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

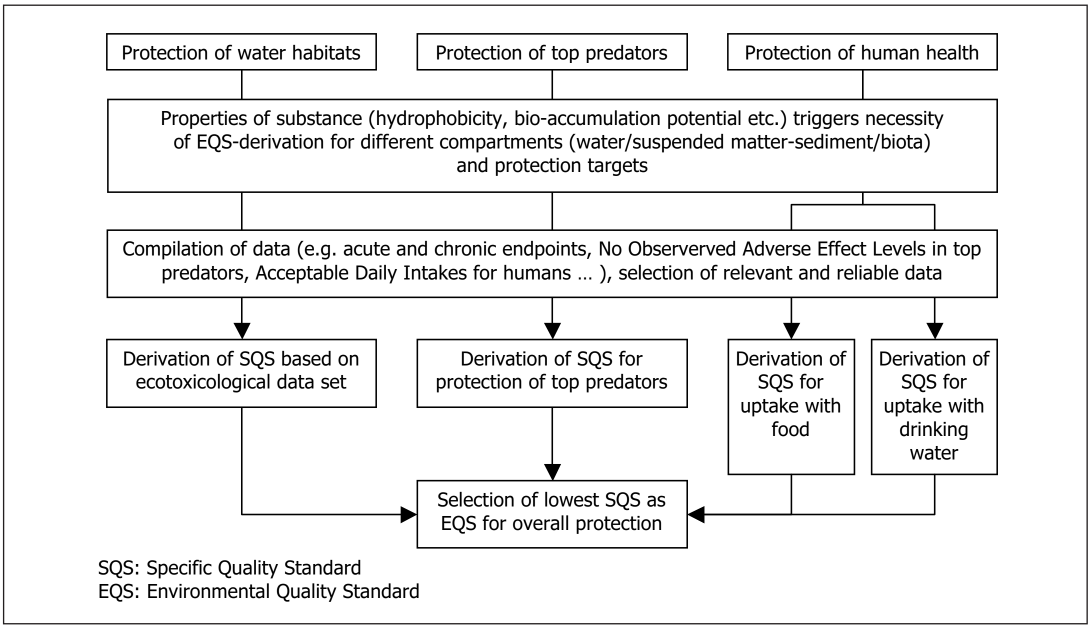

Figure 1. Overview – Derivation Method for Environmental Quality Standards under the WFD (Lepper 2005)

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 se-