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

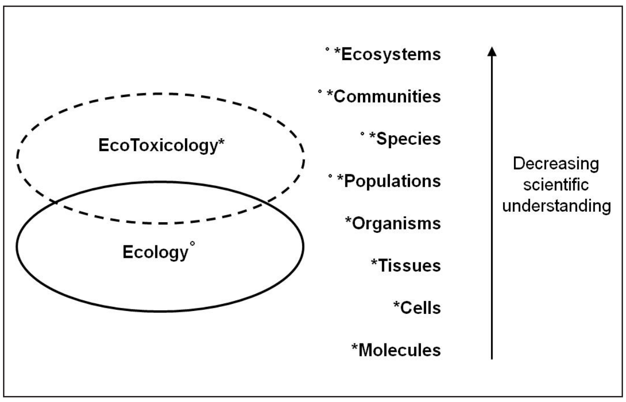

Figure 1. Levels of integration between ecology and ecotoxicology in aquatic ecosystems. Aquatic ecotoxicology seeks to increase knowledge, based on explanatory principles, about potential contaminants in aquatic ecosystems from molecules to the ecosystem level

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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