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Ecotoxicology in the Danube River Basin

Editorial

Dear Reader

Ecotoxicology has emerged in 1969 and is a relatively young scientific discipline. Defined as "ecology in the presence of toxicants" (Chapman 2002), it is truly interdisciplinary and integrates the effects of environmentally available toxic substances (stressors) across all levels of biological organisation from the molecular to individual organisms, whole communities and ecosystems. Ecotoxicological studies do not only deal with lab experiments by developing toxicity tests, but also are closely linked with in situ biomonitoring of (aquatic) ecosystems. Since humans are an integrative part of ecosystems and persistent toxic chemicals may accumulate in the food chain, ecotoxicology is of vital interest to health issues across the globe. Today, ecotoxicology gets new stimulus by elucidating the level of genoms and researching sub-lethal effects.

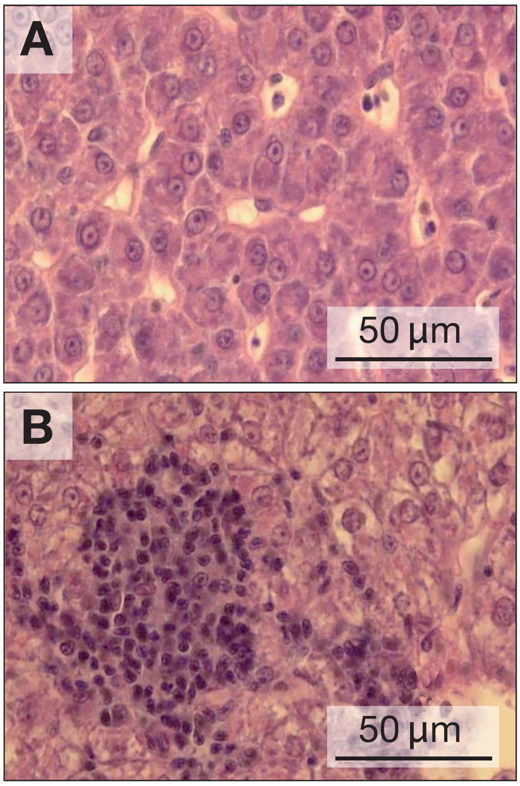

Figure 1. Histopathology of liver tissues of sneep (Chondrostoma nasus) in the Mures River (Romania) downstream of heavy metal mining sites. Figure 1A shows natural tissue with regularly shaped hepatocytes, round nuclei and eosin-positive cytoplasm from an uncontaminated site. Figure 1B shows tissue damaged by lymphocytic and macrophage infiltration, induced by accumulation of high Cadmium and Copper levels in livers. Such investigations contribute to detect contaminated rivers. (Reference: Triebskorn R et al. (2008): Monitoring pollution in River Mures, Romania, part II: Metal accumulation and histopathology in fish. Environ Monit Assess 141, 177–188)

While there are myriads of scientific literature on ecotoxicology, this topic so far had only limited attention in the Danube River Basin. This is evidenced in the actual draft Danube River Basin Management Plan (DRBMP) of the ICPDR. Given the legislative framework of the European WFD (e.g., list of priority hazardous substances, Directive 91/414/EEC regulating plant protection products, Biocidal Product Directive 98/8/EC, and REACH EC/1907/2006 – regulating registration, authorisation and restriction of chemical substances), this may change in the future. However, implementation of these Directives will need great efforts as an economically powerful chemical industry behind must change its strategy and cooperate to assess risks of toxicity and ban end-of-pipe solutions. Science, on the other hand, can contribute by elaborating threshold values and predictions of the effects of pollution, thus providing the basis for sound, efficient and effective measures and actions to restore ecosystem function and establish human health standards.

Danube News 20 provides information on the state-of-the-art of ecotoxicology, toxicity tests, legal standards, biomonitoring and human health, with specific attention to the Danube River Basin. Although human society is exposed to an ever increasing cocktail of chemical substances that change and mix in the environment, public awareness about toxicity is surprisingly low. As long as people are not killed by poisonous substances the attention in the media is small. Spectacular fish kills upon accidental spills may be reported, but long-term chronic and sub-lethal aspects are highly neglected. IAD hopes to make a valuable contribution to stimulate respective discussion and research, and to propagate an important environmental issue.

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Ecotoxicological research and its implications for important water management issues in the Danube River Basin

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IAD could certainly promote modern ecotoxicological concepts into water management. Main issues are a switch from substance to effect monitoring, a regular updating of priority pollutants lists according to new findings and an integration of sediment quality (and quantity) into river basin management plans and programmes of measures, as well as ecological impact and risk assessments across DRB.

IAD and the Danube River Basin (DRB) in ecotoxicological research

Ecotoxicology as a discipline was always rather high in the agenda of researchers, professionals and funding agencies in Europe. Not necessarily due to numerous unresolved scientific questions, but its applied aspect, i.e., potential severe environmental and particularly human health problems caused by hazardous substances. Still, the knowledge acquired during "the golden age of classic ecotoxicology" and, particularly, modern approaches and new concepts somehow fail to find their way into contemporary water management practice and risk assessment of toxic pollution. Bearing in mind that the IAD is stretching between fundamental and applied science, but is more and more determined to take an active role of real stakeholder in important water management issues within the DRB, some of the applied aspects of ecotoxicological research are stressed. The focus is on practical benefit water management can draw if basic ecotoxicological concepts are respected.

Figure 1. In vitro tests gradually replace in vivo testing – LECOTOX (University of Novi Sad) young researchers on the good track

The DRB, the most international basin in the world, covers 20 countries, whose GDP per capita ranges from 43196 US $ in Switzerland to 2984 US $ in Moldova. It is extremely difficult, if not impossible, under given circumstances, to get a consistent overview of scientific research in any discipline (particularly experimental, fully funding-dependent like ecotoxicology). The IAD Expert Group "Ecotoxicology" exists for years and Biomonitoring/Ecotoxicology has been selected as one topic of high priority. An IAD project in the field was the small-scale study on the Mures near Arad conducted in 2004 where some biomarkers and trace metal bioaccumulation in fish were combined with classic biological quality elements and water quality monitoring in search for consistent spatial pattern of pollution and its effects (Köhler et al. 2007; Triebskorn et al. 2008; Sandu et al. 2008). Many of recent and on-going big EU FP funded projects, like AquaTerra (www.attempto-projects.de/aquaterra/), Modelkey (www. modelkey.org), Liberation (www.liberation.dk), NoMiracle (http://nomiracle.jrc.ec.europa.eu), focusing on ecotoxicological research either include the DRB as a case study or involve institutions and individual researchers from the catchment, but not many affiliated to IAD. However, IAD is more actively involved in related networks, e.g. Norman (www.norman-network.net), SedNet (www.sednet.org), RiskBase (www.riskbase.info).

Shortcomings of WFD: Substance vs. effect monitoring and almost forgotten sediments

One of the driving forces for an insufficient ecological status and reduced biodiversity of freshwater and marine ecosystems is chemical stress due to environmental pollutants. In spite of the enormous number of possible contaminants in the environment, risk assessment of toxic pollution in aquatic ecosystems has been (and still is) based on few pre-selected and regularly monitored target compounds. So, it can be concluded that numerous in vivo and in vitro toxicity tests yielded a lot of data (and perhaps knowledge) on individual toxicity and mode-of-action of few chemicals *(Figure 1)*. The Water Framework Directive (WFD), sometimes considered as the "modern Bible of water managers" did not change the concept of toxic pollution monitoring and risk assessment. On the contrary – the "status quo" underpinned with e.g. the list of 33 compounds selected as priority pollutants by the European Commission and the traditional, conservative official monitoring programmes which rely on substance, rather than effect monitoring remain the accepted and widely used concept all over Europe, including the DRB. A new Directive 2008/105/EC on environmental quality standards, aiming to ensure a high level of protection against the risks of priority substances and other pollutants to the aquatic environment, was adopted in 2008 (see article of Rauchbüchl). Since about 80% of the listed priority substances are sorbed to sediment and suspended particulate matter (SPM) it has been agreed that the Member States have the opportunity to apply environmental quality standards (EQS) for sediment and/or biota instead of those for water. The guideline scheduled for 2009 should bring up the monitoring requirements for controlling the EQS. The new regulations and guidelines could be seen as an ideal vehicle for addressing the important role of sediments in watershed quality, but it is uncertain to what extent sediment quality will explicitly play a role in assessing ecological quality under the WFD as it is not mandatory. The WFD only directs Member States to monitor macrobenthic invertebrates and develop sediment quality standards, so there is clearly scope for consideration of sediment quality as an integral part of river basin management. Yet, the preliminary overview of river basin management plans (www.sednet.org) shows extreme inconsistency across Europe – neither sediment management issues became integral part of RBMP nor sediment quality assessment plays an important role in assessing ecological/chemical status.

"Pollution loads of hazardous substances are significant although the full extent cannot be evaluated to date. Currently, there are only few data available for hazardous substances such as heavy metals and pesticides" (ICPDR 2005). According to the cited Roof Report, cadmium and lead can be considered as the most serious inorganic microcontaminants in the DRB, particularly in the Lower Danube. However, sediment toxicity evaluation undertaken as a part of feasibility studies for remediation activities of transboundary watercourses showed that although heavy metal concentrations are high, bioavailability and consequently toxicity to aquatic biota is low, due to high content of clay, iron and sulphides (Dalmacija et al. 2006). The Roof Report further pointed out that levels of p, p'-DDT and Lindane in Lower Danube are often above the TNMN target values. Also, high concentrations of Atrazine in some tributaries (Sió, Sajó and Sava) should be emphasised. Significant concentrations of the EU WFD priority substances (4-isononylphenol and di [2-ethyl-hexyl] phthalate) were found in bottom sediments and suspended solids, indicating the relevance of these compounds as an indicator of industrial pollution in the Danube River. As the Roof Report is based on monitoring data, it can be concluded that the pollution of the DRB by conventional and priority pollutants is an officially recognised problem. As the current EU list of priority pollutants is short, the official monitoring programs are rather conservative and not flexible. They allow only a rough quality assessment; they say nothing or very little about bioavailability, toxicity and, hence, ecosystem risk deriving from hazardous substances; and they pay almost no attention to emerging and other substances beyond this list. The introduction of a basin relevant pollutants list to be regularly monitored might change this picture. The knowledge gaps stimulated research community to undertake a series of projects and independent studies within the DRB. Ecotoxicological assessment of sediment, suspended matter and water samples (Keiter et al. 2006) and a bioassay approach to determine the dioxin-like activity in sediment extracts (Otte at al. 2008) were conducted in search for the causes of the decline of fish catches in the Upper Danube River. A comprehensive study (Terzic et al. 2008) on 70 individual wastewater contaminants in the West Balkan Region (including pharmaceuticals, personal care products, surfactants and their degradation products, plasticizers, pesticides, insect repellents, and flame retardants) confirmed a widespread occurrence of the emerging contaminants in municipal wastewaters of the region. Due to the rather poor wastewater management practices in West Balkan countries, with less than 5% of all wastewaters being biologically treated, most of the contaminants present in wastewaters reach ambient waters and may represent a significant environmental concern.

The WFD classifies the quality status of aquatic ecosystems based on traditional hydromorphological, physicochemical, biological parameters and priority pollutant (PP) concentrations. This procedure allows a rough quality assessment, while a reliable diagnosis and prediction of toxic impacts on aquatic ecosystems and an efficient mitigation of toxic risks request an identification of the respective stressors and cause-effect relationships between chemical pollution and biodiversity decline. To date, severe gaps of knowledge impede the evaluation and mitigation of the causes for an insufficient ecological status in many aquatic ecosystems. Therefore, big EU funded projects mentioned above were initiated to establish links between chemical quality of sediments and surface waters with measurable toxic effects. This implies improved effect analysis by well

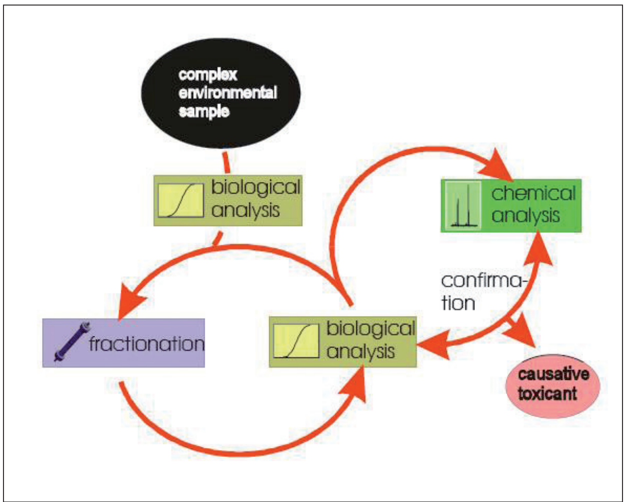

Figure 2. The EDA (effect directed analysis) based approach, e.g., a comparison of biological and chemical analysis on fractionated complex samples, allows the identification of those toxicants that actually cause effects and risks on aquatic organisms, populations, and communities. Thus, the new EDA tools are important milestones on the way to a more realistic risk assessment (taken from www.modelkey.org)

designed batteries of in vitro and in vivo tests as an early warning system to identify hazards before a decline of biodiversity is observed. Effect-based identification of key toxicants as well as analysis, modelling and assessment of bioavailability and food web accumulation are needed, as well as a better evaluation of monitoring data on contamination, toxicity and ecological quality on a basin scale *(Figure 2).* However, sound scientific concepts, models and decision support systems have to find their way to major stakeholders, water managers and even policy makers as their implementation would certainly contribute to the common European goal – achieving good ecological status.

REACH and ecotoxicogenomics

Another regulatory driver which would certainly stimulate further ecotoxicological research is the REACH Regulation (EC) No 1907/2006 - Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals. The aim of REACH is to improve the protection of human health and the environment through better and earlier identification of the intrinsic properties of chemical substances. The REACH Regulation gives greater responsibility to industry to manage the risks of chemicals and to provide safety information on the substances. Manufacturers and importers will be required to gather information on the properties of their chemical substances, which will allow their safe handling. This implies, among other, series of mandatory toxicological and ecotoxicological tests, including multiple testing on vertebrates. Recent estimates show that EU regulators by far underestimated the number of chemicals to be registered and consequently the costs and number of animal tests to be performed during registration procedure. The REACH Regulation promotes development of alternative testing methods: (Article 40) “The Commission, Member States, industry and other stakeholders should continue to contribute to the promotion of alternative test methods on an international and national level including computer supported methodologies, in vitro methodologies, such as appropriate, those based on toxicogenomics, and other relevant methodologies. The Community's strategy to promote alternative test methods is a priority…”. Alternative tests seem to be more urgent than anticipated. In line with this development of toxicogenomics, as stimulated by REACH, a completely new field of research with high potential for future application in ecological risk assessment and even monitoring emerges: ecotoxicogenomics.

Ecotoxicogenomics should describe the integration of genomics (transcriptomics, proteomics and metabolomics) into ecotoxicology and can be defined as the study of gene and protein expression in non-target organisms that is important in responses to environmental toxicant exposures. The potential of ecotoxicogenomic tools in ecological risk assessment seems great. Many of the standardized methods used to assess potential impact of chemicals on aquatic organisms rely on measuring whole-organism responses (e.g. mortality, growth, reproduction) of generally sensitive indicator species at maintained concentrations, and deriving ‘endpoints’ based on these phenomena (e.g. median lethal concentrations, no observed effect concentrations, etc.). Whilst such phenomenological approaches are useful for identifying chemicals of potential concern they provide little understanding of the mechanism of chemical toxicity. Without this understanding, it is difficult to address some of the key challenges that currently face aquatic ecotoxicology, e.g. predicting toxicant responses across the broad diversity of phylogenetic groups in aquatic ecosystems; estimating how changes at one ecological level or organisation will affect other levels (e.g. predicting population-level effects); predicting the influence of time-varying exposure on toxicant responses (Snape et al. 2004). A major advantage of functional genomic technologies, which enable measurements of thousands of transcripts, proteins and metabolites, is their „open“ nature that does not require prior assumptions about the choice of biomarkers, thus being particularly valuable to assess mechanisms of action and the effect of mixtures of chemicals where unknown biological targets may be involved. However, attention needs to be given to distinguishing between compensatory, adaptive and toxic responses, and to discovering patterns of change that are diagnostic and predictive. The biggest problem in contemporary ecotoxicogenomics lies in the enormous quantity of data produced which need to be processed and interpreted, while the bioinformatics seems not to be able to catch pace with experimental techniques. Therefore, this new discipline would certainly attract a lot of attention (and funding opportunities) in the near future by presenting a propulsive field of research, with promising outcomes, for the next couple of years.

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Standardised biological test methods for measuring toxicity of effluents and receiving waters in the Danube River Basin (DRB)

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Environmental measurements are required to determine the quality of ambient waters and the character of waste water effluents. Analytical methods are developed and evaluated to identify the concentration of chemical pollutants in drinking water, surface water, groundwater, waste water, sediment, sludge and solid waste. In addition to chemical analysis, biological test methods (biotests) are necessary to detect and quantify responses in aquatic organisms exposed to environmental stressors.

Biotests indicate a summarised response over adverse effects of all water constituents because waste water is a mixture of different chemical compounds. A set of standardised biotests with representatives of the aquatic ecosystem is available to measure acute and chronic toxicity and to use these methods in legal regulations. Some European countries (e.g. Germany, France) state that it is the national policy to enforce the prohibition to discharge toxic substances in toxic concentrations. The tests may be conducted in a central laboratory or in-site by the regulatory agency or an authorized person.

Standardisation requirements

A popular Chinese proverb says: *„Third-class companies assemble products; second-rate companies develop technologies and high-class companies set standards."* This puts "establishing standards" in perspective. Standardisation is an important and helpful requirement with benefits for products, governmental legislation and administration as well as scientific work. A sound method is the first step to „standardisation" and good scientific practice. A formal standardisation of toxicity tests as it is accomplished, e.g., by the International Organization for Standardization (ISO) is a requirement for their implementation in legal acts and ordinances to carry out objective evaluations and proceed lawfully (ISO 2009). Standards for chemical analysis and biotests are associated with rules and procedures cited in international water directives (e.g., EU-Water Framework Directive) and national water and waste water acts/ordinances. General requirements for standardisation of toxicity tests (biotests) with respect to a regulatory framework of waste water effluents are given in *Table 1.*

Table 1. Requirements for standardisation of toxicity tests (biotests) for the evaluation of waste water

Requirement	Main objectives
evidence	representative status of applied organisms or clear recording of an effect
operational aspect	test result gives a direct and evident indication about objective and quality of a waste water treatment procedure
reproducibility	results must be reproducible in intra and inter laboratory trials
accuracy	small deviations between parallels, test of reference substances as validity criteria
legitimacy	determinations and definitions referring to a test and sampling procedure
legal security	intrinsic quality assurance, data for evaluation of measurement uncertainty
compatibility	compliance with national or EU-regulations; no conflict with other laws or directives

Toxicity of environmental samples

In general, toxicity means a harmful effect of chemicals on a biological system (cell organelles, cells, organisms). Such an effect is indicated by the reaction of the biological system, for example by death, changes in behaviour, and inhibition of growth, reproduction, or functional metabolic processes (photosynthesis, respiration, luminescence). Toxicity is not an intrinsic characteristic of a substance as bioavailability is a prerequisite. Toxic effects of chemicals to water organisms may depend, e.g., on water solubility, electrolytic dissociation, which may be affected by the pH, water temperature and, last but not least, concentration (hypothesis of Paracelsus: *Dosis facit venenum).* The benefit of a biotest performed with a complex mixture like waste water is to measure a summarised and integrated hazard effect. No detailed information about a particular constituent is necessary, irrespective of the availability of methods and resources for a chemical analysis.

Chemicals can affect the environment in different ways and at different levels. Basic criteria are persistence, bioaccumulation, acute and chronic toxicity, effects on reproduction, mutagenicity and carcinogenicity. There is a standard testing frame of OECD guidelines used, for example, in the context of REACH or other formal procedures to register or evaluate chemicals (OECD 2009). A set of standardised biotests is recommended to monitor the possible ecotoxicological effects on environmental samples. This scope, for example the investigation of waste water, is covered by national (German, DIN), European (EN) and International (ISO) standards *(Figure 1).* However, novel assays are necessary to monitor advanced toxic effects on biota with a particular focus on endocrine, immunotoxic or neurotoxic effects (Teodorovic 2008).

ISO member states decided to revise some of these standards developed by the Technical Committee 147 (Water Quality), e.g., algal growth inhi-

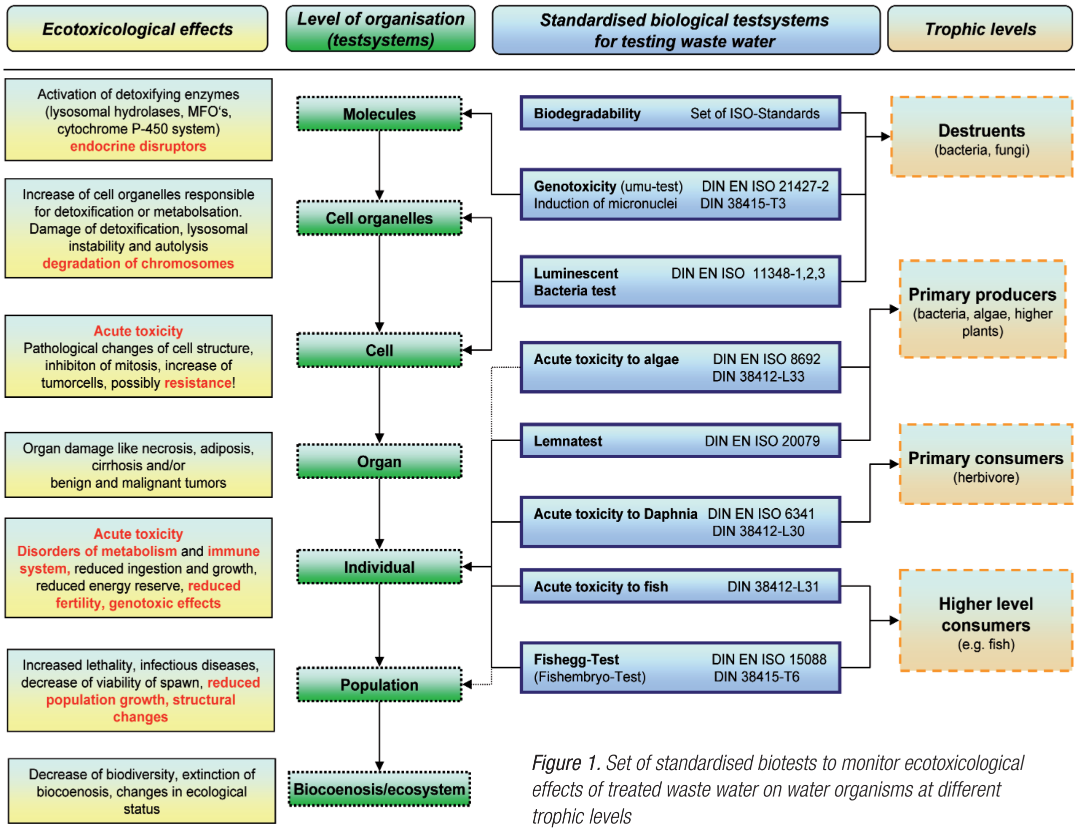

Figure 1. Set of standardised biotests to monitor ecotoxicological effects of treated waste water on water organisms at different trophic levels

bition test (ISO 8692) and acute toxicity to *Daphnia* (ISO 6341). The aim is to implement new techniques, for example to use microplates for the incubation of algae, to optimise the nutrient medium or to standardise *Daphnia* cultures.

Monitoring of industrial effluents

Aquatic toxicity tests are used worldwide to measure, predict and control the discharge of substances that might be harmful to aquatic life (US-EPA 1993). In Germany industrial effluents are periodically controlled by local authorities. Recognizing that no single test method or test organism can satisfy a comprehensive approach to environmental protection, a set of single species tests is used in Bavaria. The standardised toxicity tests referring to *Figure 1* are broadly accepted and measure toxic effects using organisms representing different trophic levels. In most cases any toxic effect indicated by one of the test systems is also shown by at least one of the other biotests. But the sensitivity varies between test systems and depends, for example, on the used organisms, the observed endpoint, the industrial branch, the constituents of the waste water tested, and the type of waste water treatment.

In the Bavarian part of the DRB the duckweed *(Lemna minor)* growth inhibition test according to ISO 20079 is applied. This test is presently used to evaluate the ecological effect of waste water originating from a flue gas scrubber of a Bavarian company. This specific effluent is treated by neutralisation and discharges into the Bavarian stretch of the River Danube. *Lemna minor* serves as a model organism for macrophytes. The plants are cultured for 7 days at different concentrations of an environmental sample, which are prepared by dilution. Industrial waste water may effect the vegetative growth of *Lemna minor*. This is indicated by a reduction of fronds (plant bodies) and/or biomass parameters like frond area, chlorophyll-a content or dry weight *(note: frond is a leaf-analogue part of a Lemna colony and a reproductive individual)*. The number of fronds is counted via observation by eye. The frond area is detected by an image analyser (Medea-AV, D-91058 Erlangen). To quantify toxic effects on frond number and/or area the average specific growth rate is calculated for both parameters and for each dilution; then, the percentage of inhibition compared to a negative control is specified. From the two biomass parameters the most sensitive will be used to calculate the final test result given as

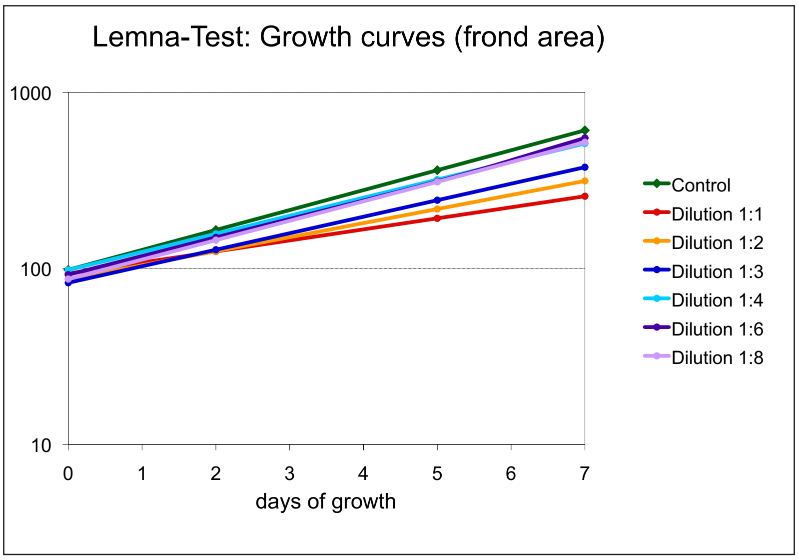

Figure 2. Effect of industrial waste water on growth of Lemna minor according to ISO 20079. Exponential growth curves at different dilutions of industrial waste water for the biomass parameter "frond area" (mm²)

EC(r)x value or according to annex B of the standard as LID (lowest ineffective dilution). The LID indicates the dilution at which an inhibition of < 10 % suggests a “no effect” concentration compared to the control. As shown in *Figure 2* the tested sample affects the growth of duckweed. The LID for water plants was calculated as dilution 1:4. Other test systems (see *Figure 1)* gave similar conclusions with a particular reference to an inexistent genotoxic effect.

From the perspective of emission control, the best available technique should be applied following the precautionary principle. Considering the huge dilution by the River Danube the predicted environmental risk caused by single discharge into the river may be negligible. If not, or if there are more discharges, an advanced treatment of waste water may be necessary or the discharge should be prohibited. This has to be stated in compliance with legal regulations as done in Germany, e.g., by the waste water ordinance and other national directives. However, national legal regulations on industrial effluents using biological test methods with regard to environmental protection as it is accepted in the upper DRB or in other European and North American countries are not yet state-of-the-art within the multinational DRB.

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Biomonitoring of aquatic pollution: from simple tradition to complex modern approaches

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Rationale of biomonitoring

Monitoring of aquatic systems refers to the systematic observation and surveillance of streams, lakes, groundwater, estuaries, coastal or marine waters. Monitoring represents a descriptive approach aiming to characterize the status and changes of aquatic systems being associated with or induced by stressors. Accordingly, monitoring programmes may aim to

- describe the condition of aquatic resources as well as spatial and/or temporal changes of the status (surveillance monitoring)
- determine the agreement of the resource condition with relevant regulations (compliance monitoring)
- evaluate associations between natural and/or anthropogenic pressures and the status of aquatic resources (impact monitoring)
- identify the causes of an impairment of the aquatic system (investigative monitoring)
- assess the effectiveness of measures enacted to improve an impaired status of the system (operational monitoring)

A major rationale for monitoring programmes is to survey pollution of aquatic systems and to evaluate the impact of pollution on aquatic resources. Approaches to monitor aquatic pollution include (1) chemical monitoring, which relies on chemical-analytical and bio-analytical tools to determine the nature and levels of chemical contaminants in water, sediments and biota, and (2) biomonitoring, which relies on the use of biological and ecological tools and parameters to characterize the quality status of an aquatic system and to assess exposure to and effects of environmental pollutants. Presence, condition and diversity of organisms respond to chemical – but also physical and biological – stressors, and biomonitoring exploits this responsiveness to indicate the cumulative response of biota. Biomonitoring is an essential foundation of ecological risk assessment as it directly addresses pollution-related biological and ecological status, while concentrations of pollutants inform only indirectly on biological and ecological effects. Advantages offered by biomonitoring include: (i) Biological elements respond to the mixture of all pollutants and to the cumulative impact, while chemical analytics – for reasons of efficiency and cost – has to focus on a sub-set of chemicals. Biological responses are also able to detect the environmental hazards caused by new emerging pollutants which may not yet be considered in chemical monitoring programmes. (ii) Biological elements provide a time-integrated response to pollutants. For instance, pesticides are often applied only during limited time periods so that chemical sampling performed at monthly or longer intervals may miss the transient presence of pesticides in the water body (particularly if the compounds are rapidly biodegraded). In contrast, a pesticide-induced change in, e.g., biological diversity, may be detectable for several weeks or even months after pesticide application. (iii) Biological elements indicate the toxicity of the active, i.e. the bioavailable and bioaccumulative fraction of chemicals, instead of refering to total levels of pollutants. A major disadvantage of biomonitoring, however, is the limited specificity of the measured parameters; this renders difficult to discriminate whether an observed biological change is due to pollution or other stressors, and, if pollution is responsible, which specific chemical substance is causative.

Biological tools for monitoring

A wide range of biological elements and responses have been used in biomonitoring to diagnose presence, intensity, sources and causes of aquatic pollution. These tools range from the molecular to the community level and include biomarkers and bioassays. Historically, biomonitoring started with indicator species which through their presence or absence reflect water quality. For reliable assessment of water quality, usually not a single indicator species, but biotic indices derived from species groups or communities are used. A biotic index takes account of the sensitivity or tolerance of individual species to pollution. The Saprobic system is one of many biotic indices that mostly rely on benthic macroinvertebrates since sampling and identification are fairly easy, the indicator value of many species for organic pollution or specific pollutant groups is known, and sediments often contain elevated levels of contaminants (e.g., Cairns & Pratt 1993).

More recently, emphasis has been placed on biomarkers. They are usually referred to as sub-organismic (molecular, biochemical, cellular, physiological) responses to pollution, although in a broader context they may also include endpoints at the organism, population and community levels (Hagger et al. 2006). A variety of biomarkers has been successfully developed and adopted to be used in national and international monitoring programmes (e.g., International Council for the Exploration of the Sea ICES, OSPAR Convention for the Protection of the Marine Environment of the North East Atlantic). The purposes biomarkers are applied for in environmental monitoring include (a) identification of exposure of organisms to chemicals (e.g., acetyl cholinesterase inhibition as marker of organophosphate exposure, induction of ethoxyresorufin-O-deethylase – EROD – activity as marker of exposure to dioxin-like chemicals), (b) surveying spatial and temporal changes in aquatic contamination levels (e.g., by analysing body burdens of contaminants in monitoring species), (c) providing early warning to environmental deterioration (e.g., disturbance of endocrine homeostasis), and (d) indicating adverse consequences of aquatic pollution (e.g., malformation of reproductive organs, such as imposex in molluscs). A well-known example of aquatic pollution detected and defined by biomarkers is endocrine disruption found by surveys on fish populations in English rivers and estuaries based on measuring the egg-yolk protein, vitellogenin, as a biomarker of exposure to estrogen receptor-binding compounds, and analysing the presence of intersex gonads, i.e. gonads containing both male and female germ cells, as a biomarker of the impact of endocrine active compounds. A comprehensive review of biomarker use in aquatic pollution monitoring is provided, e.g., by Wu et al. (2005).

The avenue of modern molecular biological methods such as reverse transcriptase polymerase chain reaction (RT-PCR), or transcriptomics, proteomics and metabolomics has strongly promoted the application of biomarkers in aquatic pollution monitoring. For instance, microarrays enable the assessment of global gene expression of aquatic organisms in response to pollution and, indeed, living at sites with different pollution levels is associated with distinctly different gene expression profiles (Goetz & McKenzie 2008). The -omic approaches have also potential in assessing the combined action of chemical, biological and physical stressors; however, the (functional) interpretation of expression patterns of such complex exposure scenarios is currently still limited.

The strength of sub-organism biomarker responses is not so much that they are faster or more sensitive than responses at the organism, population or community levels (cf. Wu et al. 2005), but it lies clearly in their use as diagnostic tools. While molecular, cellular and physiological responses are directly involved in the toxic mechanisms induced by the environmental contaminants or at least closely associated with the initial chemico-biological interactions, the causal relationship between biological change and toxicant action is getting increasingly confounded at higher levels of biological organization, due to compensatory processes and new, level-

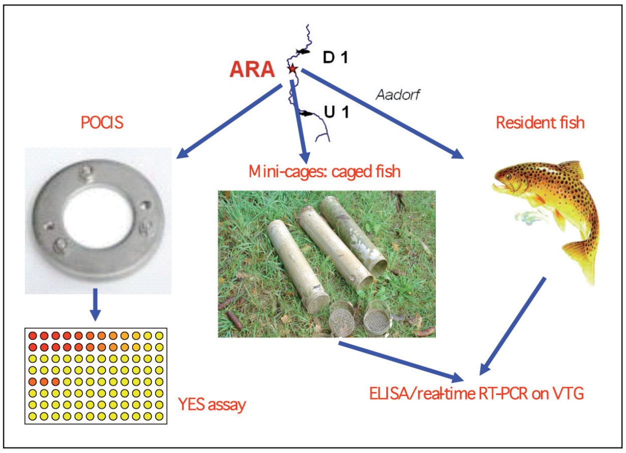

Figure 1. Integrated monitoring for estrogenic contamination of the River Lützelmurg, Switzerland. The river receives effluents from a municipal waste water treatment plant (WWTP) close to Aadorf. To evaluate whether this effluent introduces estrogen-active compounds into the river, and whether the aquatic fauna is impacted by this exposure, a combined chemical and biological monitoring was performed. For the chemical assessment, passive sampling devices (POCIS) were employed in order to obtain a time-integrated estimate of the level of estrogenic pollution. Presence of estrogenic compounds in the POCIS sample was determined by means of chemical analytics and bioanalytics, using the recombinant YEAST Estrogen Screen (YES) which contains an estrogen receptor gene coupled with a reporter gene. For the biological assessment, induction of the estrogen-responsive biomarker vitellogenin was measured in resident brown trout sampled from the river. Since the vitellogenin signal in feral fish may be confounded due to migration of the fish, in addition brown trout exposed in cages in the River Lützelmurg were analysed. Induction of vitellogenin was measured at the protein level in the blood plasma by means of Enzyme Linked Immunsorbent Assay (ELISA) and at the mRNA level in the liver by means of quantitative RT-PCR.
For details see Burki et al. 2006

specific properties. In turn, this implicates that the ecological relevance of biomarkers is limited: Demonstration of an effect at the molecular, cellular or physiological level does by no means implicate that this effect will propagate into organism or population effects. Indeed, the value of biomarkers in aquatic pollution monitoring is rather to serve as early warning signals of long-term or delayed toxicity, or as “signposts” of potential toxicity, than as predictors of ecological deterioration (Segner 2007). As a consequence, biomarkers should not be used as stand-alone tools but should be embedded in an integrated monitoring strategy combining the biomarkers with analytical (bioanalytics and chemical analytics), experimental and ecological tools *(Figure 1*, Lam & Grey 2003).

Design of biomonitoring programmes

Biomonitoring programmes on aquatic pollution should employ a suite of tools as described above. Multi-parameter biomonitoring provides the possibility of multi-variate evaluations. This reduces the risk of mis-interpretations due to problems of in site selection, natural biological variability, role of other stressors, or stochastic events.

Feasible and successful biomonitoring programmes have a clear definition of objectives and are based on conceptual models. Respecting the variability in time and space of the biota and water body to be monitored is crucial for planning frequency and number of sampling sites. A single annual sampling, for example, may have little value in assessing biological quality, especially for pollution-impacted water bodies where chemical stressors and biological properties can vary through orders of magnitude within an annual cycle. Typical sampling strategies are BACI approaches (i.e., monitoring the system “Before-and-After-Control-Impact“, Downes et al. 2002), or benchmarking using a gradient approach which relies on sampling along a presumed pollution gradient. With the latter approach, finding a non-polluted reference site can be a problem, as pristine areas are virtually non-existing in anthropogenically impacted areas such as, e.g., most parts of Europe. In this case, reference conditions may be derived from minimally or slightly disturbed water bodies, from historical data, or from modeling.

The main tool of the European Water Framework Directive (WFD) to describe the status of a water body is monitoring of various chemical, biological and ecological “quality elements”. The WFD requirements for the design of monitoring programmes represent a move away from former static approaches to a more dynamic, risk-based approach, which aims to link chemical and hydromorphological pressures to biological indicators of environmental quality. Accordingly, it is necessary to establish integrated programmes to classify water bodies using a combination of surveillance, operational and investigative monitoring (Irvine 2004). Importantly, the WFD approach to protect and restore aquatic ecosystems is based on a river basin scale, i.e. considering rivers as geographic and hydrological units. In line with this, the TransNational Monitoring Network and the Joint Danube Surveys of the ICPDR may help to improve monitoring in the Danube River Basin (www.icpdr.org).

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Environmental quality standards for hazardous substances and ecotoxicological methods stipulated by the EU WFD

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Introduction

In December 2000 the Water Framework Directive – WFD (EC 2000) of the European Union was enforced. After a long period of European water legislation determined by a patchwork of mostly use-oriented Directives and Decisions this legal act forms the basis for a new and comprehensive water policy.

After the accession of Romania and Bulgaria to the EU in 2007 the number of Member States in the Danube River Basin (DRB) has increased to 10, covering some 74 % of the basin. For the other Danube countries the WFD is not legally

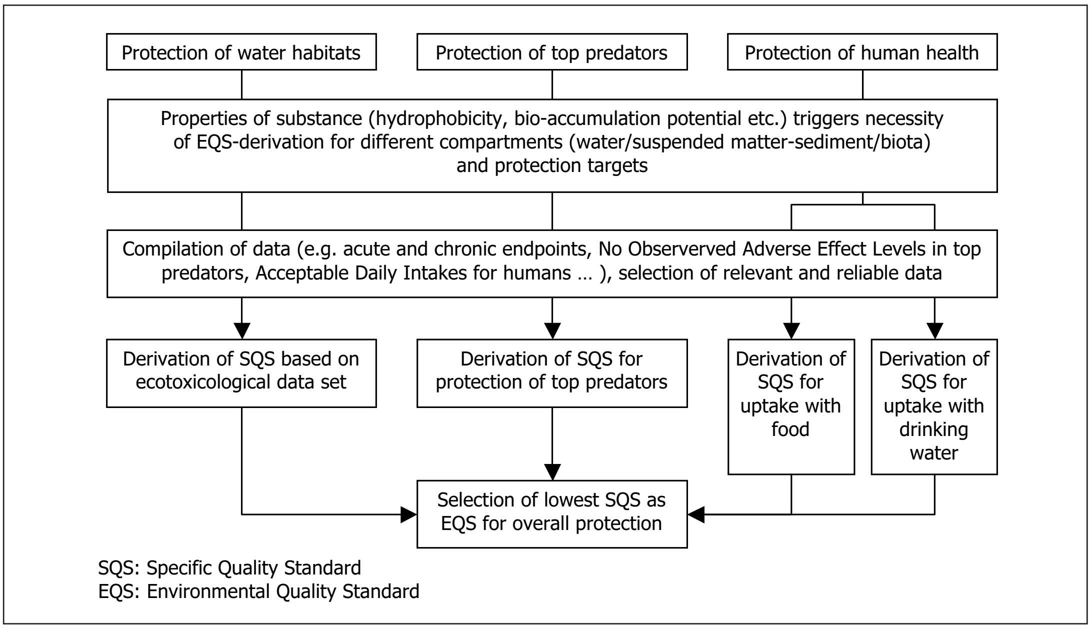

Figure 1. Overview – Derivation Method for Environmental Quality Standards under the WFD (Lepper 2005)

binding but (partly) adopted due to the need for harmonisation of the program of measures in international river basins (WFD, Article 3). Furthermore, 14 countries with a share of DRB area > 2000 km^2 (8 of them EU Member States) have signed the Danube Convention in 1994 which is implemented by the International Commission for the Protection of the Danube River (ICPDR). The WFD is the most important legal act in the DRB.

The outstanding goal of the WFD is to achieve *good status* for all surface waters and groundwater formally combined into "water bodies", coherent sub-units of the river basin district (EC 2003). A number of quality elements have to be evaluated and compared to the environmental objectives given in Annex V of the WFD. They are grouped into *ecological status* (biological, hydromorphological and physico-chemical quality elements including hazardous substances of relevance on national level) and *chemical status* (hazardous substances regulated on Community level). The combination of these two assessments leads to the overall result revealing whether a water body has achieved good status. The Environmental Quality Standards (EQS) provide concentration limits for hazardous substances mainly derived on the basis of ecotoxicological effects on aquatic organisms.

Regulation on Community level – priority substances and priority hazardous substances

The WFD defines hazardous substances as "substances or group of substances that are toxic, persistent and liable to bio-accumulate and other substances or group of substances which give rise to an equivalent level of concern". Two groups of hazardous substances are distinguished: According to the subsidiary principle, on Community level only substances shall be regulated posing a threat to a majority of European waters, therefore named Priority Substances (PS). Pollutants with only local or regional impacts have to be handled on Member State level (belonging to the quality elements of the ecological surface water status). Following Article 16 the EC submitted a proposal for a PS list ranking substances according to their risk to and via the aquatic environment due to their intrinsic properties and exposure (EC 2001) identifying 33 substances and substance groups as PS of which 11 were designated as priority hazardous substances (PHS) and 14 as PHS candidates (in the meantime this decision process has been finalised resulting in 13 PHS). For PHS, due to their extremely dangerous properties, the phase-out and cessation of discharges, emissions and losses is the mid-term goal of the WFD. For PS the WFD demands a continuous reduction of emissions into the aquatic environment.

The selection and prioritisation for PS is challenging because of the large number of potential candidates and the huge amount of data needed to assess risk and exposure. It is not surprising that this first list contains a number of well known pollutants mostly banned or limited in use and, therefore, not detectable in the aquatic environment any more. This fact was criticised by a number of stakeholders and the European Parliament. However, since 2001 the situation has changed in many respects. As a consequence of the monitoring obligations by WFD (Art. 8) and a speed-up of implementing the provisions for hazardous substances relevant on a national level (an obligation since the publication of the "dangerous substances directive" (EC 1976) and its daughter directives) a lot of new information in the Member States has been gathered.

In addition, the standardisation of ecotoxicological methods has improved data quality and reliability. Quantitative structure activity relationships (QSARs) were derived from available data and computerised as valuable tool to fill data gaps for substance properties and ecotoxicological effects. The new European chemicals law – REACH (EC 2006) – initiated the compilation of new risk data by the industry necessary to apply for the authorisation of chemicals. Although the revision of the PS list is delayed, these improved data bases may address more actual problems. The publication of the EC proposal for new PS is foreseen by the end of 2010.

Derivation of EQS according to WFD

For hazardous substances the basic principles for derivation of environmental quality objectives (EQS) are laid down in Annex V, point 1.2.6 of the WFD. The development of a detailed method was carried out by a consultant (Lepper 2002, 2005). Based on this work and after a tedious legislative procedure the EQS for PS were put into force in December 2008 (EC 2008). The directive lays down EQS for inland surface waters and other surface waters (transitional, coastal and marine waters). Both sets comprise Annual Average value – EQS (AA-EQS, protecting against long-term exposure to PS) and Maximum Allowable Concentration – EQS (MAC-EQS, protecting against short-term effects due to pollutant concentration peaks). In addition, the directive includes EQS for 8 remaining of the 17 list 1 substances (EC 1976), which have not been identified as PS. The existing standards for these substances have proved to be useful, so their regulation on Community level was maintained. The AA-EQS is compared to the annual average concentration of monthly measurements, the MAC-EQS to single values. Only if both assessments do not exceed the respective EQS values for all 41 hazardous substances the water body is assigned "good chemical status".

While MAC-EQS are based on acute ecotoxicological effects, AA-EQS take into account both chronic and acute effects. *Figure 1* gives an overview of the derivation process for freshwater AA-EQS considering the risk to the aquatic environment (water – pelagic community, sediment – benthic community), top predators via prey (biota) and humans (via drinking water and fish). For these different risk scenarios a specific quality standard (SQS) using appropriate toxicity data is derived. The lowest value is then selected as the EQS for this substance ensuring overall protection.

In a first step, on the basis of substance properties and agreed trigger criteria, it is decided which risk scenarios are relevant. For example, if the substance has no potential to bio-accumulate the risk for top predators and humans needs not to be considered. In a next step the necessary data are compiled and checked for their usability (relevance and reliability). On the basis of this filtered data set the SQS for the relevant risk scenarios is derived: The no effect concentration is identified and an appropriate Assessment Factor (AF) applied (i.e. division of the lowest concentration by AF). The AFs account for (1) uncertainties in transfer of ecotoxicological endpoints from laboratory tests to the environment, (2) completeness of data set (data gaps), (3) effects on endocrine system of aquatic organisms, and (4) synergistic toxic effects of pollutant mixtures (in part, no consolidated approach for assessment of pollution mixtures is presently available). An example for the different AFs to apply in EQS derivation for organic substances in freshwater is given in *Table 1.*

Table 1. Assessment factors to derive a Quality Standard for freshwater

Data set	Assessment Factor *
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, *Daphnia*, algae)	1000
In addition to the base set:	
One long-term NOEC (either fish or *Daphnia*)	100
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Long-term NOECs from at least three species (normally fish, *Daphnia* and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1 to be fully justified case by case
Field data or model ecosystems	reviewed on case by case basis

* a number of further details regarding the data set has to be taken into account to select the proper assessment factor, for details see Lepper (2005)
Abbreviations:
L(E)C50 Lethal (Effect) Concentration for 50% of the individuals in a toxicity test
NOEC No Observed Effect Concentration in a toxicity test
SSD Statistical extrapolation method, applicable if a large database with NOECs of a range of aquatic species is available

Taking into account endocrine disruption

The problem of endocrine disrupting substances in the environment was addressed by the EC in 1999 (EC 1999) defining an endocrine disruptor as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or (sub)populations". Well known examples are the feminisation of fish populations and the development of intersex species. Although such properties of hazardous substances were included in EQS derivation (Lepper 2005) precise instructions could not be given due the lack of agreed endpoints and international standardised methods. As an interim solution the available information was compiled in the substance data sheets (Lepper 2002) and the substance showing endocrine disrupting potential flagged for further consideration. At least for EQS in the marine environment endocrine effects via the AF should be considered.

While the discussion about the most useful endpoints for the characterisation of endocrine disruption properties of chemicals and the associated methods is still ongoing, Moltmann et al. (2007) have derived EQS for some 70 substances including endpoints for endocrine disrupting properties. The main conclusions were

Table 2. Comparison of AA-EQS for water according to Directive 2008/105/EC (EC 2008) and taking into account endocrine disruption (Moltmann 2007)

Substance	AA-EQS (EC 2008) [µg/L]	AA-EQS (Moltmann 2007) [µg/L]
p,p'-DDE	0.025 *	0.0001
4-Nonylphenol	0.3	0.0033
Tributyltin compounds (cation)	0.0002	0.0001
* AA-EQS for the sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-DDD		

- Results of in vivo test methods (e.g. induction of vitellogenin synthesis in fish, gonado-somatic index for fish) should be given preference instead of in vitro test methods (e.g. receptor binding assay, reporter gene assay) because the latter provide information on the endocrine disrupting potential but do not allow to make predictions for the intact organism
- Endpoints for endocrine disruption can be included in EQS derivation in the same manner as other ecotoxicological endpoints. Due to the fact that standardisation of methods is still missing a case by case validation of results is necessary
- Taking into account endocrine disrupting properties via endpoints reduces the limit concentration for a number of substances in comparison with existing EQS, derived according to the WFD method *(Table 2).*

Conclusions

In principle, the WFD derivation method for EQS considers all relevant risks scenarios. Practically the derivation of “right” EQS is hampered by data gaps and missing consolidated methods for the assessment of endocrine disrupting properties and pollutant mixtures. This is accounted for with the application of Assessment Factors. Despite all guidance their selection can be made within a certain range. If selected too low adverse effects may be underestimated. Selection of AF with great care can lead to unreasonable low EQS. Ecotoxicological data are steadily improving thanks to standardised methods and data generation by REACH legislation. Agreed endpoints and standardised methods for endocrine disrupting substance properties seem to be in sight leading possibly to a further lowering of limit concentrations. The effect of pollutant mixture appears to be the most difficult problem to resolve.

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Connecting aquatic ecology with toxicology – perspectives for the Danube River

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Background

The River Danube provides highly diverse ecosystems for 115 fish and 330 bird species, respectively, and supplies drinking water for riparian settlements from Germany to Romania (Sommerwerk et al. 2009). Conceptual studies have enhanced a better understanding of this highly valuable ecosystem, its ecological, economic, and societal values. Applied research linked scientific knowledge with river management (e.g., Jungwirth et al. 2002).

A key stressor/pressure is pollution by nutrients and potentially toxic substances. While point sources are mitigated by waste water treatment plants, nonpoint inputs of nutrients and contaminants are difficult to regulate because they derive from activities dispersed over wide areas of land. In aquatic ecosystems, nutrients (mostly phosphorus and nitrogen) cause diverse problems such as toxic algal blooms, loss of oxygen, fish kills, and loss of biodiversity. Contaminants such as heavy metals (Gundacker 2000; Woitke et al. 2003), persistent organic pollutants (POPs, including polychlorinated biphenyls and polybrominated diphenyl ethers; Covaci et al. 2006), and cyanobacteria-produced microcystins (Ueno et al. 1996) can cause severe and sometimes irreversible effects on aquatic biota and humans.

To prevent chemical hazards multidisciplinary scientific approaches are required to protect aquatic ecosystems from often long-lasting damage. The scientific discipline *'ecotoxicology'* connects ecological and toxicological knowledge on a cause-effect level. *Aquatic ecotoxicology* is concerned with toxic effects on organisms in various habitats and at various trophic levels, ranging from primary producers to top consumers. However, there are natural constraints of in situ ecotoxicological research because it is difficult, if at all possible, to test specific toxic cause-effect relationships in highly dynamic aquatic ecosystems. Probably because of such constraints many ecotoxicological studies of aquatic ecosystems remain descriptive, but provide valuable information from the molecule and cell level to the ecosystem level, complementing ecological investigations *(Figure 1)*.

Bioconcentration, bioaccumulation, and biomagnification

It is important to know that contaminants in aquatic ecosystems can be incorporated by organisms in three ways:

(1) *Bioconcentration* is the direct uptake of chemicals from water; through this process, the chemical concentration in the aquatic organism becomes higher than in water because uptake exceeds excretion.

(2) *Bioaccumulation* is the absorption/uptake of chemicals via food and water; this process involves biological sequestering of substances entering through respiration, food intake or skin contact and results again in a net increase of chemical concentration in aquatic organisms.

(3) *Biomagnification* goes beyond single organisms and is defined as the increase in chemical concentration with each trophic level transformation in the food chain, resulting in the highest concentrations in the upper trophic levels (i.e., top predators such as fish eating birds and humans). If a chemical is sufficiently hydrophobic or lipophilic and recalcitrant (i.e., cannot be biotransformed) it will have a tendency to biomagnify through food webs. The degree of biomagnification is evalutated by the octanol-water partition coefficient (log $K_{ow} > \sim 4$) which measures the concentration of a chemical in octanol as organic solvent and in water. It should be stressed that although a contaminant does not biomagnify, dietary exposure may still be the most important exposure route for aquatic organisms (Borgå et al. 2004).

Bioconcentration studies in organisms of the Danube are very scarce. For example, Thielen et al. (2004) studied bioconcentration of metals in the intestinal parasite *Pomphorhynchus laevis* and its fish host, the barbel *(Barbus barbus).* Gundacker (2000) examined bioaccumulation in metal-polluted habitats of the Danube around the city of Vienna, Austria, and found 20-fold higher concentrations of heavy metals (Cd, Pb, Cu, and Zn) in gastropods than bivalves. This author concluded that specific dietary source vectors for metal bioaccumulation still remain to be elucidated. In a recent study, Soeroes et al. (2005) investigated different arsenic species in freshwater mussels of the Hungarian Danube and stated that the fate and potential hazard of arsenic to other organisms at different trophic levels is still largely unknown. Finally, food web studies on contaminant biomagnification of the Danube are equally scarce *(see* Bro-Rasmussen 1996) and clearly warrant further attention.

Essential and potentially toxic compounds in aquatic food webs

Detailed ecotoxicological understanding is gained when investigating compounds that are essential and potentially toxic for aquatic organisms. *Essential* compounds are physiologically required by consumers, yet cannot be synthesized *de novo,* or cannot be synthesized in quantities sufficient to meet an organism's need for somatic growth, reproduction and survival (see Goulden & Place 1990, for daphnids; Tocher 2003, for teleost fishes). For example, some poly-unsaturated fatty acids (PUFA) and trace elements such as zinc (Zn), iron (Fe) or calcium (Ca) are considered essential, and if inadequate amounts are available in the diet the health and fitness of an organism can be reduced. *Toxic* compounds have no physiological value for organisms, but can be accumulated by consumers and may be lethal when concentrations are sufficiently high. However, essential compounds can also be toxic if concentrations are high enough or if they are converted to other molecules through cell metabolism. For example, it has been suggested that PUFA in diatoms are converted to unsaturated aldehydes which reduce egg hatching rates in marine herbivorous copepods (Miralto et al. 1999).

Lipids are amongst the most important nutritional factors that affect the fitness of aquatic organisms, supplying energy and essential compounds for general metabolic function, somatic growth, reproduction, enhanced immunocompetency, and are trophically transferred (Arts et al. 2009). However, trophic transfer of lipids (still poorly understood in the

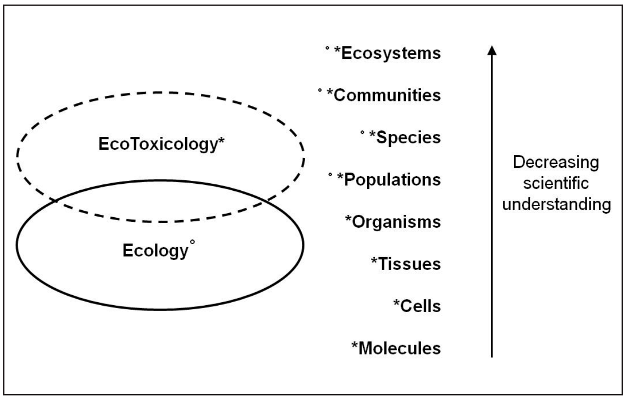

Figure 1. Levels of integration between ecology and ecotoxicology in aquatic ecosystems. Aquatic ecotoxicology seeks to increase knowledge, based on explanatory principles, about potential contaminants in aquatic ecosystems from molecules to the ecosystem level

Danube) also conveys lipophilic contaminants from resources to consumers (Borgå et al. 2004). Consequently, contaminants that bioaccumulate can counteract the mostly favorable physiological effects of essential dietary nutrients, particularly at higher trophic levels, and eventually in humans, because their trophic transfer may follow similar pathways as those of lipids (Kainz & Fisk 2009).

Biomarkers in aquatic ecotoxicology

When diet is the major conveyor of contaminants to aquatic consumers, ecotoxicologists often use tracers to indicate dietary sources of these contaminants. For example, stable isotopes of naturally occurring elements (Broman et al. 1992) and specific contaminants of concern (e.g., stable isotopes of Hg; Orihel et al. 2006) are applied to quantify bioaccumulation of contaminants to specific trophic levels within the aquatic food web. In ecotoxicology, the application of stable isotopes, $\delta^{15}N$ as an indicator of consumer trophic position (Cabana & Rasmussen 1994) and $\delta^{13}C$ as an indicator of the dietary source (Campbell et al. 2000) is widespread. As some essential fatty acids bioaccumulate along aquatic food webs, they have been used as an index of heavy metal bioaccumulation of zooplankton (Kainz et al. 2006). In a study on herring gull trophodynamics from sites across the Laurentian Great Lakes, Hebert et al. (2006) showed that egg omega-3 fatty acid concentrations correlated significantly with egg $\delta^{15}N$ values (and contaminant levels; Hebert, pers. comm.) providing further information on how food web structure influences lipid and contaminant dynamics in aquatic ecosystems. Such highly informative biomarkers have, as yet, rarely been applied in the Danube ecosystems. From an ecosystem protection point of view, studies that link effects of essential with potentially toxic substances on aquatic organisms of the Danube will greatly improve our understanding of these precious ecosystems.

Ecotoxicology – perspectives for research on the River Danube

In addition to the above mentioned field research, lab studies are required to understand how and under which conditions contaminants affect organisms. 'Classical' ecotoxicology test series are summarized elsewhere (Newman & Unger 2003) and involve, for example, toxicity tests to evaluate concentrations of contaminants resulting in death of 50% of exposed individuals by a predetermined time (LC50 test; see article of Kopf & Pluta). Other and physiologically perhaps more informative tests evaluate *sublethal effects*, which occur at concentrations below those inducing somatic death. They are most often recognized as some change in an important physiological process, somatic growth, reproduction, etc. The understanding of such sublethal effects on organisms is highly relevant because they may have lethal consequences in an ecological context, in which the individual must successfully compete with other species, avoid predation, find a mate, and/or cope with multiple stressors. Unfortunately, thus far, little is known about effects of aquatic contaminants on organisms and their cell functioning, cell composition (e.g., changes of integral membrane lipids) of organisms of the River Danube. Clearly, the field of connecting ecology with toxicology in aquatic ecosystems of the Danube is still wide open and invites ecotoxicological research to step forward and understand how many organisms are likely to adapt, or fail to adapt, to upcoming changes.

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REP LECOTOX project: An example of FP INCO project to strengthen ecotoxicological research in Eastern Europe

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FP 6 INCO, followed by FP 7 Capacities Work Programmes present an ideal chance for established but sub-optimally equipped research groups from new, candidate and non-EU member countries to fully integrate into the international scientific community (for open REGPOT calls visit http://cordis.europa.eu/fp7/dc/index.cfm?fuseaction=UserSite.capacitiesDetailsCallPage&call_id=222). The overall aim of the Capacities Programme is to enhance research and innovation throughout Europe by optimizing research infrastructure in Europe, enhancing research potential of European convergence and outermost regions, and building strategic R&D partnerships with non-EU countries.

The REP-LECOTOX project (Reinforcement of Research Potential of the Laboratory for Ecotoxicology at the University of Novi Sad Faculty of Sciences (UNSFS) http://www.lecotox.net) can be seen as one of the success stories of EU capacity building programmes used to strengthen ecotoxicological research in Eastern Europe.

Although ecotoxicological research at UNSFS dates back many years, during the 1990s it was patchy and restricted to national and regional funding, insufficient for basic consumables and chemicals needed for proper research. The overall scientific quality (and visibility) constantly failed to reach the level needed to become an equal partner in any of the European scale ecotoxicological research and networking projects. To overcome some of the deficiencies (e.g. fragmentation) LECOTOX was formally established in 2006. Having recognized the great potential of "omic" methods in ecotoxicological research and risk assessment, the multidisciplinary group of LECOTOX (consisting of ecotoxicologists, physiologists, and molecular biologists) made an initial step towards application of genomics-based tools in ecotoxicology. Mainly focused on two topics: (a) endocrine disruption/reproductive toxicity and (b) identification and characterization of aquatic toxicity, LECOTOX decided to combine ecotoxicogenomics with established conventional toxicity tests and traditional function-based biomarkers.

The REP-LECOTOX project achieved the following: upgrading and renewal of S&T equipment, reinforcement of human resources, extensive networking via workshops, exchange of scientific personnel and training of young scientists in some of the finest EU research institutions in the field of environmental research: Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany; School of Bioscience, University of Birmingham, UK and RECETOX, Masaryk University, Brno, Czech Republic. Several new methods (e.g. DarT test on zebrafish *Danio rerio* embryos) were introduced and modern ecotoxicological concepts (e.g. EDA – effect directed analysis) were adopted. These joint research activities resulted in high quality publications (e.g. Kaisarevic et al., Chemosphere, 77(7), 883-1034, 2009; http://dx.doi.org/10.1016/j.chemosphere.2009.08.042). Two workshops organized in Novi Sad, „Ecotoxicogenomics: the challenge of integrating genomics/proteomics/metabolomics into aquatic and terrestrial ecotoxicology" (June 2008) and "Trends in Ecological Risk Assessment" (September 2009) brought together key EU experts in respected fields (all invited presentations available at www.lecotox.net) and research groups from Eastern Europe *(Figure 1)*. LECOTOX scientists could participate in many important international scientific meetings (e.g. SETAC, PRIMO) and several EU initiatives/networks (e.g. COST actions, SedNet, RISKBASE).

Figure 1. 2nd REP LECOTOX Workshop "Trends in Ecological Risk Assessment", September 21–23, 2009, Novi Sad, Serbia. Invited lecturers: from the top row down, from left to right: Stefan Scholz (UFZ, Germany), Jakub Hofman (RECETOX, Czech Republic), Ivan Holoubek (RECETOX, Czech Republic), Ivana Ivancev Tumbas (UNSFS, Serbia), Ivan Grzetic (Belgrade University, Serbia), Ludek Blaha (RECETOX, Czech Republic), Marjan Ahel (Institute Rudjer Boskovic, Croatia), Jos Brils (Deltares, The Netherlands), Jussi Kukkonen (University of Joensuu, Finland), Mikhail Beketov (UFZ, Germany), Radmila Kovacevic (REP LECOTOX project coordinator, Serbia), Armand Beuf (EC DG Research), Tvrtko Smital (Institute Rudjer Boskovic, Croatia), Joop Vegter (TNO, The Netherlands), Werner Brack (UFZ, Germany), Brett Lyoons (CEFES, UK), Val Beasley (University of Illinois, USA) and Ivana Teodorovic (workshop organizer, LECOTOX, Serbia). Missing from the photo: Dimosthenis Sarigiannis (JRC, Ispra, Italy) and Katarina Krinulovic (Ministry of Environment and Spatial Planning, Serbia)

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Hydrological catchment of the River Danube

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