Advancements in effect-based water quality assessment

de Baat, M.L.

CHAPTER 1

GENERAL INTRODUCTION: EFFECT-BASED WATER QUALITY ASSESSMENT

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ML de Baat, MHS Kraak
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THE CHEMOCENE

The present age is defined by the escalating human impact on the global environment, and the current geological epoch has therefore aptly been assigned the name "Anthropocene".[1] The induction of this new era is said to have started with the industrial revolution in the second half of the eighteenth century, but the Anthropocene stands alone as a new epoch beginning sometime in the mid–20th century.[2] One of the critical markers of human-induced global change that distinguishes the Anthropocene from previous geological eras is the widespread presence of human-made chemicals in the environment.[2,3] This global chemical human signature is only expected to become more pronounced, as the number of novel chemicals is rising exponentially, with the number of chemicals registered by the chemical abstract service increasing from 20 million around the year 2000 to almost 160 million in the year 2020.[4] Although part of these substances already existed before their registration, many are and continue to be newly developed and synthesized. Currently, over 350 000 chemicals have been registered for production and use on the market globally,[5] and the exponential growth in the number of newly registered substances is indicative of the ever-increasing number of anthropogenic chemicals that can find their way into the environment. Hence, it could be argued that the turn of the millennium marked the dawn of the 'Chemocene' epoch as an integral part of the Anthropocene since the present age is defined by the presence of an unfathomable diversity of synthetic chemicals in the environment (Figure 1.1).

Once this increasingly wide variety of chemicals is emitted to the environment, many of them end up in aquatic ecosystems, where thousands of substances may be simultaneously present, resulting in complex and varying mixtures.[6] It is in these ecosystems that the resultant complex cocktails of substances exert a potential threat to human health and aquatic biodiversity.[7]

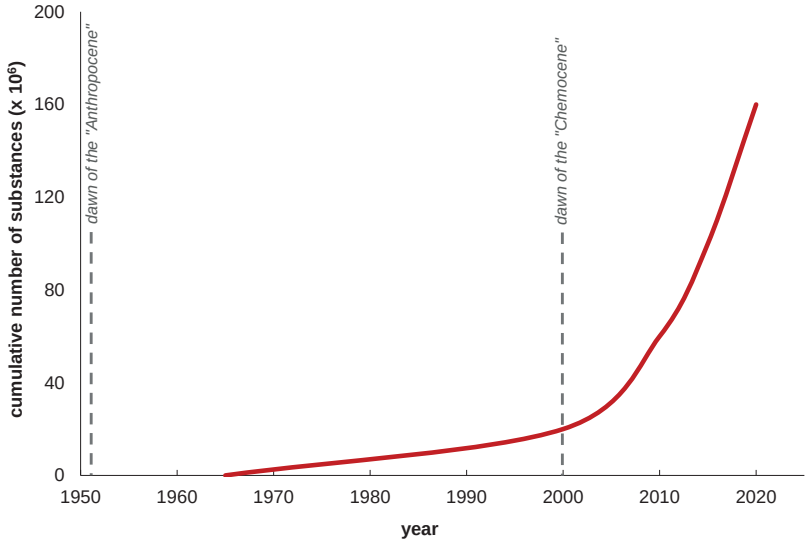

Figure 1.1. Number of chemical entities in the chemical abstract service registry over time.[4]

This threat represents substantial scientific and methodological challenges to chemical water quality assessment approaches, as current chemical policies focus on only a limited number of prioritized compounds. Therefore, there is a need for future-proof monitoring methods that allow for the impact assessment of the ever-changing complex chemical burden on aquatic ecosystems.

A PARADIGM SHIFT TOWARDS NEW MONITORING METHODS

Traditional chemical water quality assessment is based on the analysis of a list of a varying but limited number of priority substances. Nowadays, the use of many priority substances is restricted or banned, and concentrations of these legacy contaminants are decreasing.[8] At the same time, industries have switched to a plethora of alternative compounds, which may enter the aquatic environment, where they can seriously impact water quality.[9] Hence, priority substances lists will always be outdated, as the selected compounds are frequently no longer present, while many compounds with contemporary relevance to aquatic ecosystem health are not listed as priority substances. Consequently, a large portion of the toxic effects observed in surface waters cannot be attributed to measured compounds, and ecotoxicological risks to freshwater ecosystems are thus caused by changing mixtures of a myriad of (un)known and unregulated compounds.[10–12] c

Concentrations of current surface water pollutants are typically low and variable, challenging analytical methods in characterizing the composition and risks of mixtures in space and time. Time-integrative sampling methods can help overcome the challenge of low and varying compound concentrations by allowing the *in situ* sequestration and preconcentration of compounds from the aquatic environment over extended timeframes.[13] These time-integrative water samples can, in turn, be investigated with effect-based methods that employ living cells or organisms (bioanalyses or bioassays).[14–16] The combination of time-integrative monitoring and bioassays allows the determination of potential ecotoxicological risks of a wide variety of pollutants, since bioassay responses are caused by all (un)known bioavailable compounds and their metabolites in a sample, regardless of their status as priority substances.[17] Therefore, there is a need for monitoring strategies that employ a combination of time-integrative sampling methods and bioassays to identify ecotoxicological risks in surface waters.

PASSIVE SAMPLING

Conventional water sampling is performed by the instantaneous collection of an aliquot of water from the environment. Often, the water sample is then enriched by solid-phase extraction to improve the detection of compounds in subsequent analyses. However, such sampling only provides a discrete snapshot of compound concentrations in the field at the time of sampling. Alternatively, the sample enrichment can also be performed *in situ*, either by active sampling (using a pump) or by allowing the passive uptake of compounds from the water into a sampling device, which is hence known as passive sampling.[13,18] In passive sampling, a sorbing phase is exposed to the surrounding medium (e.g., water, air, sediment, soil), accumulating compounds

over extended periods. This way, fluctuations in compound concentrations are integrated while the water sample is simultaneously concentrated, leading to improved detection of compounds in chemical analysis and of effects in bioassays.[18,19] There are, however, certain pitfalls when combining passive sampling and bioassays.[20] First, the composition of the mixture extracted from the passive samplers is not identical to the one that organisms are exposed to in the field. Second, since the compounds causing significant responses in the bioassays commonly remain unknown, it is not possible to derive exact concentrations of causative compounds in the water phase based on bioassay responses to passive sampler extracts.

A wide variety of passive sampling devices is available nowadays, which can be classified into two main groups according to the mass transfer regimes in which they operate.[13] 'Integrative passive samplers' are designed for operation in the kinetic uptake regime, in which the uptake of compounds into the sampler proceeds (pseudo-)linearly with time. Contrastingly, 'equilibrium passive samplers' reach thermodynamic equilibrium with the surrounding medium. Both classes of samplers can provide time-weighted average concentrations of compounds present in the environment.[13] In general, integrative samplers are more frequently applied for the detection of polar (hydrophilic) organic compounds and metals, while equilibrium samplers are usually applied for the detection of non-polar (hydrophobic) compounds.[19] The selection of the appropriate passive samplers, or combinations of multiple sampler types, for the monitoring of the wide variety of present-day environmental pollutants, is, however, under debate and is an emerging field of research.[13,18] Despite these ongoing debates, extracts from both classes of passive samplers are increasingly applied in combination with (batteries of) bioassays in the ecotoxicological risk assessment of surface waters.[14,15]

BIOASSAY BATTERIES

The assessment of chemical water quality based on responses in bioassays is commonly known as effect-based monitoring. The regular application of effect-based monitoring largely relies on the ease of use, endpoint specificity, costs and size of the used bioassays, as well as on the ability to interpret the observed responses. To ensure sensitivity to a wide range of potential chemical stressors and high ecological relevance, while still providing specific endpoint sensitivity, bioanalytical test batteries should ideally encompass endpoints that allow the detection of molecular initiating events, as well as adverse outcomes on whole organisms or even populations.[14,21] To achieve this, a successful bioassay battery should include laboratory-based whole-organism *in vivo* as well as mechanism-specific *in vitro* assays. Additionally, *in situ* whole organism assays can be included to represent highly realistic field exposure scenarios. Adverse effects in the whole-organism bioassays point to general toxic pressure and represent a high ecological relevance. The *in vitro* or small-scale *in vivo* assays with specific drivers of adverse effects allow for the focused identification and subsequent confirmation of (groups of) toxic compounds with specific modes of action. To curate a bioassay battery that meets the abovementioned criteria, bioassay selection can be based on the adverse outcome pathway (AOP) concept that describes the relationships between molecular initiating events and adverse

outcomes.[22] Combining different types of bioassays ranging from whole organism tests to *in vitro* assays targeting specific modes of action can thus greatly aid in narrowing down the number of candidate compound(s) that cause the identified environmental risks.[23] For example, if bioanalytical responses at a higher organizational level are observed (the orange and black pathways in Figure 1.2), responses in specific molecular pathways (blue, green, grey and red in Figure 1.2) can help to identify certain (groups of) compounds responsible for the observed effects. However, given the broad diversity of bioassays that is currently available, it is necessary to establish a limited yet effective suite of assays for application in water quality monitoring.[24] Whether or not a bioassay response is representative of an environmental protection goal should be corroborated by field-based studies that consider the ecological status at the investigated locations. Hence, empirical findings that demonstrate the suitability of bioassays for chemical water quality assessment are required to curate bioassay batteries that are fit-for-purpose while remaining feasible in terms of infrastructural demands and costs. Ultimately, the appropriate composition of bioassay batteries depends on the purpose of the study, the environmental matrix of interest, and the availability of (newly developed) bioassays and the ability to reliably interpret their responses.[24,25] Sediments are a particularly underrepresented compartment in chemical water quality assessment strategies despite their relevance to aquatic ecosystem health,[26,27] and the integration of effect-based sediment quality assessment methods is urgently needed.[28,29]

BIOASSAY EFFECT EXPRESSION

The effect of an environmental sample in a bioassay is quantified as toxic units (TU) for toxicity in *in vivo* assays and as bioanalytical equivalent (BEQ) concentrations for responses in *in vitro* bioassays. TU are determined with the relative enrichment factor (REF) of a sample needed to measure a significant effect in the bioassay. The BEQ concentrations represent the joint toxic potency of all (un)known chemicals present in the sample that have the same mode of action as the reference compound and act concentration-additively.[17] The BEQ concentrations are expressed as the concentration of a reference compound that causes an effect equal to the entire mixture of compounds present in an environmental sample. Figure 1.3 depicts a typical dose-response curve for a molecular *in vitro* assay that is indicative of the presence of compounds with a specific mode of action. A specific water sample induced an effect of 38% in this assay, equivalent to the effect of approximately 0.02 nM bioanalytical equivalents.

EFFECT-BASED TRIGGER VALUES

The combination of advanced sample enrichment techniques like passive sampling with increasingly sensitive bioassays allows the detection of effects even in environments with very low contaminant levels. This means that bioanalytical signals do not always indicate unacceptable contamination levels, and it is thus necessary to define thresholds that differentiate between acceptable and poor environmental quality. These thresholds are defined as effect-based trigger values (EBT) and are essential in the evaluation of the significance of the effects observed in

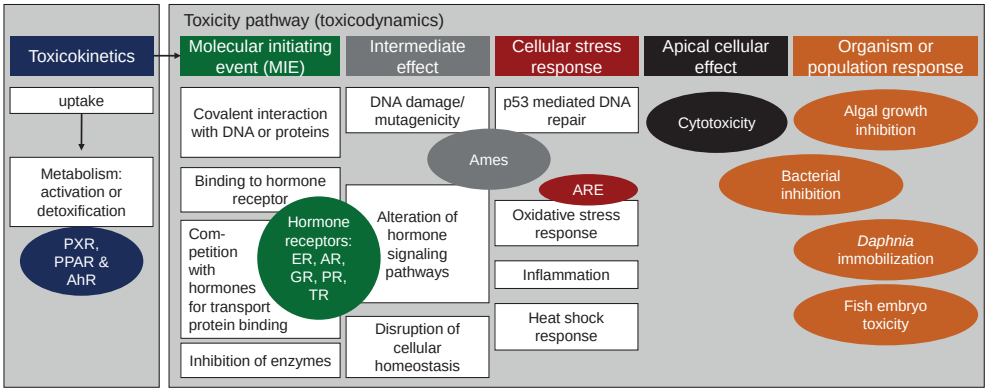

Figure 1.2. Design of a bioassay panel (ovals) based on adverse outcome pathways (boxes), from toxicokinetics via molecular and cellular responses to population responses. Redrawn from Escher *et al.* (2018).[30]

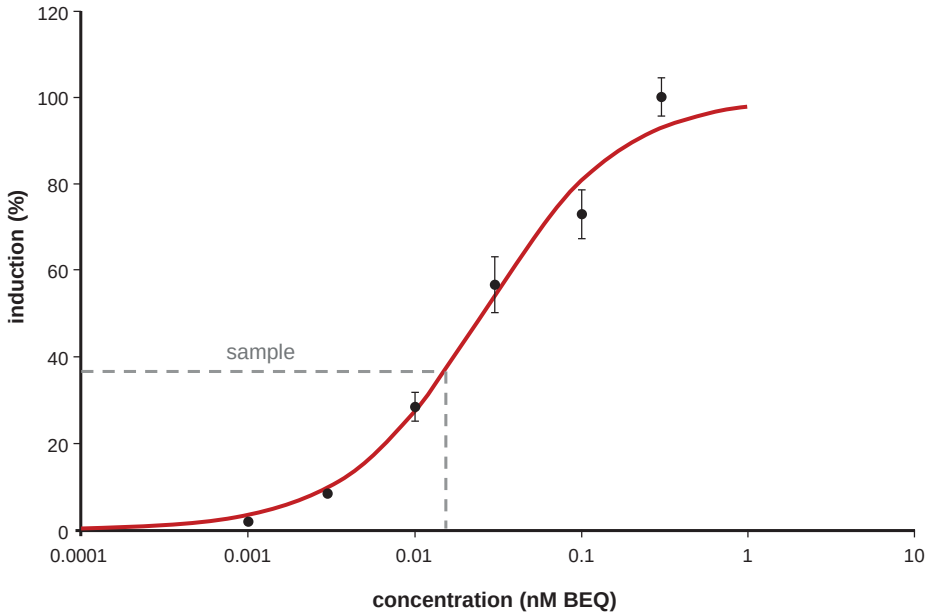

Figure 1.3. Dose response relationship for a reference compound in an *in vitro* bioassay. The dashed lines show that a specific water sample induced an effect of 38%, representing approximately 0.02 nM bioanalytical equivalents (BEQ).

bioassay batteries.[30] Similar to what environmental quality standards represent for single compounds, EBTs indicate predicted no-risk levels for mixtures of compounds that are present in environmental samples.[31] Hence, the exceedance of an EBT indicates a potential ecotoxicological risk, and further investigations into the source and causative compounds of the observed

toxicity are recommended at locations where EBTs are exceeded.[30] In line with bioassays, EBTs are expressed as TU or as BEQ concentrations of model compounds for the respective bioassay. Since most bioassays have different characteristics and correspond to different modes of action, the correlation of observed bioassay responses to EBTs offers a standardization that allows for the quantitative comparison between responses of bioassays in a battery. The derivation of EBTs is a novel field of research that has gained traction in recent years due to its relevance for the interpretation of bioassay responses in effect-based water quality assessment.[21,30–32] Multiple partly overlapping philosophies on EBT derivation have arisen, two of which have recently experienced considerable application in water quality assessment. The approach by Van der Oost *et al.* (2017) [21] integrates all available effect concentrations for compounds in a bioassay to derive an EBT. Alternatively, Escher *et al.* (2018)[30] established a common derivation method for EBTs based on environmental quality standards for regulated chemicals. Interestingly, when applied to the same bioassays, these approaches often yield similar EBTs, while for some bioassays they result in divergent EBTs. Despite ongoing debate on EBT derivation approaches, bioassay batteries are increasingly applied in the assessment of chemical surface water quality and the ranking of contaminated sites based thereon.

RANKING OF CONTAMINATED SITES BASED ON EFFECT-BASED RISK ASSESSMENT

The ecotoxicity profiles resulting from bioassay battery responses to environmental samples allow for the calculation and ranking of cumulative ecotoxicological risks for the investigated locations.[14,15] In the example given in Figure 1.4, a battery of 20 bioassays was subjected to passive sampler extracts (metals and non-polar and polar organic compounds) from six surface water locations. All bioassay responses were divided by the corresponding EBT to obtain effect-based risk quotients. The cumulative ecotoxicological risk per location was then calculated by summing the separate effect-based risk quotients. The resulting cumulative ecotoxicological risk score allows the ranking of the selected sites based on the presence of ecotoxicological risks rather than on the presence of a limited number of target compounds. This, in turn, permits water authorities to invest money where it matters most: the identification of compounds causing adverse effects at locations with indicated ecotoxicological risks. Although initially the compounds causing the observed EBT exceedances will not be known, this can subsequently be elucidated with targeted or non-target chemical analysis.[23] Hence, the use of bioanalytical tools does not absolve of the need for chemical analysis for environmental quality assessment. Rather, the advantages of bioanalysis illustrate the continued need for powerful chemical non-target analysis for the identification of previously unknown contaminants that contribute to ecotoxicological risks in the aquatic environment.[33,34]

MOTIVATION

Over the past two decades, coincident with the dawn of the Chemocene, fundamental steps have been taken in the scientific underpinning of technological innovations for effect-

		bioassay	A	B	C	D	E	F
in vivo	non-polar	bact. bioluminescence	0.1	0.1	0.0	0.1	0.1	0.0
		Daphnia	0.0	0.0	0.0	0.0	0.2	0.1
		PAM	0.0	0.0	0.0	0.0	0.0	0.0
	polar	bact. bioluminescence	0.1	0.1	0.1	0.1	2.0	1.0
		Daphnia	0.0	0.0	0.0	0.0	0.0	1.5
		PAM	0.0	0.0	0.0	0.0	0.0	0.9
	metals	bact. bioluminescence	0.2	0.3	0.5	0.7	3.1	0.7
		Daphnia	0.0	0.0	0.0	0.0	0.0	0.0
		PAM	0.0	0.0	0.0	0.0	0.0	0.0
in vitro	non-polar	cytotox	0.0	0.0	0.0	0.0	0.0	0.0
		DR	0.0	0.0	0.0	0.0	0.0	0.0
		PAH	0.1	0.1	0.5	0.4	1.0	0.6
		PPARγ	0.0	0.0	0.0	0.0	0.0	0.0
		PXR	1.4	0.7	0.9	2.4	3.7	2.9
		Nrf2	0.4	0.2	0.4	0.5	0.5	0.9
		p53	0.1	0.3	0.1	0.2	0.7	0.5
	polar	cytotox	0.2	0.4	0.6	0.5	0.1	0.1
		ERα	0.1	0.5	1.4	1.9	9.2	13.7
		anti-AR	1.2	3.0	3.2	3.8	1.8	0.7
		anti-PR	0.5	1.1	3.5	3.0	0.5	0.9
Σ effect-based risk quotient			**4.3**	**6.7**	**11.3**	**13.5**	**22.8**	**24.4**

Figure 1.4. Heat map depicting the effect-based risk quotients (bioassay response/effect-based trigger value) of 20 bioassays to passive sampler extracts from six surface water locations (A-F). Effect-based risk quotients ≥ 1 indicate potential ecotoxicological risks, and the Σ effect-based risk quotients indicate the cumulative ecotoxicological risks at the investigated locations.

based water quality assessment approaches and the interpretation of the obtained results. Due to their advantages, there has been increasing interest in the implementation of effect-based methods in regulatory water quality assessment frameworks, like the European Water Framework Directive.[35] Nonetheless, their regular implementation is still in its infancy, and scientific knowledge gaps exist that need to be addressed to unleash the full potential of effect-based methods in chemical water quality assessment, which motivated the present project. High priorities for the scientific advancement of effect-based methods include the i) effective sampling and extraction of all bioavailable potentially toxic compounds, ii) representative dosing of water extracts to bioassays, iii) appropriate composition of bioassay batteries, iv) translation of bioassay responses to environmental risks, v) inclusion of the sediment compartment, and vi) identification of bioassay battery response profiles characteristic of specific contamination sources.

AIM AND OBJECTIVES

The present research aimed to fuel the paradigm shift towards new chemical aquatic environmental quality monitoring methods by providing a scientific basis for the advancement of effect-based water quality assessment. This aim was translated into the following objectives:

1. To gain insight into the state-of-science of effect-based surface water quality assessment and its potential for implementation into regular chemical water quality monitoring.
2. To determine the influence of passive sampler design on bioassay battery responses and to develop a passive sampling strategy that ensures the monitoring of a wide range of potentially toxic compounds.
3. To replace non-responsive bioassays in existing batteries with relevant and sensitive high-throughput alternatives.
4. To develop an effect-based approach that allows the incorporation of chemical sediment quality assessment into water quality monitoring.
5. To curate a selection of bioassays that together cover those endpoints that are relevant to and representative of aquatic ecosystem health.

THESIS OUTLINE

To gain insight into the state-of-science of effect-based surface water quality assessment and its potential for implementation into regular water quality monitoring, the aim of **chapter 2** was to perform an effect-based nationwide water quality assessment to identify ecotoxicological risks in a wide variety of surface waters. To this end, passive sampling of polar and non-polar organic compounds was combined with a battery of *in situ*, *in vivo* and *in vitro* bioassays for 45 surface water locations. The applied bioassay battery was selected such that it could identify the risks posed by a wide range of chemical pollutants and their transformation products, while simultaneously allowing for targeted identification of groups of compounds that cause specific effects. Bioassay responses were compared to effect-based trigger values to identify potential ecotoxicological risks at the investigated locations. Based on the results, considerations regarding future improvements of effect-based monitoring were given, which were addressed in the chapters 3-6.

The design of integrative passive samplers can affect the accumulation of compounds and therewith the bioassay responses. **Chapter 3** aimed to determine the effects of sampler housing and sorbent type on bioassay responses to polar passive sampler extracts. To this end, four integrative passive sampler configurations, resulting from the combination of two housings with two sorbents, were simultaneously exposed at reference and contaminated surface water locations. To measure the toxicity of the accumulated polar organic compounds, a battery of five bioassays was exposed to the extracts.

Since algal photosynthesis is a sensitive process that can be applied to identify the presence of hazardous herbicides in surface water, the aim of **chapter 4** was to employ an algal photosynthesis bioassay to assess surface water toxicity to algae and to identify the compounds causing the observed effects. To this end, *Raphidocelis subcapitata* was exposed to surface water grab samples and after 4.5 h photosynthetic efficiency was determined using PAM fluorometry.

Sediment quality assessment methods that consider the risks caused by the combined action of all sediment-associated contaminants to benthic biota are still underrepresented in water quality assessment strategies. **Chapter 5** aimed to integrate effect-monitoring and chemical

profiling of sediment contamination. To this end, 28-day life cycle bioassays with *Chironomus riparius* using intact whole sediment cores from contaminated sites were performed in tandem with explorative chemical profiling of bioavailable concentrations of groups of legacy and emerging sediment contaminants to investigate ecotoxicological risks to benthic biota.

It was hypothesized that the refined insights and methodological improvements obtained in the previous chapters would contribute to an improved strategy for the assessment of the aggregated risk of all bioavailable micropollutants present in the aquatic environment. **Chapter 6** aimed to advance effect-based water quality assessment by implementing the developed methodological improvements and to gain insight into contamination source-specific bioanalytical responses. To this end, passive sampling of non-polar and polar organic compounds and metals was applied at 14 surface water locations that were characterized by two major anthropogenic contamination sources, agriculture and WWTP effluent, as well as reference sites with an expected low impact from micropollutants. A revised battery of 20 *in vivo* and *in vitro* bioassays was exposed to the passive sampler extracts, and the bioanalytical responses were compared to effect-based trigger values to identify potential ecotoxicological risks.

Finally, in the synthesis (**chapter 7**), the current state of knowledge on effect-based methods is discussed, and the findings obtained in this thesis are placed into a wider scientific and societal perspective.