Advancements in effect-based water quality assessment

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CHAPTER 7

SYNTHESIS: TOWARDS HOLISTIC CHEMICAL WATER QUALITY ASSESSMENT

THE FULL POTENTIAL OF EFFECT-BASED WATER QUALITY ASSESSMENT

The present research aimed to fuel the paradigm shift towards new chemical water quality monitoring methods by providing a scientific basis for the advancement of effect-based water quality assessment. The exploration of the state-of-science of effect-based methods in a nationwide field-based study (chapter 2) identified the potential for improvements in the sampling methodology, the effect-based tools that are applied, and the interpretation of the obtained results. Here, it is postulated that the full potential of effect-based water quality assessment is achieved when:

I. The sampling methods capture all relevant contaminants from the water
II. The environmental samples are transferred as representative mixtures at environmentally relevant concentrations to the bioassays
III. The bioassay battery represents all toxicity endpoints relevant to aquatic ecosystem health
IV. The defined thresholds for the interpretation of bioassay responses are indicative of ecotoxicological risks in the environment

The present synthesis examines to what extent these requirements for successful effect-based water quality assessment have been met by the present work and offers perspectives on how the unattained requirements may be met in the future.

I – SAMPLING OF RELEVANT CONTAMINANTS

Bioassays respond to all bioactive chemicals present in an environmental sample, regardless of *a priori* selection or identification of compounds.[17] This offers the potential for the toxicity detection of all compounds present in the environment but simultaneously highlights the need for effective sampling methodologies that ensure the efficient recovery of the potentially toxic substances from aquatic media.[20] In the present research, multiple passive sampling methods were applied, often simultaneously, to enable the sequestration of an as wide as possible variety of contaminants at the investigated locations. The combination of integrative and equilibrium passive samplers, which was also applied in previous effect-based water quality assessments,[14,15] allowed the sampling of organic compounds ranging from the polar to the non-polar spectrum (chapters 2 and 6). However, the design of integrative samplers was shown to strongly affect the amount and diversity of polar organic compounds that are sequestered from the surrounding water (chapter 3), underlining that scientifically supported choices in passive sampler configurations are required to ensure the detection of an as wide as possible range of contaminants.

Currently applied passive sampling strategies can and should be further expanded to include environmental compartments and compound groups that are relevant to aquatic ecosystem health but currently underrepresented in effect-based water quality assessment efforts. In this thesis, passive sampling was applied for the bioavailability-based chemical profiling of sediments in tandem with whole sediment bioassays (chapter 5). The observed ubiquity of

sediment toxicity underlined the relevance of sediment as a sink and source of contaminants in aquatic ecosystems and simultaneously emphasized the value of effect-based tools in sediment quality assessment. The applied approach can be further advanced by performing the passive sampling of sediments with polymeric materials with a high sorption capacity that allows for the subsequent solvent spiking or passive dosing to *in vivo* and *in vitro* bioassays. This would provide a high-throughput sediment quality assessment approach that can readily be integrated with effect-based chemical water quality monitoring strategies. Furthermore, the passive sampler array applied in the water column was amended with diffusive gradients in thin films (DGT) to include inorganic contaminants, i.e. metals, in the bioanalytical assessment of chemical water quality (chapter 6). Metal pollution is ubiquitous in aquatic ecosystems, yet metals have only rarely been included in effect-based water quality assessment strategies.[82] The application of the inorganic DGT extracts in *in vivo* bioassays indeed identified potential ecotoxicological risks caused by metals and was efficiently integrated with the effect-based assessment of organic contaminants.

The simultaneous application of the presently explored sampling methods at field locations represents a promising approach to the integrated assessment of a very wide range of contaminants, present in all abiotic aquatic environmental compartments. In the future, the sampling strategy can be even further expanded to include highly polar and/or ionizable compounds, which represent an important class of contaminants of emerging concern (CECs),[85,102] in the water column, the sediment, but also in biota. This can be achieved by the application of passive samplers that house mixtures of polymeric sorbents that target contaminants with particular chemical characteristics, allowing the sequestration of a broad spectrum of potentially toxic substances from the aquatic environment.

II – REPRESENTATIVE TRANSFER OF ENVIRONMENTAL SAMPLES INTO BIOASSAYS

The dosing of realistic environmental mixtures of contaminants to bioassays is important for an accurate representation of contaminant exposure levels that organisms would experience in the field.[20] The most direct way of representative dosing is conducting bioassays directly in an unmanipulated environmental sample (chapters 4 and 5). However, this is only possible for contaminated locations or very sensitive test endpoints and can introduce confounding factors that can either mask or enhance bioassay responses.[20] Passive sampling offers an elegant alternative to the use of direct exposure to or total extraction of samples as it provides enriched and relatively clean environmental extracts.[19] The extraction of chemicals accumulated in field-exposed passive samplers and subsequent solvent spiking into bioassays is simple and efficient. However, the back-calculation of bioassay responses to environmental concentrations in surface waters requires the estimation of sampled water volumes. The approaches to this are fundamentally different for equilibrium and integrative passive samplers.

For equilibrium passive samplers, sampled volumes can be estimated based on the dissipation of performance reference compounds from the samplers during the exposure period (chapters 2

and 6).[50] This offers a close approximation of sampled water volumes that takes the environmental factors determining uptake rates at the field locations into account. Nonetheless, the extraction of passive samplers and subsequent solvent spiking manipulates the original mixture composition in the bioassay setup. Moreover, after any single spiking event the freely dissolved concentrations of contaminants in the bioassay decrease over time, especially for non-polar compounds, due to the sorption of compounds to biomass and test vessels.[20] These drawbacks of solvent spiking of equilibrium passive sampler extracts to bioassays can be overcome by passive dosing, in which an absorptive polymer is used as a partitioning source of the extracts to bioassays.[20] This keeps the freely dissolved contaminant concentrations in the test setup constant, while simultaneously recreating the composition of the original mixture of compounds in the environment.[20] Such passive dosing setups have been developed even for miniaturized *in vitro* test systems, and are hence suited for application in high-throughput screening.[209] Passive dosing approaches for equilibrium passive samplers are currently only limited by the long equilibration times and the low water solubility of very hydrophobic compounds, but recent methodological advancements allow the accelerated passive dosing to bioassays, even of complex mixtures of hydrophobic compounds.[210] The combination of equilibrium passive sampling and passive dosing thus offers a promising approach to recreate environmentally relevant exposure to contaminant mixtures in future effect-based chemical water quality assessment strategies.

For integrative passive samplers, which operate in the kinetic uptake regime, passive dosing does not reproduce the actual environmental contaminant mixtures since the samplers have not reached equilibrium with the surrounding matrix.[20] Moreover, field-correction methods that apply performance reference compounds to estimate sampled water volumes are not reliable for integrative passive samplers because the uptake and release of compounds are often non-isotropic.[51] Thus, the closest estimation of compound concentrations obtained using integrative passive samplers is based on compound-specific sampling rates, which are derived from laboratory or field-based calibration studies.[52] This approach can readily be applied for chemical analysis of passive sampler extracts, but this is not possible for bioassays as the mixtures of compounds jointly causing the bioassay responses are not known. Therefore, bioassay responses can be corrected for reported average sampling rates of the used samplers to represent the exposure level that organisms would experience in the field (chapters 2 and 6).[14,52] However, the use of average sampling rates does not take the variability in sampling rates for individual compounds into account, nor the environmental factors that influence the uptake rates at the field locations. This may result in a lack of precision in sampling rate estimations for integrative samplers, leading to uncertainties in the back-calculation of bioassay responses to compound concentrations in the environment.

The use of active-advection samplers can substantially decrease the uncertainties related to sampling rate estimations for integrative samplers.[13] A multitude of devices for the active sampling of surface water has been developed in recent years.[211–214] However, these active sampling devices all depend on an electric power supply for their successful deployment, and their acquisition and use entail considerable costs compared with passive sampling devices. This

makes them markedly less appealing for use in regular monitoring efforts, especially in remote and/or developing regions.

The limitations discussed above highlight the urgent need for approaches to accurately estimate sampling rates for integrative passive samplers and the subsequent representative dosing of the extracts to bioassays. Alternatively, or additionally, the polymeric sorbents used in integrative passive samplers can be applied for the equilibrium sorption of organic compounds from environmental matrices.[93,215,216] The application of polymeric sorbents for the equilibrium sorption and subsequent passive dosing of polar compounds to bioassays was, at the time of writing, not yet reported in the scientific literature. However, the sorption of compounds to polymeric sorbents was shown to be reversible,[216] especially for polar compounds,[93] offering promising applicability of such sorbents for passive dosing of polar compounds to bioassays. As such, passive samplers targeting organic compounds in the more polar range (log $K_{OW} < 3$) could be combined with passive dosing approaches to reliably assess the potential ecotoxicological risks of the increasingly relevant polar organic compounds in aquatic ecosystems.[215]

III – BIOASSAY BATTERIES REPRESENTATIVE OF AQUATIC ECOSYSTEM HEALTH

The composition of bioassay batteries determines which effects can potentially be detected in effect-based chemical water quality assessment.[25] To meet the goal of safeguarding the ecological integrity of aquatic ecosystems against environmental pollution, bioassay batteries should function as early-warning systems for adverse effects of chemicals on natural populations. Therefore, the batteries should be curated to comprise indicator bioassays that represent relevant endpoints. With this aim in mind, the relevance of bioassays for application in effect-based chemical water quality assessment can be determined based on several criteria which are outlined below.

First, the responsiveness of the bioassays to real-world pollutant mixtures should be verified by exposing candidate assays to field-obtained samples.[24,217] Knowledge of the (groups of) compounds that elicit effects in these bioassays will strongly aid the subsequent identification of the causative compounds when responses to environmental samples are observed. Second, the battery should cover a wide range of endpoints to allow the toxicity assessment for as many of the sampled chemicals as possible, while simultaneously ensuring the relevance for ecological health. This can be achieved by aligning the selected bioassays to relevant steps at different levels in adverse outcome pathways (chapter 1).[22,25] Finally, to ensure their function as early-warning tools, bioassays should respond to lower toxic pressures than natural populations of organisms in the investigated environment (see also section IV). This optimal situation is conceptually depicted in Figure 7.1A, where highly sensitive *in vitro* endpoints respond first to increasing toxic pressures, followed by responses of *in vivo* endpoints, and eventually by adverse effects on natural populations. An example of a set of bioassays with which this situation is achieved is offered by the combination of receptor-mediated estrogen receptor and zebrafish embryo bioassays. Several studies have shown that highly specific assays for estrogenic activity respond most sensitively to environmental samples, followed by zebrafish embryo toxicity test.[32,218] In

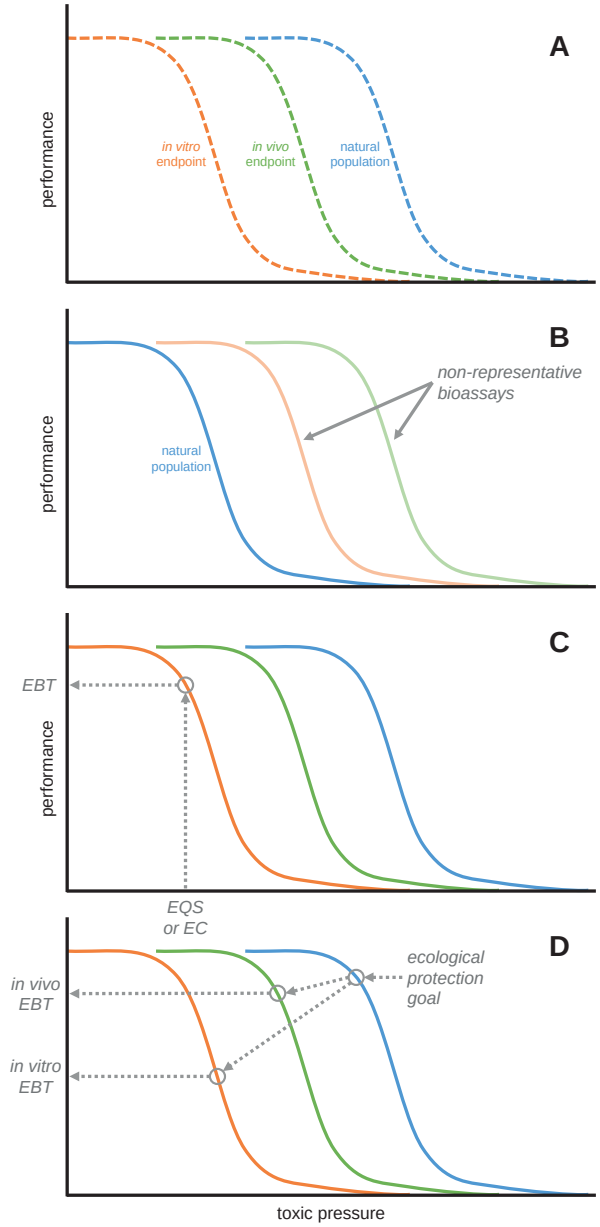

Figure 7.1. Conceptual depiction of the relationship between toxic pressure in the environment and the performance of natural populations of organisms and *in vivo* and *in vitro* bioassay endpoints. Ideally, responses in bioassays are observed at lower toxic pressures than adverse effects on natural populations (panel A). Non-representative bioassays will respond at higher toxic pressures than natural populations, making them unfit for effect-based chemical water quality assessment (panel B). Current strategies for effect-based trigger value (EBT) derivation are based on environmental quality standards (EQS) or effect concentrations (EC) of compounds in bioassays (panel C). Future EBT derivation can take ecological protection goals of natural populations as point of departure by defining when bioassay responses are indicative of adverse effects on natural populations (panel D).

another study, the *in vitro* yeast estrogen screen test was shown to be a sensitive indicator of intersex in wild fish populations exposed to wastewater treatment plant effluent.[111] Hence, highly sensitive *in vitro* estrogen receptor assays, but also sensitive *in vivo* zebrafish embryo assays, are promising indicator bioassays for endocrine disruption in wild populations of fish. Whether other bioassays indeed also provide such early warnings for adverse population effects can be determined by the collation of simultaneous measurements of bioanalytical responses and the status of natural populations of interest at field locations.

Examples of bioassays that would not be fit-for-purpose are i) those that respond to environmental contamination at higher toxic pressures than the relevant natural populations do, ii) assays representing endpoints that are not indicative of adverse effects in natural populations, or iii) assays that are unresponsive to toxic environmental samples for other reasons than the former two, including a lack of standardization and methodological constraints (Figure 7.1B). As such, they cannot be used as early-warning tools for adverse effects on natural populations and they should, therefore, not be applied in effect-based water quality assessment. Other reasons to exclude bioassays from batteries include high costs and/or infrastructural demands and the inability to reliably interpret their responses in relation to aquatic ecosystem health.

Based on the criteria outlined above, the present research identified several currently employed bioassays that appeared unfit for application in batteries for effect-based water quality assessment, and which were, therefore, excluded from revised batteries or replaced with more responsive and relevant alternative assays in subsequent research efforts. Three exemplary cases of these advancements are discussed here:

1. The *in situ Daphnia magna* bioassay that was employed in chapter 2, although responsive, was excluded from further bioassay batteries due to the inability to reliably interpret the observed responses. Generally, the extended field exposure of organisms represents a highly-realistic environmental exposure scenario, yet it also introduces many confounding factors that may affect the performance of the organisms and which makes it difficult to isolate the effects of micropollutants in these bioassays from the effects of other stressors.
2. The algal growth inhibition assay performed in chapter 2 appeared unresponsive at the majority of the investigated locations, triggering its replacement with a short-term (4.5 h) algal photosynthesis bioassay (chapter 4) in the bioassay battery applied in chapter 6. Although the algal photosynthesis bioassay did not elucidate any potential ecotoxicological risks in this final measuring campaign, responses were observed at a substantially higher percentage of the locations than for the algal growth inhibition assay in the first measuring campaign. Hence, this replacement appears to have introduced a more sensitive endpoint representative of herbicide activity, while simultaneously presenting a high-throughput test setup that can readily be applied in large-scale measurement campaigns.
3. The inclusion of the *anti*-PR CALUX assay in bioassay batteries (chapters 3 and 6) resulted in the detection of responses for all the investigated locations. Furthermore, in

the final field campaign this assay allowed the detection of potential ecotoxicological risks at half of the locations. This confirmed the responsiveness of this assay to environmental samples and highlights the relevance of this test for application in effect-based water quality assessment.

Considering infrastructural demands, costs, and the potential for high-throughput capacity, *in vitro* bioassays offer convincing advantages over *in vivo* bioassays. Moreover, the use of cell-based bioassays is also preferable to the use of whole organisms from an ethical perspective. For these reasons, a shift from the application of primarily *in vivo* to *in vitro* bioassays in environmental quality assessment has been proposed.[219] However, the reliable translation of responses of *in vitro* bioassays to ecotoxicological risks in the field remains challenging as receptor binding or cellular responses will not always lead to whole organism and population responses.[24] This is because repair and defense mechanisms that are activated between molecular initiating events detected by an *in vitro* assay and adverse effects in whole organisms may prevent such adverse effects. Therefore, responses in *in vivo* tests are more directly relatable to the ecological status of a water body, and may therefore as of yet be irreplaceable for the translation of bioassay responses to effects on natural populations of aquatic biota.[73] Nonetheless, the molecular initiating events covered by *in vitro* assays provide crucial information on potential adverse effects and especially on the groups of compounds responsible for the effects observed at higher levels. Hence, bioassay batteries that can comprehensively assess water quality will constitute a combination of representative *in vitro* and *in vivo* assays (chapters 2 and 6).

Vice versa, the application of bioassay batteries that cover a wide range of endpoints can be used to gain insight into contamination source-specific bioanalytical response profiles. However, such characteristic response profiles could not be revealed in a very heterogeneous set of locations receiving a very diffuse and mixed loading of micropollutants (chapter 2). The application of a revised bioassay battery at a selection of locations with much more well-defined contamination sources did, on the other hand, allow the identification of contamination source-specific ecotoxicological profiles (chapter 6). This represents valuable information since bioassay responses that are characteristic of particular sources of surface water contamination can serve as diagnostic indicators of specific types of pollution. As such, they can aid targeted mitigation efforts for risk abatement, also at locations at risk from the presence of more complex mixtures of micropollutants.

IV – EFFECT-BASED TRIGGER VALUES THAT ACCURATELY INDICATE ECOTOXICOLOGICAL RISKS

Effect-based trigger values (EBT) are valuable tools in the interpretation of potential ecotoxicological risks as indicated by bioanalytical responses.[21,30,31] In this way, they act similar to environmental quality standards (EQS) in traditional chemical water quality monitoring. The currently available and most commonly used EBTs for effect-based chemical water quality assessment are based on either EQS for regulated chemicals or on all reported effect

concentrations (EC) for compounds in the used bioassays (Figure 7.1C; chapter 1).[21,30] Hence, similar to EQSs, EBTs can be reinterpreted over time as more toxicity data becomes available or when alternative methods for their derivation are developed.[32] Nonetheless, the present EBTs are selected based on anthropogenic criteria like EQSs, that lack environmental realism, and that, consequently, biological organisms do not necessarily abide by. Therefore, these EBTs are very useful in legislation for the implementation of effect-based chemical water quality assessment, but due to their lack of environmental realism, the normalization of bioassay-derived data to EBTs renders the data less suitable to gain insight into the relationship between toxicity and aquatic ecosystem health.

An alternative approach to the interpretation of bioassay responses that would allow for unbiased investigations into the relation with adverse effects in natural populations is yet to be developed, and a promising approach is, therefore, proposed here. The first step would be to define a response range, derived from big data on responses for each bioassay in a battery to environmental samples. The minimum reported value can be defined as 0, representing a background level of toxicity, and the highest reported value, defined as 1, represents the maximum naturally occurring activity for that bioassay. Hereafter, each bioassay response can be expressed as a value between 0 and 1, where a higher value represents a higher response, and responses are calibrated to an environmentally relevant range of toxic pressures. This approach allows for summation of the responses of all bioassays in a battery, however without the interpretation of risk based on *a priori* defined thresholds, and thus allows for unbiased correlation analyses with ecological data.

Next, the environmentally calibrated bioassay responses can be applied in an as of yet underexplored approach to EBT derivation. The here postulated approach builds upon the foundation of the derivation of the "background BEQ" as laid by Van der Oost *et al.*,[21] and is outlined below. Keeping in mind the function of effect-based methods as early-warning systems for adverse effects of chemicals on natural populations, there has been remarkably little attention to the health of wildlife populations in the assessment of chemical impacts on the environment.[220,221] However, since EBTs should ultimately safeguard the health of natural populations, rather than using proxies for adverse effects like EQSs or ECs, sufficiently strong adverse effects on natural populations of aquatic biota can be defined as thresholds for environmental quality. As such, these thresholds represent the ecological protection goals that EBTs strive to achieve much more directly. Once these ecological protection goals have been defined in a quantifiable way, field studies that combine simultaneous measurements of bioanalytical responses and the status of natural populations of interest can provide the bioassay response levels that correlate with exceedances of the defined threshold for environmental quality, resulting in ecologically derived EBTs for all bioassays in a battery (Figure 7.1D). This can be done for all possible combinations of bioassays and ecological protection goals. The (combination of) bioassay(s) that is/are the most sensitive and accurate predictor of the exceedance of the ecological threshold is then appropriate as an early-warning tool for a specific ecological protection goal. This way, the point-of-departure of EBT derivation is switched from EQSs or ECs to ecological protection goals (Figure 7.1D).

This newly proposed approach to EBT derivation is currently limited by the availability of data on both bioassay responses and ecological quality parameters measured simultaneously at the same locations. However, given the increasing application of effect-based methods for chemical water quality assessment in large-scale research efforts like the SOLUTIONS project,[222] but also in the research presented in this thesis, a growing amount of work is available that can help to meet these data requirements. Another foreseeable challenge to the here outlined approach is the formulation of ecological protection goals. Intrinsically, the goals will differ between environmental compartments, ecosystems, and geographic regions. This requires decisions and consensus on defining variables between aquatic ecologists and ecotoxicologists, water managers, and authorities, which may represent the biggest challenge in the regular application of effect-based water quality assessment yet. Finally, it must be noted that a deteriorated ecological status of aquatic ecosystems is often not solely attributable to chemical pollution.[220] However, the required simultaneous measurements of bioanalytical responses and the status of wildlife populations also offer the opportunity for improved insight into the multi-stress acting on aquatic ecosystems. When combined with measurements of other co-occurring important stressors like eutrophication and habitat degradation, such research provides datasets that can help isolate the contribution of toxicity to the overall ecosystem degradation from the multiple other stressors that are ubiquitously present in human-impacted waters. Despite existing challenges, the here postulated novel, more ecologically relevant, approach to EBT derivation would strongly aid in achieving the ultimate goal of EBTs: to protect natural populations of organisms in the aquatic environment.

FUTURE CHEMICAL WATER QUALITY ASSESSMENT

The present thesis provided deliberations on sampling and dosing methodologies of aquatic environmental samples to bioassays, the appropriate composition of bioassay batteries, and the interpretation of bioassay battery responses for effect-based chemical water quality assessment. The attained advancements, but also the identified knowledge gaps and the here proposed ecologically relevant EBT derivation approach, will hopefully serve as a roadmap for the future application of effect-based tools in regular chemical water quality assessment.

In an ideal future, chemical water quality monitoring will result in a comprehensive assessment of the 'exposome of the natural aquatic environment', where (semi)continuous simultaneous biological and chemical analyses reveal the chemicals that (jointly) pose a threat to the biotic environment.[9,223,224] The widespread application of such chemo-biological sentinels of aquatic environmental health will provide high-resolution information that will make targeted and effective chemical risk abatement possible. Finally, this will also allow the complete and effective integration of effect-based methods with the "One Health" principle, that aims to ensure non-toxicity to the living environment.