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

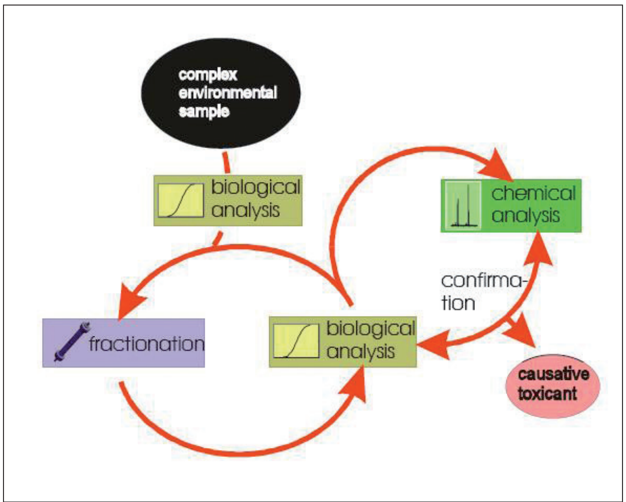

Figure 2. The EDA (effect directed analysis) based approach, e.g., a comparison of biological and chemical analysis on fractionated complex samples, allows the identification of those toxicants that actually cause effects and risks on aquatic organisms, populations, and communities. Thus, the new EDA 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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