

The stations with the highest concentrations of pesticides are those of Flumen in Sariñena, Alcanadre in Ontiñena, Arba de Luesia in Tauste and Clamor Amarga in Zaidín.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index   I

Linking SPATIOtemporal variations of diuron contamination to biofilm induced tolerance in a river

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Biofilms represent useful potential early warning indicators for monitoring of rivers. When assessing the effects of toxicants on natural microbial communities, special attention must be given in the distinction between these effects and those resulting from other environmental parameters. Pollution induced community tolerance (PICT) approaches offer possibilities to partially isolate effects of individual toxicants within a complex ecosystem by studying shifts in community sensitivity. To validate the pertinence of PICT methodology for risk assessment, the aim of our study was to investigate if diuron tolerance levels induced in photoautrophic biofilm communities were proportional to their previous *in situ* exposure level to this herbicide. A field survey was conducted for 9 months at two sites located in a river chronically contaminated by diuron. Spatiotemporal variations of diuron tolerance capacities within photoautotrophic communities were estimated monthly from short-term photosynthesis bioassays. Even if we observed a possible influence of three co-varying environmental variables (nitrates, conductivity and temperature) in diuron tolerance induction processes, statistical analysis clearly demonstrated that the main factor explaining variation in diuron sensitivity was the diuron exposure level during biofilm colonization periods. A remarkable exponential correlation between EC_{50} values and *in situ* diuron concentrations was thus recorded, confirming that PICT can serve as a relevant tool for environmental monitoring of rivers in complement to other existing bioindicator methods.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Multi-biomarker responses of benthic macroinvertebrate species to diagnose the ecological status of polluted rivers.

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Biological indexes of benthic macroinvertebrate species are currently used world wide to measure river water quality with ecological criteria.. These indexes assign a global ecological status of the biotic community, but do not detect specific effects of water pollutants at environmental relevant concentrations. Here we provide experimental data on biochemical responses of transplanted and field collected individuals of two taxa: *Echinogammarus* sp. and *Hydropsyche exocellata* across a gradient of polluted sites. The study was performed in the LLobregat, the Besòs and the Ebro river basins (NE, Spain). A multi-biomarker approach including up to ten different markers was used to assess effects of a broad range of pollutant sources such as glyphosate, pharmaceuticals, organochlorine compounds and mercury. Biological responses included biotransformation and antioxidant enzymes and markers of tissue damage. The results obtained evidenced the following: (1) a good repeatability of biomarker responses within sites across seasons, (2) greater biotransformation and antioxidant enzyme activities in polluted sites and in tolerant species, (3) a higher responses of field collected relative to transplanted organisms and (4) the feasibility of biomarkers to differentiate and hence indentify major pollutant sources affecting river biota. Furthermore, likewise biological indexes, biomarkers responses measured in populations located in reference and contaminated sites were also able to discriminate different degrees of pollution. Therefore, our results indicate that multi-biomarker responses of macroinvertebrate benthic species provide useful and complementary information than those obtained with biotic indices and are necessary to characterize the ecological status of Mediterranean river ecosystems. This is especially interesting in moderately polluted sites, where stressors are already affecting communities but not too strongly to be detected by biotic indexes. Multi-biomarker responses thus can be used as warning signals alerting that if no changes in the increasing trends of pollutants are produced, the ecological status of moderate polluted areas may even decrease in the future. This study was funded by the Spanish projects CGL2008-01898 and CGL2007-64551/HID and the Diputació de Barcelona ECOSTRIMED programm (www.ecostrimed.net)

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Patterns in diatom community structure in rivers submitted to heavy metal inputs in different countries and consequences for biomonitoring

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A large database of river diatoms (comprising more than 450 taxa) was constituted based on field surveys carried out in different countries (France, Spain, Switzerland, Canada, Vietnam), in rivers exposed to various loads of heavy metals in the water. After taxonomy harmonization, the patterns in diatom community structure were investigated for 163 samples, all collected from hard substrates.

The biotypology (*i.e.* structuration of the diatom dataset) indicates that the species are influenced by the hydroecoregional context as well as metal inputs. The most structuring environmental parameters are investigated, and discriminating analyses are used to determine the relevance of some particular species (e.g. *Eolimna minima*, *Achnanthidium minutissimum*) as well as teratological forms for the biomonitoring of heavy metal pollutions.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index I

Pesticide gradients in rivers: fish-based investigative field monitoring

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By 2015, surface waters of all European member states must reach high or good chemical and ecological status, as stated in the current Water Framework Directive. Thus, the present extent of the contamination of aquatic environments due to anthropogenic activities calls for active assessment of the impact on exposed organisms.

In order to verify whether different levels of pesticides - mostly herbicides - found in rivers in South-West France are affecting fish populations, we had a look at their health status and traits. Classification of sampling sites regarding pesticide concentrations are based on data from annual surveys performed by the local water agency. Different classifications of the sampling sites are obtained according to which toxicity indexes are taken into account (eg. total msPAF, multi-substance Potentially Affected Fraction (of species), msPAF per Toxic Mode of Action, and Toxic Units).

An apriori selection of the sites was followed by electrofishing of chub (Leuciscus cephalus) and gudgeon (Gobio gobio) in autumn 2008. Our aim was to verify whether fish from polluted sites, in comparison to those from more pristine ones, present: lower nutritional status (condition factor), signs of deteriorated health (organo-somatic indexes), constrained growth, signs of toxic chemical stress (morphometric changes and fluctuating asymmetry), and higher body concentrations of pesticides.

Chubs from more polluted sites (higher msPAF and TU levels) presented higher gonado-somatic indices and lower condition factors, whilst the hepato-somatic index did not reveal any particular tendency along the gradient. Increased levels of pesticides and a number of hepatic histological changes were detected in chubs from more contaminated rivers. Gudgeon from more polluted sites appeared to have less external parasites and present, for example, significantly larger body height and significantly smaller eyes and dorsal fin. These results will be further developed and corrected for gudgeon population genetic diversity.

Results and conclusions from our study will additionally be placed within the framework of a general decision making process for water management.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Effect Directed Analysis of a benthic food chain using genotoxicity screening

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European surface waters are reported to be contaminated with direct- and indirect-acting mutagenic chemicals in many studies (Vahl et al. 1997, Keiter et al. 2006, Barceló et al. 2007, Higley et al. 2009 etc.). In order to efficiently identify mutagens and assess their presence in our freshwater systems Effect Directed Analysis (EDA) is a promising tool of investigative monitoring in the context of the Water Framework Directive monitoring programs.

EDA was applied to trace genotoxic activity in biotic and abiotic samples representing a benthic food chain from the aquatic environment.

Samples obtained from the Dutch Delta area, the Western Scheldt, were tested for genotoxicity. These included sediment, suspended particulate matter (SPM), worms, shrimps, cockles and a flatfish, the flounder. The abiotic samples (sediment, SPM) and the whole body homogenate of the biotic samples were extracted and cleaned up with a combination of Dialysis, Gel Permeation Chromatography (GPC) and Normal-Phase Liquid Chromatography (NP-HPLC). This stepwise sample treatment method to remove lipids and other interferences was developed and validated earlier. The extracts were tested in the AMES fluctuation test to detect point mutations and in the Comet assay to detect DNA damage induced by the chemicals in the extracts.

The first genotoxicity screening of the extracts revealed high cytotoxicity in the bioassays, despite the elaborate sample treatment procedure. To avoid the potential masking effect of cytotoxicity on the genotoxic endpoints, the samples were further fractionated on a silica column in order to remove cytotoxic constituents of the sample and to enable a proper genotoxicity screening. The additional fractionation then revealed weak genotoxic effects in certain fractions of some of the samples, as could be expected given the high reactivity of genotoxic substances in general.

Keywords: food chain, Effect Directed Analysis (EDA), genotoxicity, suborganismic bioassays

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Effect Directed Analysis performed on European river sediment with emphasis on the identification of androgen disrupting compounds

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In most areas influenced by anthropogenic activity, samples can contain a complex mixture of substances that may exhibit toxicological effects, such as endocrine effects, in organisms. Effect Directed Analysis (EDA) studies employ bioassay-directed fractionation techniques to be able to identify those fractions containing toxic compounds, and hence perform a toxicity characterization of the sample. The study presented here focuses on a sample that was selected after a toxicity screening of a number of river sediments within the Modelkey EU-project (SSPI-CT-2003-511237-2).

The selected active sample originates from the tributary Schijn to the river Scheldt in Belgium. The bioassay used to direct the analysis is the **C**hemically **A**ctivated **Lu**ciferase gene e**X**pression assay for androgen detection (AR-CALUX®), for both agonistic and antagonistic responses.

The aim of this EDA study is to identify androgen-disrupting compounds responsible for the bioassay responses. This is performed in several steps: bioassays on whole extract, first (reversed phase LC) and second fractions (normal phase LC), chemical analysis, identification of compounds, analytical confirmation and finally toxicity confirmation. The application of clean up and fractionation strategies and the use of various analytical chemical identification techniques will be presented, as well as a discussion how to interpret agonistic and antagonistic effects in EDAs. It was shown that a whole extract screening is not sufficient to reveal androgenic effects due to suppression of the agonistic effect by presence of antagonistic compounds.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Identification of androgen disrupting compounds in effect directed analysed river sediment with an LTQ-Orbitrap

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Attention regarding the presence of endocrine disrupting compounds in the environment has been high the last decades. These compounds range from natural and synthetic hormones to industrial chemicals. In areas influenced by anthropogenic activity, samples can contain a complex mixture of these compounds. Effect Directed Analysis (EDA) studies employ bioassay-directed fractionation techniques to be able to identify fractions containing active compounds, and hence decrease the complexity of the sample matrix before chemical analysis of the fraction. The identification of key toxicants and the final confirmation of toxicity and identity are critical for the success of EDA studies.

In this study an EDA was performed on a sediment sample from the river Schijn in Belgium and the androgenic activity was determined in the AR-CALUX® bioassay. The aim was to identify the androgenic compounds, both agonistic and antagonistic, causing effects in the active fractions of the EDA. The chemical analysis was performed on an LTQ-Orbitrap mass spectrometer. Commercially available software was used to sieve the active and the non-active fractions to discriminate the peaks of interest. The criteria were (1) a ratio of >100 between peak intensity in non-active and active fractions, (2) the suggested chemical formulas extracted from the accurate mass should be present in the NIST spectral database, (3) a CAS-number to be able to purchase the compounds for confirmation studies.

Around one fourth of the discriminated peaks were tentatively identified, e.g. anabolic steroids, antidepressants, musk fragrances and flavouring agents. The results suggest that working with an accurate mass instrument is a powerful tool in the task to identify unknown compounds in a complex.

ISBN 978-84-8458-311-0

Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index   I

Biodegradation of Pharmaceuticals during Wastewater Treatment and the Evaluation of Proteomics for Metabolic Pathway Elucidation

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Currently an increasing awareness of the presence of pharmaceuticals in domestic wastewater exists, which coupled with the potential risks associated with their release into the environment ensures the need to gain knowledge on their fate during wastewater treatment. With the improved analytical tools, a wide range of pharmaceuticals have been detected in raw domestic wastewater as well as in receiving ecosystems. Although there exists a great deal of uncertainty concerning the potential effects of these compounds on the aquatic ecosystems, the precautionary principle will likely give rise to more stringent legislations on wastewater treatment in the near future.

Many studies have shown greatly fluctuating elimination efficiencies for a wide range of pharmaceuticals, which have been often ascribed to biodegradation. However, only for a few compounds biodegradation mechanisms have been elucidated so far, and often these mechanisms have been found in lab scale experiments at relative high pharmaceutical concentrations. The study of biodegradation mechanisms of pharmaceuticals at environmental relevant concentrations is highly challenging due to the difficulties of their analysis. Knowledge on the fate of pharmaceuticals in wastewater treatment facilities requires knowledge on the biodegradation mechanisms and rate limiting conversions within the biodegradation pathways to enable the estimation of potential accumulation of intermediates.

In this work we focus on the involvement of specific trophic groups of microorganisms and elucidation of biodegradation mechanisms. The behavior of selected pharmaceuticals, carbamazepine, clofibric acid, diclofenac, ibuprofen and naproxen, was studied in a lab-scale SBR reactor. Removal rates under different operational conditions of a pharmaceutical gives information on the involvement of specific trophic groups of microorganisms for their degradation. This knowledge could ultimately aid in the optimization of the biological wastewater treatment for the simultaneous removal of the bulk pollutants and specific micropollutants such as pharmaceuticals, which requires new approaches in wastewater treatment. Even though there are many reports on biodegradation of pharmaceuticals, information on the employed mechanisms or biodegradation pathways are largely lacking. Since these pollutants occur at trace levels, their biological degradation remains subject to many uncertainties. As a result, the use of conventional microbiological techniques will likely remain ineffective for the elucidation of biodegradation mechanisms. In this study we evaluate the use of protein fingerprinting, in order to observe the differential expression of proteins under different growth conditions, i.e. in the presence and absence of a specific pharmaceutical. Proteomics, a relatively new approach in environmental microbiology, studies protein properties and permits us to identify key proteins and associated changes

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**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index I

under specific conditions. Since nearly all enzymes involved in biodegradation are proteins, changes in the protein fingerprint can be ascribed to the presence of the administered pharmaceutical. The protein fingerprints are obtained by protein separation in two dimensions, i.e. first by isoelectric point and subsequently by molecular weight, in 2D-gel electrophoresis. Afterwards, the differently expressed proteins, i.e. either their presence or absence as well as significant changes in expression level, are analyzed for their peptide composition and compared with peptide compositions of known proteins in online accessible protein databases. Ultimately, the involvement of specific enzymes enables the determination of biodegradation pathways, which in a later stadium will be affirmed if possible by analysis for biodegradation intermediates.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index   I

Uptake and effects of 1,2,3,5,7-pentachloronaphthalene in an aquatic food chain: from sediment via benthic organisms (*Lumbriculus variegatus*) to rainbow trout (*Oncorhynchus mykiss*)

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One of the many stressors in river systems are chemical pollutants. It is important to learn more about the mode of action of specific pollutants in aquatic organisms to be able to link adverse effects in a river system to the right causes. Having this knowledge will help water managers in taking effective measures to improve a declined ecological status of a river system.

In this study we focus on effects of hydrophobic toxicants which are present in sediment. Although binding to sediment decreases the bioavailability, benthic organisms that live in and feed on sediment, can bioaccumulate those toxicants and make them bioavailable again for organisms higher in the food chain, like fish. By further biomagnification, internal concentrations in the fish can reach levels at which the toxicant causes adverse effects on the individual fish. If for example growth or reproduction of individual fish are affected, this might in the end have consequences for the whole fish population. Uptake and effects of 1,2,3,5,7-pentachloronaphthalene (PeCN52) were followed in a simplified food chain consisting of three compartments: sediment – benthic worms (*Lumbriculus variegatus*) – rainbow trout (*Oncorhynchus mykiss*). PeCN52 is a congener of the group of polychlorinated naphthalenes, which are persistent and widely distributed pollutants: in Elbe River sediments concentrations have been measured in the range of µg/kg dry weight (dw).

At first a bioaccumulation study was performed in which worms were exposed for 28 days to sediment spiked with PeCN52. The resulting bioaccumulation factor of 35.3 (dw/dw) demonstrated that worms, when serving as food source, can transfer PeCN52 from sediment to higher levels in the food chain. Therefore, in a following study, juvenile trout were exposed to live contaminated worms. Worms were exposed in water with PeCN52 present at aqueous solubility to produce a large amount of worms with a constant burden of PeCN52. Trout were held individually and fed daily with worms. Four concentration levels (0 - 9 - 18 and 36 µg/g food) were tested. From the total intake of PeCN52 via worms, 60% was measured in trout tissue after 28 days. Despite this high bioaccumulation, no significant effects were found on mortality, behaviour, growth, and liver somatic index of juvenile trout. Expression of CYP1A and P-glycoprotein, both involved in the defence mechanism of fish, were measured in liver, brain and gut of trout through real time RT-PCR. Exposure to PeCN52 resulted in a concentration dependent induction of CYP1A, but no effect was found on P-glycoprotein expression. These results add to our knowledge on transfer of hydrophobic toxicants in a food chain, amd make it possible to model the flux of PeCN52 in a food chain.

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index I

The use of a new set of fluvial biofilms biomarkers to assess the effects of metals: contribution to the water framework directive application

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It is well know that some aquatic environments are contaminated by heavy metals from diverse kind of wastes. At present there are not many biomarkers of metal pollution and many of the biotic indices which are in use are mostly based on structural changes occurring at community level which are expected to integrate biotic responses over relatively long periods of time (from weeks to months depending on the life time of the organisms investigated). Thus, it is of great interest to develop new metal toxicity bioindicators focusing on both early responses and chronic effects in order to complete the information provided by the available ones. This will improve our understanding of the causes of ecosystem damage as required by the water framework directive (WFD, 2000/60/EC).

In rivers and streams, the biofilm (also known as phytobenthos or periphyton) has been widely used as bioindicator of pollution due its capacity to detect early effects produced by toxic substances, as are heavy metals, providing an ecotoxicology approach at community level with high ecological relevance.

Metals interfere with the metabolism of organisms at different stages. The pulse amplitude modulation (PAM) fluorometry has been widely used to assess the direct and indirect functional effects of toxicants on photosynthetic organisms. Antioxidant enzyme activities (AEA) have been studied as well, giving to the main conclusions that AEA can be used as "early warning systems" because they have a functional response detectable before than structural changes (i.e. algal biomass or species composition). Moreover, these enzyme activities might also be biomarkers of adaptation, since their activation is expected to contribute to metal detoxification under chronic exposure.

The aim of this study was to investigate metal toxicity on fluvial biofilms using functional (PAM), metabolic (AEA) as well as structural (diatom species composition) endpoints. To reach this goal, a biofilm translocation experiment was performed in the Osor River, a tributary of the Ter river located in a former mining area. This river presents high levels of dissolved zinc (Zn) and iron (Fe) reaching up to 600 µg Zn/L and 750 µg Fe/L after the entrance of a mining source.

Biofilms were translocated from non-polluted to polluted sites in order to evaluate the different responses obtained over a metal concentration gradient at different temporal scales (from hours to several weeks of exposure).

In spite of the high temporal variability observed, we identified several functional and metabolic parameters related with the metal gradient. Metal concentrations found in Osor River affected the fluvial biofilm causing transitory physiological responses (short exposure) and structural and functional alterations (chronic exposure). Moreover, at the end of the experiment (after 5 weeks of exposure), dominant diatom species were also related with

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Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index   I

the metal gradient, showing more teratological forms and a smaller biovolume at the metal-polluted sites.

Our results suggest that PAM and AEA of fluvial biofilms can be used as early-warning tools as well as biomarkers of adaptation in metal polluted rivers complementing the information provided by diatom studies (including taxonomical and morphological attributes).

ISBN 978-84-8458-311-0

**Emerging and Priority Pollutants:
Bringing Science into River Management Plans**
25-26 March 2010, Girona

Abstracts Poster presentations

Index   I

Predicting the effects of toxic substances upon functional diversity of North-American fish species

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The aim of this study was to assess the effects of chemicals and mixtures upon the functional diversity of fifty seven native fish species collected at 2000 sampling sites over a broad geographical area, the state of Ohio (USA). The functional diversity approach holds much potential for making quantitative prediction of the representation organismal groups along gradients of environmental alteration caused by humans than the typical taxonomic approach. Abundance data collected were used to weight the occurrence of 'biological' traits, such as trophic ecology, reproductive strategy, locomotion/morphology, body length and 'ecological' traits, such as preferences for substrate, geomorphic and stream size. Two different artificial neural network algorithms were used in this study: a self-organizing map (SOM) and a multilayer perceptron (MLP). A SOM was applied in order to determine trait assemblage types, and a MLP was used to predict assemblages using different predictors, including: landcover, macrohabitat characteristics, classical water chemistry, and exposure and potential risks to a large variety of chemicals. Our results suggest that fish community function is primarily structured by large-scale differences in habitat supporting the findings of previous studies. Moreover, we demonstrate a significant link between fish traits assemblages and chemical exposure and risks showing that this specific stress factor may act as a filter selecting particular trait types and assemblages.

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Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index |

Investigating the Environmental Fate of Emerging and Priority Contaminants - Identification of Transformation Products

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In recent years, research has focused on the development of sensitive and reliable analytical methods for detecting the presence of organic contaminants (i.e. pharmaceuticals, personal care products, biocides, plasticizers, perfluorinated compounds, etc.) in various environmental matrices. However, research on the fate of these emerging and priority contaminants in watersheds is largely unknown.

Fate studies provide valuable information about the partitioning capabilities and degradation potential of the target compounds. In addition, how the compound might react during various treatment processes, and therefore potential mechanisms of removal. Previous research has investigated the removal efficiencies of various conventional and advanced treatment processes to limit these compounds from entering the aquatic environment as well as drinking water supplies. However, limited research has investigated what compounds are formed after the application of various treatments (whether biological or chemical) if complete elimination is not obtained, and if these unknown products are considered a threat to ecological health.

Research conducted within the Marie Curie Research Training Network KEYBIOEFFECTS (MRTN-CT-2006-035695) investigates the biotransformation of a group of contaminants, iodinated X-ray contrast media (ICM), which are commonly detected in WWTP effluents, surface water, and groundwater. In particular, research focused on an approach to elucidate the chemical structures of biotransformation products of ICM in aerobic water-soil and water-river sediment batch systems. This multi-step approach enabled the identification of a total of 34 TPs of three non-ionic ICM (iohexol, iomeprol and iopamidol) in these batch systems. The concept involved the use of semi-preparative HPLC-UV, LC ESI tandem/linear ion trap MS and NMR.

The development of a LC-ESI tandem MS method in combination with SPE techniques allowed for the newly identified TPs to be detected in real environmental samples. Concentrations of the TPs have been detected up to the low μg/L range in surface waters. The significance of these biotransformation products in the environment is still unknown, but the approach is a crucial step in attempting to resolve and identify what key unknown compounds might be responsible for the effects observed in aquatic and terrestrial ecosystems.

Water toxicity assessment in Catalan rivers (NE Spain) using Species Sensitivity Distribution and Artificial Neural Networks

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In compliance with the requirements of the EU Water Framework Directive, monitoring controls of the ecological and chemical status of Catalan river basins have been carried out by the Catalan Water Agency (NE Spain), following the calendar set by the Directive.

The large amount of data collected and the complex relationships among the monitored variables make difficult data interpretation in terms of toxic impact, especially considering that even pollutants at very low concentration might contribute to the total toxicity of a mixture.

The whole dataset of chemical controls carried out during years 2007-2008 (232 sampling stations and 60 pollutants) have been analysed using sequential advanced modelling techniques.

Data on concentration of contaminants in water were pre-treated in order to calculate the bioavailable fraction, depending on substance properties and local environmental conditions.

The resulting values were used to predict the potential impact on aquatic biota of toxic substances in complex mixtures and to identify hot spots. Exposure assessment with Species Sensitivity Distribution (SSD) and mixture toxicity rules were used to compute the multi-substances Potentially Affected Fraction (msPAF).

In order to understand and visualize the spatial distribution of the toxic risk, the Self Organising Maps (SOM) method, an unsupervised algorithm of an artificial neural network model, was applied on the output data of these models.

Principal Component Analysis (PCA) was performed on top of Neural Network results in order to identify main influential variables which account for the pollution trends.

Finally, predicted toxic impacts on biota have been linked and correlated to experimental data on biota quality indexes (IBMWP, IPS) and to other physico-chemical variables.

Hot spots and contamination patterns have been identified in order to give indications to water managers to assess chemical risk on a basin scale.

Keywords: Aquatic toxicity - Risk Assessment- Species Sensitivity Distribution- Artificial Neural Networks

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Emerging and Priority Pollutants:
Bringing Science into River Management Plans
25-26 March 2010, Girona

Abstracts Poster presentations

Index I

Are biotic indices sensitive to river micropollutants? A comparison of metrics based on diatoms and macroinvertebrates.

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Many field studies have shown that the presence of toxicants results in predictable changes in river benthic communities. Biotic indices based on macroinvertebrates and diatoms are frequently used to diagnose the ecological quality in watercourses, but few works assess their effectiveness as biomonitors of the concentration of micropollutants. This work reports the results from a biological survey performed in 188 sites in the Duero River Basin (NW Spain). 19 diatom and 6 macroinvertebrate indices were calculated an compared with the concentration of 37 different pollutants by means of correlation analysis. More than a half of the analyzed chemical variables correlated significantly with at least one biotic index. Sládeček's diatom index and the number of macroinvertebrate families exhibited particularly high correlation coefficients. Methods based on macroinvertebrates gave a better performance for the detection of biocides, while diatom indices showed stronger correlations with potentially toxic elements such as heavy metals. All biotic indices and, particularly, diatom indices, were especially sensitive to the concentration of fats and oils and trichloroethene, while anionic and nitrogen-derived compounds achieved the lowest correlation values. Results show that both macroinvertebrate and diatom indices can be reliable methods for the surveillance of river toxicants.