

4th NORMAN workshop

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

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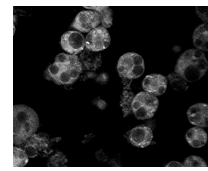
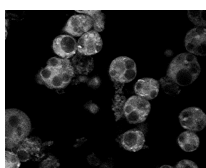
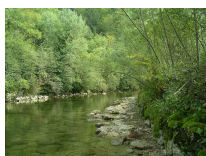


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Integrated chemical and biomonitoring strategies for risk assessment of emerging substances

Report on the 4th thematic workshop of the EU Project NORMAN, held 17-18 March 2008 at Lyon, France

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1. Organisation and objectives

The 4th Norman workshop, the last of the thematic workshops in the European project NORMAN¹ gathered over 100 participants from 19 different countries and different fields of expertise. The objectives were to present existing protocols and experiences in the development of integrated chemical and biological strategies and to discuss the concrete possibilities and limitations for their application in the framework of the current legislation (in particular, the Water Framework Directive) to complement chemical-driven risk assessment approaches.

The workshop programme included 19 presentations and 15 posters (available on the Norman workshop website: <http://norman.ineris.fr/public/workshops/workshopss.htm>), which were organised around the three following topics:

- Prioritisation of emerging substances for field monitoring and risk assessment (*Chaired by Valeria DULIO and Marina COQUERY*)
- Effects-driven approaches for field monitoring and risk assessment of emerging pollutants (*Chaired by Pim LEONARDS and Werner BRACK*)
- Integrated approaches within risk assessment strategies for monitoring risks from emerging pollutants at local and large scales (*Chaired by Jeanne GARRIC and Eric VINDIMIAN [morning] and by Dick VETHAAK and Bo JACOBSEN [afternoon]*)

This report highlights the main points expressed during the workshop, the different tools potentially applicable to complement chemical-driven approaches and improve risk assessment, and also briefly describes major on-going programmes currently developing and applying integrated monitoring strategies in freshwater and marine environments. Lastly it presents major conclusions on integrated monitoring methods and strategies relating to the risk assessment process, research needs and recommendations for policy makers.

2. Summary of topics

2.1. *Prioritisation of emerging substances for field monitoring and risk assessment*

Research and development in analytical methods, monitoring of occurrence, experimental fate studies and hazard and risk assessment have led to more significant knowledge and understanding during the last two decades. This has resulted in regulations targeted on chemicals in the environment via EU Directives, international conventions and others. At the same time it has led to the recognition of several emerging substances due either to new chemicals or new knowledge on occurrence and risks of existing substances.

Emerging substances can be defined as substances that are currently not included in routine monitoring programs at the European level. They may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects, public perception and on monitoring data regarding their occurrence in the various environmental compartments. Emerging pollutants often originate from consumer products and by-products used every day in homes and farms, or by business and industry. They include: household-cleaning products, fragrances, over-the-counter and prescription drugs, veterinary medicines, disinfectants, pesticides, pathogens and nanoparticles.

Existing hazard and risk assessment strategies of emerging substances suffer from the lack of accurate information about their fate in different aquatic environments and their effects on aquatic ecosystems. Little information is available about the point sources of these chemicals and little is known about their fate processes at a local level. Moreover, for ecosystems, predicted no-effect concentration (PNEC) values provide insufficient information about the environmental risk: assessment with organisms is done at the individual level and do not represent biodiversity or the long-term impact on populations.

Monitoring data accessibility on emerging substances is another obstacle. The data either do not exist or are difficult to access from industries due for example to protective purposes. REACH should be helpful to overcome this obstacle, however this system is currently limited to industrial compounds (e.g. excludes pharmaceutical products and biocides), and the type and amount of available, unrestricted information is unknown.

Finally, because of the vast number of emerging substances, some prioritization is needed. It is therefore necessary to re-examine existing protocols and monitoring strategies for environmental risk assessment and integrated chemical and biological methods that should help policy-makers better structure their decisions about prioritizing emerging substances and about authorizing the use of new ones.

Among others, the following suggestions were made to improve data prioritisation methodology for risk assessment:

- Integration of biomarker data (exposure biomarkers provide an integrated measure of exposure over a time period, they allow to take into account bioavailability and allow a better understanding of the link between effects and exposure)
- Use of QSAR modelling (useful for gathering knowledge and making predictions about chemicals)
- Better communication with stakeholders to explain policies

- Risk assessment exercises conducted by mixed panels including stakeholders.

Two presentations addressed prioritisation methodologies for risk assessment and shortcomings of today's knowledge. The first one described a prioritization methodology for monitoring pharmaceutical products, and the second one focused on household products and pesticides.

- The methodology developed for human pharmaceutical products and their metabolites, involves three steps: 1) classification of compounds based on modified EMEA² predicted environmental concentrations (PEC), 2) a case-by-case review of all available biological data (e.g. pharmacological, ecotoxicological), and 3) selection of molecules with the same chemical structure and the same mechanism of action. The method was applied to a French situation by the research institute CEMAGREF (*presented by Besse*) and allowed to prioritize 120 parent molecules and 30 metabolites. The final priority list groups 40 parent compounds and 14 metabolites. Among the 40 parent molecules, 21 have already been detected in the aquatic environment at expected concentration levels, which indicates a good agreement between the theoretical approach and the environmental measurements.
- Within the framework of the Finnish research project VESKA³ (*presented by Mannio*), a screening model has been developed to identify sources and concentration of WFD priority substances and nationally-relevant organic pollutants (e.g. industrial/household compounds and pesticides) in aquatic environments in 12 Finnish cities and 30 agricultural areas. The model consists of a preliminary risk analysis to identify the compounds to be studied. Those of concern are subjected to further screening by selecting compounds based on their use (e.g. factories, industries, agriculture) and their properties (e.g. bioconcentration factor, water solubility). Then emission sites are selected to screen for compounds in different matrices (e.g. wastewater treatment plants (WWTP) sludge, WWTP effluent, sediment, biological material). Pesticides and organotins were easy to detect. However, screening methods did not provide the necessary information for certain chemicals with several, poorly well-know sources, such as industrial and household substances. In other words they were difficult to "catch" since source identification (site, timing) was not always clear. Using integrated (chemical & biological) monitoring should help to better define the risks of these substances.

2.2. Strategies to complement chemical-driven risk assessment approaches

Emerging substances have been traditionally identified using a source specific approach: evaluating volumes of chemicals used and determining, through calculations, quantities potentially released to the environment. Analytical chemistry is used to target the compounds of interest and to record their occurrence in the environment. However this approach does not sufficiently cover unintentionally produced chemicals (by-products, metabolites) that may cause adverse effects even at low concentrations. Two presentations (*presented by Thomas, Brack*) described an approach that targets toxicants based on their effects in environmental samples. This approach, known as effect-directed analysis (EDA), combines effect testing with a sequential reduction of sample complexity by fractionation techniques and subsequent chemical analysis of toxic fractions. The approach can be used to identify compounds that have the potential to affect biological systems in the environment and thus provide data to complement risk assessments and hazardous substance monitoring strategies.

EDA involves the 3 following tiers:

(i) *Toxicity characterization*: Characterization of the toxicity of bulk samples with biotests and assignment to types of toxicants (metals, lipophilic organic toxicants...) by simple sample manipulations.

(ii) *Toxicity identification*: Identification of candidate toxicants that may be responsible for measured effects by combining chemical extraction, fractionation, bioassays and chemical analysis.

(iii) *Toxicity confirmation*: Qualitative (chemical structure) and quantitative (contribution to measured effect) confirmation of candidate toxicants as cause of the effect. For hazard confirmation, effects on higher levels of biological organization (e.g. communities) under realistic exposure conditions may be analyzed.

Studies on EDA development and implementation have been conducted in UK estuaries and offshore effluents in the North Sea to identify the chemical compounds at the origin of detected endocrine disruption effects (*Thomas*) and in Germany to identify industrial compounds found in sediment (*Brack, MODELKEY⁴*.)

As far as biological analysis is concerned, bioassays are not the only available tools. Below are examples of biological methods that could be integrated in the effect-driven analysis:

• **Environmental Specimen Bank (ESB⁵) for effect-driven analysis** (*presented by Rüdell*)

As a part of a German environmental programme since 1985, this project involves collecting and storing biota samples from freshwater, marine and terrestrial environments.

The application of *direct-effects assessment* on ESB is an ideal tool in identifying effects of emerging substances. Up until now, the following biological analyses used for ESB screening have been: biomarkers (e.g. vitellogenin) and reproductive impairment (e.g. gonad disorders). DNA and RNA analysis of homogenized standard ESB samples is also possible allowing investigations of temporal trends and spatial comparisons (e.g. anthropogenic influenced vs. pristine regions). Effect data can be assessed together with exposure data from monitoring of samples from the same site and sampling period. Moreover, special effect studies can be performed with routine specimens or with specimens sampled additionally to the normal ESB sampling campaigns.

As for *exposure assessment*, ESB also offers the following advantages:

- Concentration trends can be identified by analysis of appropriate biota samples from different levels of the trophic system allowing the identification of emerging pollutants (e.g. methyltriclosan)
- Even small temporal changes or slight regional differences of concentrations become obvious due to standardized samples
- The monitoring data can be used as a basis for the justification of political measures (e.g. banning of pollutants with accumulation potential)
- Monitoring results allow the assessment of results of political measures taken in the past (e.g. use restrictions for TBT)

• ***In vivo* measurements on small model organisms using *in vitro* tools** (*presented by Lemkine*) In collaboration with Veolia Water's research station "Anjou Recherche"⁶, Watchfrog⁷ has been investigating the use of small model organisms (i.e. amphibians and fish) combining *in vivo* and *in vitro* methods to address the OECD testing criteria for the risk assessment of

endocrine chemicals in mixtures and complex liquid matrices. In other words, this innovative method incorporates *in vivo* significance with early vertebrate stages, providing quick results about target chemicals. Further more, it's a tool that could be adapted for *in situ* tests for semi-robotized monitoring at industrial sites and WWTPs. A fluorescent-based screening in genetically modified amphibian larvae can mean the presence of one or more pollutants or a reaction in an organ (tissue or muscles). This low-cost and rapid testing method not only provides predictive results about effects to aquatic environments.

2.3. Integrating chemical and biological monitoring strategies : existing protocols and application experiences

Over the past several years, and even decades, different international and European programmes have been developing analytical and sampling methods combining both chemistry and biology in order to improve the risk assessment process of emerging substances and their effects to marine, estuarine and freshwater environments.

2.3.1 Marine environment

The marine environment is particularly exposed to increasing human activities, consequently making these ecosystems more and more vulnerable to contaminants, which may accumulate along the food chain effecting higher organisms, including humans. We know today how to determine at what concentrations some major emerging substances occur in the marine environment, but to what extent are they a hazard to the ecosystem? To answer this question, on-going programmes are developing and implementing monitoring and assessment approaches for Mediterranean (MEDPOL), North-East Atlantic (ICES/OSPAR), and Arctic regions (AMAP, RESPIL).

• MEDPOL (presented in session II by Martinez-Gomez)

The programme for the assessment and control of pollution in Mediterranean regions (MEDPOL⁸), is the first operational programme under the Mediterranean Action Plan (MAP), which contributes to the Barcelona Convention and is supported by the UNEP. MEDPOL is entering its 4th phase and involves integrated monitoring strategies to develop Mediterranean marine pollution indicators (MPIs) such as biological indicators (i.e. community diversity), biomarkers (i.e. lysosomal membrane stability) and chemical indicators (i.e. hazardous substances, eutrophication). These indicators would be a basis for preparing marine environment assessments to develop and implement protection and conservation policies.

The Marine Contamination and Biological Effects (MCBE) research group, belonging to the Spanish Institute of Oceanography (IEO), has been contributing to the UNEP MAP MEDPOL Programme these last decades, performing annual biomonitoring activities along the Spanish Mediterranean waters. Main objectives are: 1) the determination of spatial distribution and temporal trends of selected contaminants in target organisms (mussels and red mullet) and sediments in coastal, hot spots and reference areas; 2) the integrated assessment of the chemical contamination in some selected sites/areas chemically well characterized as hot-spots (by measuring contaminant content in target organisms and sediments) and biomarkers of biological effects of contaminants and secondary parameters (in target organisms).

The programme is also working on the application of a 2-tier approach using caged mussels to evaluate organism-health status and compounds responsible for the pollutant-induced stress syndrome. It considers lysosomal membrane stability (LMS), "stress-on-stress", and mortality as the main mussel biomarkers. Caged mussels have been proposed in hopes to standardize and

facilitate result comparisons between different areas. However the use of native mussels is preferred for studying temporal trends in chemical concentrations and long-term biological effects.

• **JAMP-OSPAR** (presented by Hylland).

The on-going Joint Assessment and Monitoring Programme (JAMP) is a monitoring strategy formed under the OSPAR⁹ commission. There is currently a joint ICES/OSPAR process to develop guidelines and a programme for integrated chemical and biological monitoring for the North East Atlantic marine environment (WKIMON¹⁰). This process has included inputs from national programmes within European countries (e.g. Germany, UK, NL, France, Norway and Sweden).

In developing the integrated assessment, the following issues are being addressed:

- (i) measuring biological effects in parallel to the measurement of chemical concentrations
- (ii) choosing the appropriate compartments, contaminants and analytical methods
- (iii) developing assessment criteria for each parameter and endpoint (e.g. for bioassays, EROD, fish diseases...)
- (iv) developing an integrated framework within which the results of the assessment of the different components – for sediment (chemistry, characteristics, bioassay...), for biota (tissue chemistry, biological effects for different types of organisms,) and for water (water chemistry, hydrography, bioassays...) – can be weighed and combined.

• **PRAGMA and RESPIL** (presented by Baussant).

PRAGMA¹¹ (2005-2008) and RESPIL¹² (on-going) are two EU funded projects [2006 & 2007 Community framework for cooperation in the field of accidental or deliberate marine pollution¹³] focusing on adaptive integrating monitoring methodologies to evaluate environmental impacts of accidental spills of hazardous and noxious substances. These recent projects aim to propose biological monitoring methods to complement chemical analysis and to test them in the laboratory and in the field mesocosms. These alternative methods, which can be implemented in a 2-tier risk assessment, include: biosensors, bioassays, and biomarkers (histological and histopathological). Biosensors can be used in the 1st tier of the assessment to screen DNA, for example in fish bile, or the valve movement of mussels. The biomarkers and the chemical analysis would be used for more in-depth screening in the 2nd tier for toxicity confirmation. How can these methods be implemented to assess recovery post-spill and help decision-making by EU regulators and stakeholders? The last stage of the RESPIL project currently involves the development of a simple environmental index (based on biological data), which can provide valuable information for decision-makers, then the integration of the methodologies in EU environmental guidelines.

• **AMAP** (presented in session II by Reiersen)

The Arctic Monitoring and Assessment Programme (AMAP¹⁴) formed in the 1990s of multidisciplinary expert groups from the Arctic and non-Arctic countries, aims to examine, monitor and assess the effects and trends of pollutants in biota and humans of the Arctic area. Results have been reported for decision makers and public interest and are used to advise decision makers on actions to be taken to improve the Arctic environment. The pollutants of concern are persistent organic pollutants (POPs), heavy metals (Hq), radionuclides, petroleum hydrocarbons,

acidification and Arctic haze. The monitoring and assessment issues also include climate change, UV and ozone. Bioaccumulation and effects of these pollutants have been observed in the higher levels of the food chain up to the indigenous peoples. Due to the monitoring and data collection, it has been observed that pollutant concentrations (e.g. PCBs, DDT) have decreased over the years. However, emerging chemicals (e.g. brominated flame retardants, PFCs...) have been increasingly measured in the environment. Data management is a key issue since data is collected from national programmes.

• **Other strategies under development:**

(i) Package of methods relevant to monitoring for new emerging substances: passive sampling devices and bioanalysis (integrated methods to link the WFD and the Marine Strategy Directive (MSD)) (presented by Vethaak)

For some emerging substances, such as endocrine disrupters, integrated methods are being studied under ICES/OSPAR that momentarily focus on endpoints in male fish (VTG, intersex gonads) and snails (imposex/intersex), as no specific methods have been developed for many other marine invertebrate phyla. For other known emerging substances potentially having other unknown effects (e.g. brominated flame retardants, PFOS) few biological effect methods are available, and for nanoparticles both chemical and biological methods are lacking. Passive sampling devices (e.g. silicone rubber) are a solution under development as an integrated tool in monitoring some of the above substances in water and assessment of their availability in sediments. It can collect extracts of chemicals for either chemical analysis or bioanalysis. Another useful tool to obtain a first hazard identification of known and unknown emerging substances is the application of integrated bioanalysis. This method includes the application of a battery of short-term low-volume screening assays (*in vitro* and *in vivo*) on environmental extracts (water, sediment). It's cost-effective, rapid, and logistically and technically feasible. It can be applied in freshwater, brackish and marine environments. The combined use of bioanalysis and chemical measurements can be used for preliminary assessment and hazard identification of complex mixtures of toxicants and unanalysed toxicants (e.g. TIE, EDA). Though assessing for biological effects is not required in the WFD, it should be integrated to complement chemical analyses. This would enable the effects of substances other than the selected priority chemicals to be monitored as well. In this connection, there are advantages to be gained for combining the use of passive sampler extracts and bioanalyses as an important link between the WFD and MSD.

(ii) "Biomarker Bridges" (or biomarker response distributions) (presented by Sanni)

This tool could be a helpful in validating the environmental risk of hydrocarbon discharges and in monitoring early effects of emerging pollutants in arctic species by using caged or wild caught organisms. This tool can integrate predictive risk assessment with biomonitoring to obtain coherent assessment schemes. This implies the correlation of (predicted) responses of chemical constituents of oily discharges to biological responses (*in-situ*). Also the tool can provide an early indication of the effects of emerging substances due to the biomarker response signals (genotoxic stress, oxidative stress, LMS...). Finally it has been shown that it may contribute to the identification of environmental indicators for risk-based environmental management of the Barents Sea and other Arctic waters.

2.3.2 Freshwater environment

Programmes for development of integrated monitoring methods in freshwater environments currently remain limited at a local and national level. Many possibilities are being investigated. However many factors need to be taken into account before harmonising and standardising

them at a European level. Different elements are being studied: passive samplers (mimicking living organisms), biological monitoring to be combined with chemical analysis in WWTP, and biological indicators for evaluating water quality.

- **Passive samplers** (*presented by Vermeirssen*). Passive samplers are useful tools recently developed to improve sampling and monitoring of chemicals in the environment. They are placed for example in a river to collect chemicals over periods of days to weeks providing data about low levels of pollution and about potential bioaccumulation. When the uptake kinetics are known, time weighted average concentrations can be calculated.

The following describes studies conducted by Eawag¹⁵ in Swiss rivers and effluents, testing the two polymer-based samplers:

- 1) The polar organic chemical integrative sampler (POCIS), mainly focused on estrogenic substances. POCIS data correlated well with average concentrations assessed with repeated grab samples. The yeast oestrogen screen (YES) was applied to the POCIS sample extracts. The bioassay data correlated well with the amount of steroidal estrogens measured with chemical analysis. POCIS data also correlated with the amount of estrogenic activity accumulated in the bile of male fish.
- 2) The Chemcatcher where mainly pharmaceuticals and pesticides were analysed. Results from the samples analysed biologically (algae-based assay) and chemically (herbicides) showed a good correlation between the two approaches.

Passive samplers for polar organic compounds already provide valuable data on the identification of chemicals in water. However, improvements are needed to standardise field experiments, as results are significantly influenced by environmental conditions. This method should allow for the calculation of accurate average concentrations and to extend passive sampling to a routine level.

- ***In vitro* bioassays for freshwater sediment.**

A combined chemical and biological approach (*presented by Ait-Aissa*) is under investigation to know more about endocrine disrupting chemicals entering French rivers and sediment from different sources. The approach begins by detecting endocrine disruption in fish with estrogenicity and androgenicity biomarkers, then applying *in vitro* bioassays to detect endocrine activities in natural, sediment samples, and finally chemical identification of the active endocrines by applying bioassay-directed analysis (HPLC fractionation, affinity columns with purified nuclear receptors) on sediment extracts. Such an approach could be useful for assessing endocrine activity in aquatic ecosystems, which will be evaluated within the framework of the project SURVAQUA¹⁶ (funded by the French national research programme on endocrine disrupters).

- ***In vitro* bioassays for integrated monitoring in water treatment utilities.**

Over the past decade many efforts have been made to monitor occurrence of a number of hazardous substances (regulated chemicals and emerging pollutants) in drinking water and in treated wastewater effluents. The treatment techniques include both conventional and more advanced technologies (membranes, ozonation...). However, what are the concentrations of suspected emerging chemicals, and do these concentrations present a risk to ecosystems and human health? Integrated monitoring is a promising tool to answer such questions

In United States drinking water facilities (*presented by Snyder*), pharmaceutical products and their metabolites are being detected in nanogram concentrations with robust analytical methods. Estrogenicity was detected at higher concentrations in beverages than in drinking

waters. Nevertheless, drinking water facilities demand more research to reassure citizens that their drinking water is in fact safe.

As for wastewater facilities, the Avedoere WWTP in Denmark (*presented by Jacobsen*) conducted, in collaboration with the national monitoring programme NOVANA, a combined chemical and biological analyses to evaluate effluent quality and conventional tertiary treatment efficiency. Biomonitoring screening effluents (e.g. exposing mussels) allowed to detect chemicals, such as brominated flame retardants, undetected with chemical analysis alone. It also showed the lack of bioaccumulation of other compounds, such as Hg, Cd, Cu, and TBT in natural mussels in the receiving bay. Toxicity of influent and effluent was measured with algal bioassays. As expected from the chemical analyses no toxicity could be detected in the effluent. Influent toxicity could be explained by the concentrations of heavy metals, whereas the organic substances measured did not contribute significantly. In another study with exposure of fish in controlled dilutions of the effluent, response for estrogenicity based on P-content in blood serum (as a measure for vitellogenin biomarker) was significant in adult flounder but neither in juvenile trout nor silver eel.

- **Linking ecological status (SPEAR index) to chemical pollution (Toxic Units)** (*presented by von der Ohe, MODELKEY project*)

Specific indicators are needed to discriminate between different stressors responsible for insufficient ecological status. The SPEcies At Risk (SPEAR) index is an example for such an indicator, which has been developed to detect the effects of pesticides on the community structure of benthic invertebrate communities. SPEAR is based on the physiological sensitivity of invertebrate species exposed to organic compounds and on additional life history information. It is computed as a ratio of the total abundance of sensitive species (species at risk) and the total abundance of all species and thus, meets the requirements of an indicator for the WFD. In an investigation of study sites in French and Finnish streams, SPEAR was highly correlated with Toxic Units, a measure of the expected mixture toxicity of the analysed pesticides on *Daphnia magna*. A significant decrease in the relative abundance of species classified “at risk” had also been observed at sites with medium and high Toxic Units up to 60%.

3. Key questions and discussion

To better target emerging substances within chemical and biological monitoring programmes, the following key questions were addressed:

- To what extent do all relevant data on emerging substances in the environment need to be collected, stored and made available?
 - Should this be a responsibility of Member States?
 - How can private entities be convinced to contribute and share their knowledge?
- How far are we today from the application of bioanalytical methods and integrated assessment approaches for evaluating the ecological status under the WFD?

- How can the use of bioanalytical methods be improved for chemical risk assessment in the field?
- Do these strategies allow to discriminate pollution as a cause of ecological degradation from other causes?
- How are risks likely to evolve in the future with temperature and climatic changes?
- What can be done to convince policy makers to adopt monitoring strategies based on the use of cost-effective chemical and biological methods and modelling? How can acceptance of these methodologies by authorities be improved?

In response to these complex uncertainties, relevant suggestions and remarks have been summarised below under three categories: limitations, research needs, the implications of NORMAN, and recommendations.

1. Limitations of monitoring strategies for risk assessment

- The availability or the exchange of data between manufacturers and research entities is an important obstacle to overcome. REACH could be a solution as it plans to establish a risk communication system with which each company has to register chemical information (production volumes, use, fate, transport...). However, it's not clear to what extent this information will be accessible for researchers, especially knowing the data from private companies are protected. Furthermore, as already stressed, REACH is currently limited to industrial chemicals and doesn't consider compounds such as pharmaceutical and biocides.
- There remains a large gap to fill before practical application of integrated chemical and biological methods at a routine level.
- Another limitation addresses the assessment of ecological status of an aquatic environment and the selection of sensitive species: the assessment should take into consideration more environmental properties, the impact on each individual species, and their habitats.
- For river basin level approaches, a guidance document for Member States that explains how to evaluate the ecological status and how to identify river basin specific contaminants would be essential.

2. Research needs for prioritisation of data and the implementation of integrated monitoring methods

Data availability:

- It is essential to establish efficient data collection systems and make them available at the European level.
- Screening techniques to detect nanoparticles and their environmental impacts need to be better developed.

Tools for integrating biological and chemical monitoring:

- The major problem for water managers will be the multi-stressor situation that they will face if they want to identify the driving force at the origin of insufficient ecological status before implementing mitigation measures. A multitude of 'old' and emerging compounds together with eutrophication, hydromorphological problems, invasive species and so forth may contribute to ecological impairment. The development of approaches to identify the major stressors, to analyse combined effects, to link causes with effects and to understand the ecology of recovery are major research needs of water managers. Approaches linking bioassays, biomarkers and ecotoxicogenomics with effect-directed analysis need to be developed to identify potentially hazardous compounds emerging in the environment. Then they should be integrated in monitoring approaches to identify causal links between (emerging) toxicants and hazards.
- Passive samplers and extraction techniques: more research is needed to understand the impact of environmental conditions on sampling. Moreover research is also needed on finding a possible link between passive sampling and accumulation by living organisms and identification of toxic effects, biotransformation of contaminants and how these tools can be implemented to quantify or identify biological effects.
- Environmental Specimen bank: can this method be systematically extended to the European level?
- Effect-driven approaches: although an ideal method to know more about emerging substances and their impact in the environment, more studies are required in linking biological effects to chemical analysis, especially before application at a routine level.
- Biosensors: this cost-effective tool could be useful in identifying priority compounds under the WFD.
- Need to make a link between effects and bioaccumulation and biomagnification.
- Need to better understand the mode of actions of emerging substances.

Harmonisation of methods

A better harmonisation of the monitoring programmes and results obtained by the different regions / countries [i.e. EU Mediterranean countries, through their MED POL biomonitoring programmes, Atlantic-, Baltic sea countries through the Co-ordinated Environmental Monitoring Programme (CEMP¹⁷) and the Helsinki Commission (HELCOM¹⁸)] will reduce the costs caused by some overlapping monitoring activities and will allow a more realistic picture of the marine ecosystem health status. Despite differences among national/regional programmes, combination of synthetic indices for contaminants, biological effects and biology into generic assessment frameworks are under development and may be a solution to improve comparisons of results among regions.

- Develop a list of priority effects including thresholds and standard measurement methods to be used.
- Chemical analytical methods are basically harmonized. However biological methods for harmonisation need improvement. Also, QA/QC in biological analysis must be promoted.

3. Implication of NORMAN

Scientific knowledge continues to progress and competent authorities have to anticipate and prevent future risks. Because of the increasing number of reports on emerging substances in

the environment, identification, hazard characterisation and prioritisation of these compounds is needed in to identify the emerging substances that exhibit the highest risk in the European environment. Currently, existing hazard and risk assessment strategies of emerging substances suffer from a lack of accurate information about point sources, occurrence, routes of exposure, fate, and their effects on organisms.

During the NORMAN project, the network has developed three web-based databases, including EMPODAT, a database on monitoring data and results from bioassays and biomonitoring experiments in the field, which should contribute to improve current gaps in terms of accessibility and exploitation of the information on emerging substances.

A validation protocol for chemical and biological monitoring methods has been developed by the NORMAN experts to respond to the need for a common European approach to methods validation, which is the first step for good comparability and reliability of the data to be used in risk assessment.

Four workshops have been organised by NORMAN during the course of the project and the NORMAN network intends to continue these activities as a permanent, self-sustaining network to encourage the debate and contribute to decision-making.

The conclusions and recommendations of this workshop, and those from the previous events, will be further evaluated by the network and used to define the main topics for a research agenda on emerging substances under the European Framework Programme for Research and Development.

4. Final recommendations

Many of the programmes presented at this workshop seek to facilitate decision-making processes about detecting and monitoring emerging pollutants in view of measures to protect and conserve ecosystems and human health. An integrated programme should include not only water components but sediment and biota as well. The assessment of environmental impacts of contaminants requires both chemical analysis and characterisation of biological effects. Finally, assessment frameworks need to be transparent and should include all relevant ecosystems.

There is a need for consistent pan-European screening programmes designed for preliminary assessment and identification of hazard of yet unknown emerging chemicals. These programmes could include, amongst other techniques, novel tools like ecotoxicogenomics and metabolomics, *in vitro* and *in vivo* bioassays and biomarkers in aquatic organisms together with bioinformatic and statistical clustering methods and TIE/EDA approaches to identify causal compounds. In this connection, there are advantages to be gained in the combined use of passive sampler extracts and bioanalyses as an important linkage between the WFD and MSD. Both methods are generic and can be applied to a wide variety of environments.

One key issue for effective communication is the simplification of the message from the scientific community to the decision-makers, for example by developing simple indices. However, assessment frameworks need to be transparent and should include all relevant ecosystems components.

The communication message to policy-makers should explain why the application of integrated chemical-biological methods is more cost effective. The method itself is actually not cheaper.

However, the benefit would come from the fact that these methods would allow to better understand the reasons for not attaining a good ecological status (finding the causes of the effects observed in the ecosystems at the community level in the field). A better understanding of the causes is the only way to apply effective corrective measures and avoid waste of resources.

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