Integrated Environmental Assessment and Management — Volume 15, Number 1—pp. 126–134
Received: 13 March 2018 | Returned for Revision: 21 May 2018 | Accepted: 21 August 2018

Environmental Management

Risk-Based Approach in the Revised European Union Drinking Water Legislation: Opportunities for Bioanalytical Tools

Milou ML Dingemans,† Kirsten A Baken,† Ron van der Oost,‡ Merijn Schriks,§ and Annemarie P van Wezel†‖*

†*KWR Watercycle Research Institute, Nieuwegein, The Netherlands*
‡*Waternet Institute for the Urban Water Cycle, Amsterdam, The Netherlands*
§*Vitens Drinking Water Company, Zwolle, The Netherlands*
‖*Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, The Netherlands*

ABSTRACT

A plethora of in vitro bioassays are developed in the context of chemical risk assessment and clinical diagnostics to test effects on different biological processes. Such assays can also be implemented in effect-based monitoring (EBM) of (drinking) water quality alongside chemical analyses. Effects-based monitoring can provide insight into risks for the environment and human health associated with exposure to (unknown) complex, low-level mixtures of micropollutants, which fits in the risk-based approach that was recently introduced in the European Drinking Water Directive. Some challenges remain, in particular those related to selection and interpretation of bioassays. For water quality assessment, carcinogenesis, adverse effects on reproduction and development, effects on xenobiotic metabolism, modulation of hormone systems, DNA reactivity, and adaptive stress responses are considered the most relevant toxicological endpoints. An evaluation procedure of the applicability and performance of in vitro bioassays for water quality monitoring, based on existing information, has been developed, which can be expanded with guidelines for experimental evaluations. In addition, a methodology for the interpretation of in vitro monitoring data is required, because the sensitivity of specific in vitro bioassays in combination with sample concentration may lead to responses of chemicals (far) below exposure concentrations that are relevant for human health effects. Different approaches are proposed to derive effect-based trigger values (EBTs), including EBTs based on (1) relative ecotoxicity potency, (2) health-based threshold values for chronic exposure in humans and kinetics of reference chemicals, and (3) read-across from (drinking) water guideline values. Effects-based trigger values need to be chosen carefully in order to be sufficiently but not overly conservative to indicate potential health effects. Consensus on the crucial steps in the selection and interpretation of in vitro bioassay data will facilitate implementation and legal embedding in the context of water quality monitoring of such assays in EBM strategies. *Integr Environ Assess Manag* 2019;15:126–134. © 2018 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals, Inc. on behalf of Society of Environmental Toxicology & Chemistry (SETAC)

Keywords: Effect-based monitoring (EBM) Effect-based trigger values (EBT) Chemical water quality Mixtures Health risk

INTRODUCTION

Trace amounts of natural and anthropogenic chemicals are present in drinking water and its sources. Extensive monitoring programs are in place to protect drinking water consumers from adverse health effects of chemical contaminants. Chemical water quality is currently mostly assessed by analysis of concentrations of individual chemicals. It is increasingly recognized that targeted chemical monitoring cannot account for the presence of unknown chemicals in aquatic environments, e.g., new, emerging chemicals or transformation products (Brack et al. 2015; Maruya et al. 2016). For risk assessment, concentrations in water are related to (provisional) guideline values included in national and international water quality legislation (Baken et al. 2018). When derived for health safety purposes, such guideline values are often based on the acceptable daily intake of a chemical, which is usually derived by regulatory agencies from exposure levels resulting in adverse effects in experimental animal toxicity studies. Health-based threshold values are extrapolated from these exposure levels by using a number of extrapolation factors to compensate for species differences, inter-individual differences, exposure duration, route-to-route extrapolation, mixture toxicity, and other uncertainties or data gaps in toxicological effects.

Many efforts are currently being undertaken to support the derivation of human health-based threshold values from toxicological and kinetic data obtained in in vitro bioassays (National Research Council 2007; Allen et al. 2014; Wetmore

* Address correspondence to annemarie.van.wezel@kwrwater.nl
Published 24 August 2018 on wileyonlinelibrary.com/journal/ieam.

et al. 2015; Bell et al. 2018). Initiatives are developed to include such effect-based tools in (drinking) water quality monitoring programs in a complementary manner to target chemical analyses. As chemicals cause a wide range of biological effects and most bioassays only respond to chemicals with a particular mechanism of action, a test battery of bioassays covering a range of endpoints commonly responsive to drinking water is needed to monitor water quality. In the Netherlands, bioanalytical tools are already in use at some water utilities, mainly those that abstract surface water for drinking water production. In vitro bioassays have been demonstrated to be sufficiently sensitive to detect the effects of chemicals, in some cases below the detection limits of chemical analyses (e.g., Chapman et al. 2011). When chemical identification is challenging, in vitro bioassay responses of water samples with known origin but unknown composition may give insight in the presence of (emerging) chemicals with certain biological activities. Another advantage over substance-specific monitoring is that bioassays measure the complete effect of the low-level mixture of chemicals present, which may be different than the combined effects of the individual micropollutants. While the concentrations of individual chemicals may be below guideline values, it cannot be excluded that an effect is caused by unintentional mixtures of many chemicals at low concentrations (Silva et al. 2002).

Parallel chemical analysis, e.g., using nontarget high-resolution mass spectrometry (Hollender et al. 2017), prevents (new) chemicals that do not give a response in the selected in vitro bioassays from being overlooked. The complementary contributions of both analyses for human health risk assessment have been demonstrated in several case studies on water treatment efficiency and the chemical quality of drinking water (Pablos et al. 2009; Zegura et al. 2009; Macova et al. 2010; Schriks et al. 2010; Kienle et al. 2011; Macova et al. 2011; Conley et al. 2017; Vughs et al. 2018). This paper describes the state of the art of the different crucial steps that are required for the selection, implementation, and interpretation of in vitro bioassays as bioanalytical tools in effect-based water quality monitoring. A number of possible future developments and recommendations for efforts needed for the successful implementation of in vitro bioassays for water quality monitoring are presented.

SELECTION OF EFFECT-BASED TOOLS

At sufficiently high levels, exposure to chemicals may result in various biological effects on cells and molecules that may ultimately result in adverse effects. Depending on the route of exposure and types of effects, different organ or physiological systems may be affected. In the scientific field of toxicology, such relationships between the interaction of a chemical on a cellular target and the key events that are triggered on organ or organism level are described in toxicity pathways and adverse outcome pathways (Ankley et al. 2010; Vinken 2013; Villeneuve et al. 2014). Toxic effects can be investigated in different types of biological systems, ranging from isolated biological molecules such as enzymes or receptors, to intact organisms or even populations. A chemical can only activate a toxicity pathway if the involved molecular and cellular entities are present in the exposed organism or test system. Chemicals may activate multiple toxicity pathways simultaneously. The activation of a toxicity pathway depends on the exposure level, exposure duration, and timing of exposure, for example during sensitive windows in development.

A wide range of mode-of-action categories has been observed for water-relevant chemicals (Busch et al. 2016). Responses of water concentrates (extracts) in bioassays can be used as indicators for the presence of particular (groups of) chemicals that may cause adverse health effects. For rapid screening of potential toxicological effects of chemicals in water, in vitro (for example reporter gene) and small-scale in vivo (such as algae, daphnids, and fish embryos) models are preferred over testing in large intact organisms (in vivo) (e.g., Escher and Leusch 2011; Hamers et al. 2013; Leusch and Snyder 2015; Prasse et al. 2015; Wernersson et al. 2015). In vitro and small-scale in vivo assays are preferred over in vivo studies because of cost and time efficiency, because smaller sample volumes are sufficient, and because in vitro assays give information on specific toxicity pathways (National Research Council 2007). Because there are many in vitro bioassays available (Richard et al. 2016) that can be used to test effects on different biological processes in different types of models (e.g., cells from different organs or species), it is necessary to select the most relevant set of bioassays for water quality monitoring. Although a broad selection of toxic endpoints will gain a more complete picture of possible effects of unexpected or unknown chemicals (Escher and Leusch 2011), time and cost-effectiveness should also be included in these considerations.

In the European Union (EU) Seventh Framework Programme (FP7) DEMEAU project, the most relevant modes of action and corresponding toxicological endpoints for application in drinking water and its sources were selected on the bioactivity of chemicals present in different types of water samples in available high-throughput bioassays, as indicated by a large interlaboratory study using 103 different in vitro bioassays studying a broad range of toxicity pathways (Escher et al. 2014; Table 1). This study demonstrated that the most responsive toxicity pathways were related to xenobiotic metabolism, modulation of hormone systems, reactivity, and adaptive stress responses. Although endocrine effects in this study were mainly induced by (treated) wastewater and storm water (residential run off), other studies demonstrated hormone system–related responses induced by drinking water (Conley et al. 2017; Van Zijl et al. 2017; Shi et al. 2018). Both endocrine disruption and DNA reactivity may underlie carcinogenesis and be related to reproduction and developmental effects, health effects with considerable impact on quality of life (GBD 2016 DALYs and HALE Collaborators 2017).

New endpoints of interest (such as sensitive endpoints for developmental toxicity, neurotoxicity, or immunotoxicity; Health Council of the Netherlands 2014) or detection of emerging chemicals with alternative mechanisms of action

Table 1. Health effects and related toxicity mechanisms relevant for drinking water safety assessment. Modified from the report of Schriks et al. (2015). Information on the specific bioassays (methods, evaluation, and ranking) can also be found in the original DEMEAU report (Schriks et al. 2015).

Health effects	Toxicity mechanism
Xenobiotic metabolism	pregnane x receptor activation
	aryl hydrocarbon receptor activation
Modulation of hormone systems	estrogenicity
	antiandrogenicity
	glucocorticoid activity
Reactivity	gene mutation
	chromosomal mutation
	DNA damage response
Adaptive stress responses	endoplasmic reticulum stress
	heat shock
	hypoxia
	inflammation
	metal stress
	oxidative stress
Reproductive and developmental toxicity	preimplantation toxicity
	nonmechanistic assays (in vivo) including early life stages
	mechanistic assays to be included (several in early research and development stage)
Neurotoxicity	most relevant mechanisms to be determined
Immunotoxicity	most relevant mechanisms to be determined

may urge expansion of the in vitro bioassay battery. To choose the most useful candidate bioassays for testing potential risks for a particular health effect or related mechanisms, a scoring matrix has been designed in the DEMEAU project to evaluate bioassays for their applicability and performance (Schriks et al. 2015). The scores are based on information obtained from scientific literature, bioassay suppliers, or expert judgment. Bioassays for the prioritized health effects (Table 1) were scored and ranked. It should be noted that new information on technical specifications and applications of specific assays may (have) become available, which urges periodic updates of the scores. Also, if desired, more emphasis may be placed on specific parameters in the scoring methodology. It was also observed that in vitro bioassays to study potential effects on neurotoxicity, immunotoxicity, reproduction, and development that fulfill the criteria for applicability and performance are scarce. Current developments and innovations in these particular fields are of interest for water quality monitoring.

Both mechanistic (in vitro) bioassays and nonspecific assays (in vivo) can be used to study such potential effects. Because of the complexity of intact organisms, including early life stages, in vivo assays allow a simultaneous evaluation of (the chemical effect on) many different physiological processes. An example is the use of the fish embryo toxicity assay (Zhang et al. 2017). Although application of mechanistic in vitro bioassays may be more feasible in a monitoring context, they can only detect specific endpoints related to neurotoxicity, immunotoxicity, reproduction, and development, while other effects potentially contributing to such health outcomes may not be detected if nonspecific (in vivo) assays are not included. In addition to the scoring of individual test systems, another consideration in the selection of in vitro bioassays for effect-based water quality monitoring is that the use of a set of similar bioassays (for different endpoints) improves efficiency and quality control. The eventual bioassay panel should cover the various types of toxic action (i.e., nonspecific, specific, and reactive toxicity), be cost-effective in terms of equipment and consumables, and include in vitro bioassays that perform well and can be implemented without high-tech laboratory requirements or specialist knowledge (Van der Oost et al. 2017a).

EMPIRICAL EVALUATION OF A CANDIDATE BIOASSAY

A particular bioassay can be selected as a candidate bioassay for EBM on the basis of its coverage of a relevant toxicity mechanism and appropriate performance and ease of use based on available information in literature and from providers. Another selection reason could be to replace another assay that measures comparable endpoints. Such a candidate bioassay should be empirically evaluated for the specific application. Aspects to assess for such evaluations include sensitivity and reproducibility for effects of realistic environmental mixtures of chemicals in low concentrations. It needs to be established whether the candidate bioassay is compatible to test the effects of realistic low-level mixtures in (concentrated) water extracts without interference of matrix effects, and if the sensitivity of the bioassay is sufficient to detect possible effects of chemicals at concentrations present in drinking water and its sources. For example, a recent study by Leusch et al. (2017) has demonstrated that the sensitivity of in vitro bioassays for endocrine activity ranges widely. Available bioassays have sufficient sensitivity for androgenic and estrogenic activity in drinking water. The sensitivity of assays for progestagenic and glucocorticoid activity is lower but still suitable to detect activity in surface and waste water. Sensitivity is even less for assays to detect thyroid activity, and there is no standardization in the methods used to test antagonistic activity.

Concentration-dependent effects of relevant chemicals at relevant exposure concentrations can be used to confirm a causal relationship between the exposure and an effect (Fedak et al. 2015). It is critical that complete dose responses are pursued in these evaluations to allow concentration-response curve fitting from which the minimum concentration

 DOI: 10.1002/ieam.4096

of a chemical that can be detected in the assays can be interpolated. The comparison of effective concentrations of target chemicals in a particular assay to those in other bioassays for the same or other endpoints may also be used to prioritize bioassays for drinking water quality. Varying deviations between freely dissolved (bioavailable) concentration and nominal concentration depending on in vitro bioassay format (e.g., medium constituents) may explain differences in sensitivity between in vitro bioassays (Fischer et al. 2017), and this aspect should be included in the evaluation of in vitro bioassays. It may be possible to establish a standard protocol to determine the sensitivity of individual in vitro bioassays to detect effects of (relevant) regulated chemicals at their respective guideline values. Including a list of reference chemicals (e.g., based on the list included in the report by Busch et al. [2016]) and their expected mode of action can aid in the comparison between in vitro bioassays.

The evaluation of an in vitro bioassay should also include an assessment of the intra- and interday variability in in vitro bioassay results. Such an analysis, using environmentally relevant mixtures of estrogenic compounds, was performed for a set of in vitro bioassays for estrogenicity, demonstrating differences in precision and repeatability (Kunz et al. 2017). As soon as the usefulness of an in vitro bioassay for EBM is established on the criteria described above (in an order that suits the urgency of information needed to draw such a conclusion), harmonized standard procedures can further improve reproducibility. For several bioassays it has already been demonstrated that comparable results can be achieved in interlaboratory studies (Escher et al. 2014; Mehinto et al. 2015; Di Paolo et al. 2016).

EFFECT-BASED TRIGGER VALUES (EBTs)

Because of the high sensitivity of in vitro bioassays, responses can be expected (far) below exposure concentrations that are relevant for potential effects on human health. When responses of water samples with unknown composition are observed above the level of quantification (e.g., a significant difference from the negative control), EBTs are needed to establish whether the response observed in a bioassay may be linked to a potential adverse health outcome. It is critical that EBTs are sufficiently conservative to serve as indicators of potential health effects but are not overly conservative to prevent unnecessary studies that are conducted to investigate further if preventive or remediating actions are needed. A number of different strategies to derive EBTs are proposed, either based on human health-related threshold and guideline values or on ecotoxicity data. Effect-based trigger values can differ between bioassays for the same mechanism of action owing to differences in sensitivity and bioassay-specific relative potencies.

In the approach by Brand et al. (2013), EBTs are based on health-based threshold values for chronic exposure in humans but are corrected for bioavailability based on absorption from the gastrointestinal tract and protein binding in blood by using realistic worst-case kinetic factors (derived from literature or estimated with in silico tools) and exposure assumptions. Effect-based trigger values were derived for a number of chemical-activated luciferase gene expression (CALUX) bioassays for endocrine activity expressed as equivalents of a reference compound. The acceptable daily intake (ADI) of the reference chemical of the CALUX assay of interest was corrected for bioavailability by multiplying with the estimated oral bioavailable fraction, which passes the intestinal transport barriers and escapes first-pass metabolism by the intestine and liver. A correction also was applied for the free internal concentration by multiplying with the fraction unbound to plasma proteins. The resulting internal ADI of the reference chemical was used to calculate the safe external (oral) equivalent exposure to water-relevant chemicals with the same endocrine activity, by dividing by the highest observed values for oral bioavailable fraction and fraction unbound to plasma proteins in the relevant set of chemicals. In cases where no ADI was available for the reference chemical, the ADI of another relevant chemical was used and corrected based on the respective relative potency in the assay. The safe external equivalent exposure was used to calculate the EBT in equivalents of the reference chemical, by multiplying with average body weight, dividing by average water consumption, and taking the default allocation factor for drinking water into account.

Escher et al. (2015) proposed a read-across approach in EBTs that is based on existing water quality guidelines. Effect concentrations in bioassays are matched to existing chemical guideline values and the relevant reference chemicals. Effect concentrations in bioassays and guidance values were collected for regulated chemicals. Relevant chemicals were selected on the basis of their effect concentrations within an order of magnitude of the guideline values. Relative effect potencies (REPs) in the bioassay were used to convert guideline values to bioanalytical equivalents (BEQs), and these were included in cumulative distributions per bioassay. The fifth percentile in the distribution was selected as the EBT BEQ for that assay. By directly relating regulatory guideline values to EBTs based on effective concentrations of the regulated chemicals (not necessarily with causal physiological relationships), in vitro bioassays are used as an analytical tool to detect combined effects of complex low-level mixtures in water.

Effect-based trigger values have also been derived for ecological risks. Although these may seem less relevant for drinking water, ecological risks in sources for drinking water production may be considered as an early warning system. This is in line with the OneHealth paradigm, an interdisciplinary effort to attain optimal health for people, animals, and the environment. With this paradigm, bioassay data could be used both to protect human and environmental health, and data by water managers and drinking water utilities can be shared. Moreover, certain approaches to derive ecological EBT may be adapted to derive EBTs for potential human health risks. Based on the assumption that responses in in vitro bioassays for estrogenicity are caused by a limited number of active chemicals, assay-specific safe concentrations of estrogen equivalents have been derived directly with

the environmental quality standards of these chemicals (Jarošová et al. 2014; Kunz et al. 2015). This method cannot be applied on bioassays with "promiscuous" endpoints, i.e., with many different chemicals causing a response. Escher et al. (2018) proposed an approach to derive EBTs from European environmental quality standards (EQS) for a large number of bioassays, by including additional mixture considerations for the "promiscuous" endpoints. The derived tentative EBTs were compared with observed environmental effects. It is assumed that these EBTs are also protective for human health because EU EQS are generally much lower than drinking water guidelines.

Environmental EBTs were derived by van der Oost et al. (2017a) for a number of in vitro bioassays that are included in the Smart Integrated Monitoring (SIMONI) strategy, which is part of the conceptual framework of the Ecological Key Factors for the ecological assessment of water quality issues. For unknown mixtures in water samples, the BEQ can be experimentally determined by investigating which amount of a reference compound causes the same effect in this bioassay. To this aim, a concentration-response curve of the reference chemical should always be measured in parallel to the uncharacterized samples. To interpret whether the BEQ of such an unknown mixture indicates an environmental health hazard, EBT BEQs were derived for each in vitro bioassay on the responses (and relative potencies) of a reference chemical and other relevant chemicals. The relative potencies of these chemicals in the bioassay of interest were used to calculate toxic equivalents for observed in vivo ecotoxic effects found in literature. A BEQ value representing an exposure situation that will negatively affect at most 5% of the species in an ecosystem (HC5 BEQ) was derived from species-sensitivity distributions performed with toxicity data for a specific group of chemicals converted to BEQ values. The derived EBTs were also benchmarked to bioassay responses measured for water from ecologically clean sites in the Netherlands, to ensure that a realistic EBT BEQ above this background BEQ was derived. A variant of this approach, based on toxicity data, possibly with the inclusion of biokinetic parameters, could also be developed in the near future to estimate effects on human health.

Different parameters are used for the calculation of EBTs in the different approaches, and toxicokinetics are not taken into account in every approach for human EBT. Therefore, it can be expected that the different approaches derive (slightly) different EBTs. Even though toxic equivalency approaches are based on the theory that chemicals act via a single well-defined mechanism such as receptor activation, agonistic and antagonistic mechanisms can occur simultaneously (Leusch et al. 2017). Effects may therefore deviate from those expected from concentration addition, and this may in particular be the case in more complex integrated (heterogeneous) in vitro bioassays. It is therefore critical that EBTs be calibrated with realistic water samples, preferably of known composition, to ensure that the EBTs are exceeded only at polluted sites.

INTERNATIONAL REGULATIONS AND IMPLEMENTATION IN THE NETHERLANDS

In vivo (aquatic) bioassays with intact organisms are used as standard tools for characterizing water quality and are well accepted by surface water quality regulators in many regions of the world, including the EU (Power and Boumphrey 2004). The regulatory framework of drinking water quality concerns the quality of water intended for human consumption. In water quality regulations, only chemical quality standards are set. Examples of regulations are the Drinking Water Directive in the European Union, The Safe Drinking Water Act in the United States, the Drinking Water Protection Act in Canada, and the Australian Drinking Water Guidelines. Currently, these drinking water frameworks do not include the use of in vitro bioassays for water quality assessment. However, bioassays are specifically mentioned as a promising approach in the Australian Guidelines for Water Recycling (National Water Quality Management Strategy 2008) and World Health Organization (WHO) guidelines for potable reuse (WHO 2017).

In the recent revision of the EU Drinking Water Directive (European Commission 2018) amendments are included that allow risk-based monitoring approaches, provided that they ensure full protection of public health. Member states are expected to transpose these amendments in national legislation. It is expected that effect-based tools might also be included in the imminent revision of the EU Water Framework Directive (Brack et al. 2017) and Guidelines on Integrating Water Reuse into Water Planning and Management in the context of the Water Framework Directive (WFD), which is currently an informal consensus position between EU member states and relevant stakeholders (EU Water directors 2016). A practical framework to apply bioanalytical tools for routine and recycled water quality monitoring has been proposed (Leusch and Snyder 2015). The value of effect-based tools, from examples of their use as diagnostic research tools for hazard identification and testing of treatment efficiency of (novel) drinking water treatment methodologies, has also been recognized by the Directorate-General (DG) Environment of the European Commission, and effect-based tools and methods are currently being evaluated in the Common Implementation Strategy Work Programme. To optimize the chances of inclusion of effect-based tools in water quality regulations, collaboration between researchers and policy makers is needed to obtain (1) more knowledge on the correlation between results from chemical analyses and in vitro bioassays (distinguishing different types of bioassays that either respond to a number of specific, highly active chemicals, or to many less active chemicals) (Escher et al. 2018), (2) evaluation and harmonization of candidate in vitro bioassays to be used as bioanalytical tool, and (3) consensus on the derivation and application of EBTs to interpret results that are obtained in in vitro effect-based tools.

Even though there are no formal regulations in place yet, bioassays are already being used for water quality monitoring in the Netherlands at Dutch drinking water utilities and water

 DOI: 10.1002/ieam.4096

laboratories, in particular those that produce drinking water from infiltrated surface water. A panel of in vitro bioassays is included in the SIMONI strategy for water quality assessment that is regularly applied by many Dutch water boards (Van der Oost et al. 2017a, 2017b). The selection of in vitro bioassays in the SIMONI panel was based on Waternet research and earlier studies in which in vitro bioassays were evaluated for their use in water quality monitoring (Willemsen et al. 1995; van der Linden et al. 2008; Macova et al. 2011; Escher et al. 2014). It has recently also been proposed to use in vitro bioassays for specific toxic mechanisms (activation of the aryl hydrocarbon receptor and androgen receptor antagonism) to monitor the presence of all dioxin-like compounds in drinking water by their total activity instead of measuring concentration of a limited number of such chemicals (Houtman et al. 2017).

Dutch Association of River Waterworks RIWA has already replaced the analysis of estrogenic compounds in surface water with the ER-CALUX bioassay (van der Hoek et al. 2015), and other CALUX assays are being considered to monitor the water quality of the Rhine and Meuse. It was recently explored whether in vitro bioassays included in the US Environmental Protection Agency (USEPA) ToxCast database could be used to detect chemicals for which group standards (maximum summed concentrations) are included in the European and/or Dutch Drinking Water Directives. For some of the groups of chemicals, suitable ToxCast assays could be identified although sensitivity and specificity should be improved to be able to detect guideline value concentrations in water (Louisse et al. 2018). In conclusion, the value of in vitro bioassays is generally acknowledged in the Dutch water sector, but the actual use is currently limited, mainly because there is a common knowledge gap with regard to the practice and interpretation of in vitro bioassay data. To successfully implement bioassays for water quality monitoring, water laboratories need to add biological test systems, including appropriate concentration methods, to their repertoire. It may be challenging to select the appropriate set of bioassays, taking into account differences in water sources and applied water treatment technologies, and EBTs to interpret potential risks, but there is ample knowledge in this field that is ready to be applied. Several current Dutch research projects (e.g., projects EMERCHE and RoutinEDA, Zwart et al. [2018]) support the implementation of bioanalytical tools for water quality monitoring.

DISCUSSION AND FUTURE PERSPECTIVES

In vitro bioassays are valuable tools that can be used in water quality monitoring alongside chemical analysis. The choice for a particular in vitro bioassay as a bioanalytical tool for effect-based water quality monitoring is based on (1) relevant potential health effects after exposure to chemicals via water and (2) the performance and applicability of the method. As there is a wide range of possible endpoints, models, and technologies, it is critical to appropriately evaluate candidate bioassays for applicability and sensitivity before implementation and to establish EBTs to determine the relevance of observed effects of water samples of unknown composition. The implementation of in vitro assays can thus be further supported by the development of protocols and workflows for the evaluation, use, and implementation of in vitro bioassays for water quality monitoring and guidance for the interpretation of data (i.e., EBTs). A number of other challenges remain, and these are discussed below.

Improved efficiency of effect-based tools

The increased support to use in vitro cellular models in bioassays for human health hazard and risk characterization chemicals in food and the environment has resulted in a rapid development of new in vitro bioassays in academic research and biotechnology businesses. For the implementation of an in vitro bioassay as a bioanalytical tool in water quality monitoring, the throughput of a bioassay should be sufficient to process water samples collected in routine monitoring sample campaigns. Not all in vitro bioassays are compatible with high-throughput methods, which was a parameter included in the DEMEAU scoring matrix. However, automation of an in vitro bioassay to achieve a high-throughput mode for cost-effective and rapid measurements may be promising, and it is therefore recommended to explore possibilities to optimize the efficiency of in vitro bioassays by innovative methods such as miniaturization and automation.

Optimization of sample preparation

When chemical and effect-based analyses are used in a complementary fashion to achieve the best possible insight in health risks associated with the presence of micropollutants in drinking water and its sources, it is recommended to apply both techniques in the same (type of) water concentrate. To detect effects of environmental water samples in in vitro bioassays, specific sample preparation techniques for preconcentration and sample clean-up are required that extract chemicals within a wide range of physicochemical properties, enrich concentrates to a sufficient extent, and use solvents that do not interfere with biological responses in effect-based analyses. These aspects of sample preparation are less critical in current analytical methods. Nevertheless, for efficiency and to allow a quantitative comparison between the collected data, a water sample concentration method that can be used for both chemical-analytical analysis and effect-based testing in in vitro bioassays is preferred. The volume of a concentrate should suffice to be tested in a battery of bioanalytical tools, which may call for large-volume solid-phase (LVSPE) extraction. It was demonstrated that using large-volume solid-phase extraction of organic chemicals with different physicochemical properties results in less than 30% loss of effect in bioassays applied for water monitoring, and the authors emphasize the need for appropriate extraction procedure controls (Neale et al. 2018). It has been demonstrated that an extraction method developed for bioassays for genotoxicity and endocrine receptor activation could also be used for chemical analyses (Kolkman et al. 2013). Efforts are on-going to optimize this method by using different SPE sorbents. The impacts of different SPE approaches on the presence of different (types

of) chemicals in the tested concentrate needs to be more firmly established, as it is known that different types of chemicals behave differently in SPE extraction (Osorio et al. 2018).

In vitro data for human health risk assessment

A more mechanistic manner of interpreting in vitro bioassay data can be based on a better understanding (also quantitatively) of the physiological link between cellular and molecular effects in vitro and potentially associated downstream adverse outcomes in the intact organism. This knowledge may be used to determine whether an effect in an in vitro bioassay can be considered acceptable. This is in particular relevant for adaptive stress responses that are the cellular reactions that occur after exposure to various stressors. These responses are detected at lower concentrations than adverse effects and can be reversible (Zhang et al. 2015). An example of such a stressor is oxidative stress. A wide range of chemicals can cause cellular oxidative stress by causing an imbalance between the amount of reactive oxygen species and the protective antioxidant reaction. To prevent reactive damage on proteins, lipids, and DNA, oxidative stress response pathways (regulated by nuclear factor proteins Nrf2 and keap1) are activated; this activation results in the production of proteins with antioxidant and detoxifying capacity. The oxidative stress response has been demonstrated to be a sensitive indicator of environmental exposure to a wide range of chemicals, as well as transformation products and disinfection-by-products (Escher et al. 2013). The quantitative relationship between adaptive stress responses and adverse effects on human health (either reversible or irreversible) is not yet clear. In particular for these types of effect, it is interesting in which effect range the intact organism (humans) may adapt with protective mechanisms, and at what effect size (unacceptable) irreversible adverse effects can occur. Additionally, ADME (absorption, distribution, metabolism, elimination) processes are critical factors in the potential for health effects of chemicals, and the inclusion of an assessment of the effect of ADME is well established in the pharmaceutical industry and can be used to improve the predictivity of in vitro bioassays.

Uncertainties will remain

Effect-based trigger values are used to interpret responses induced by water samples of unknown chemical composition and to prioritize samples for further chemical analysis and health risk assessment. In this manner, EBTs unlock bioassay data for water quality regulation, risk management, and the organization of abatement processes in drinking water production. Because not every bioassay responds to every possible chemical, a certain degree of uncertainty remains in the interpretation of in vitro bioassay data. Part of this uncertainty may be reduced by using toxicity information for individual chemicals to form hypotheses on which chemicals could be responsible for observed bioassay responses ("chemical fingerprinting"). For this aim, toxicity databases such as the USEPA ToxCast database can be used (Richard et al. 2016). However, countless chemicals are present in water, and for many of these chemicals toxicity data may not (yet) be available. However, it should be noted that uncertainties also exist in a framework using analytical chemistry only. These include the effect of metabolites and a plethora of unknown substances that are not included in the analyses. In addition, for all methods there will be loss of chemicals during sample preparation. Also, in classical chemical-per-chemical risk assessment, certain assumptions cannot be avoided. For example, the drinking water guideline values are based on experimental data extrapolated from other organisms. In addition, guideline values of chemicals in water (which are monitored with analytical chemistry) are designed to prevent adverse health effects, but generally do not take into account potential effects of combined exposure. Although the use of in vitro bioassays is therefore a valuable addition to the available tools for monitoring water quality (as these detect the combined effect of the chemicals in a complex environmental mixture), a number of uncertainties remain when studying effects of unknown substances in in vitro bioassays. In most cases it is not known which compounds of a complex mixture of micropollutants are responsible for the observed response in an in vitro bioassay. Depending on the in vitro bioassay model and the mechanisms of action of the constituents of the mixture, the complete toxic effect of a mixture can also be underestimated (if different mechanisms are summarized in an organ response) or overestimated (if adaptive mechanisms are in place). This argues for the use of a wide range of in vitro bioassays (with regard to mechanisms) and the pursuit of the most efficient combination of analytical chemistry and in vitro bioassays.

CONCLUSION

Bioanalytical tools are powerful instruments to gain insight in the water quality of different parts of the water cycle. With tailor-made sets of in vitro bioassays, potential effects of low-level chemical mixtures in water on either human health or ecological status can be assessed. This approach is increasingly applied by drinking water producers, water regulators, and environmental scientist worldwide. Possibilities for EBM are also increasing in national and international water quality regulations. In addition to applications related to quality control of drinking water sources and production, and management of surface waters including effluents, it is expected that bioanalytical tools can also be successfully applied in quality control processes related to water reuse. Challenges include the most efficient implementation of bioanalytical tools for each specific situation. This concerns in particular the selection of bioassays based on consistent criteria (Schriks et al. 2015), implementation of bioanalytical tools in combination with analytical chemistry (for example for effect-directed analysis), and the interpretation of measured responses in in vitro bioassays by using harmonized EBTs.

Acknowledgment—This work was conducted within the framework of the Joint Research Program of the Dutch water

 DOI: 10.1002/ieam.4096

companies (BTO; project 400554-180). We acknowledge the EU commission for funding under the European Union's Seventh Framework Programme, the DEMEAU project grant agreement no. 308339, and the SOLUTIONS project under contract number 603437. The authors kindly acknowledge the critical comments of Tineke van der Velden-Slootweg and Varvara Kokkali on the work in this project.

Disclaimer—The authors declare no conflicts of interest.

Data Accessibility—All used information can be obtained by contacting the first author Milou Dingemans (milou.dingemans@kwrwater.nl) or the corresponding author Annemarie van Wezel (annemarie.van.wezel@kwrwater.nl).

REFERENCES

Allen TE, Goodman JM, Gutsell S, Russell PJ. 2014. Defining molecular initiating events in the adverse outcome pathway framework for risk assessment. *Chem Res Toxicol* 27(12):2100–12.

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, et al. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29(3):730–41.

Baken KA, Sjerps RMA, Schriks M, van Wezel AP. 2018. Toxicological risk assessment and prioritization of drinking water relevant contaminants of emerging concern. *Environ Int* 118:293–303.

Bell SM, Chang X, Wambaugh JF, Allen DG, Bartels M, Brouwer KLR, Casey WM, Choksi N, Ferguson SS, Fraczkiewicz G, et al. 2018. In vitro to in vivo extrapolation for high throughput prioritization and decision making. *Toxicol In Vitro* 47:213–227.

Brack W, Altenburger R, Schüürmann G, Krauss M, López Herráez D, van Gils J, Slobodnik J, Munthe J, Gawlik BM, van Wezel A, et al. 2015. The SOLUTIONS project: Challenges and responses for present and future emerging pollutants in land and water resources management. *Sci Total Environ* 503–504:22–31.

Brack W, Dulio V, Ågerstrand M, Allan I, Altenburger R, Brinkmann M, Bunke D, Burgess RM, Cousins I, Escher BI, et al. 2017. Towards the review of the European Union Water Framework Directive: Recommendations for more efficient assessment and management of chemical contamination in European surface water resources. *Sci Total Environ* 576:720–737.

Brand W, de Jongh CM, van der Linden SC, Mennes W, Puijker LM, van Leeuwen CJ, van Wezel AP, Schriks M, Heringa MB. 2013. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. *Environ Int* 55:109–118.

Busch W, Schmidt S, Kühne R, Schulze T, Krauss M, Altenburger R. 2016. Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring. *Environ Toxicol Chem* 5(8):1887–1899.

Chapman HF, Leusch FDL, Prochazka E, Cumming J, Ross V, Humpage A, Froscio S, Laingam S, Khan SJ, Trinh T, McDonald JA. 2011. A national approach to health risk assessment, risk communication and management of chemical hazards from recycled water. Waterlines report 48. Canberra (AU): National Water Commission. 255 p.

Conley JM, Evans N, Mash H, Rosenblum L, Schenck K, Glassmeyer S, Furlong ET, Kolpin DW, Wilson VS. 2017. Comparison of in vitro estrogenic activity and estrogen concentrations in source and treated waters from 25 U.S. drinking water treatment plants. *Sci Total Environ* 579:1610–1617.

Di Paolo C, Ottermanns R, Keiter S, Ait-Aissa S, Bluhm K, Brack W, Breitholtz M, Buchinger S, Carere M, Chalon C, et al. 2016. Bioassay battery interlaboratory investigation of emerging contaminants in spiked water extracts—Towards the implementation of bioanalytical monitoring tools in water quality assessment and monitoring. *Water Res* 104:473–484.

[EC] European Commission. 2018. Review of the directive. Brussels (BE): European Commission. [cited 2018 August 2]. http://ec.europa.eu/environment/water/water-drink/pdf/revised_drinking_water_directive.pdf

Escher B, Leusch F. 2011. Bioanalytical tools in water quality assessment. London (UK): IWA Publishing. 272 p.

Escher BI, Aït-Aïssa S, Behnisch PA, Brack W, Brion F, Brouwer A, Buchinger S, Crawford SE, Du Pasquier D, Hamers T, et al. 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci Total Environ* 628–629:748–765.

Escher BI, Allinson M, Altenburger R, Bain PA, Balaguer P, Busch W, Crago J, Denslow ND, Dopp E, Hilscherova K, et al. 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ Sci Technol* 48(3):1940–56.

Escher BI, Neale PA, Leusch FD. 2015. Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. *Water Res* 81:137–48.

Escher BI, van Daele C, Dutt M, Tang JY, Altenburger R. 2013. Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. *Environ Sci Technol* 47(13):7002–11.

EU Water Directors. 2016. Guidelines on integrating water reuse into water planning and management in the context of the WFD. Brussels (BE): European Commission. [cited 2018 August 2]. http://ec.europa.eu/environment/water/pdf/Guidelines_on_water_reuse.pdf

Fedak KM, Bernal A, Capshaw ZA, Gross S. 2015. Applying the Bradford Hill criteria in the 21st century: How data integration has changed causal inference in molecular epidemiology. *Emerg Themes Epidemiol* 12:14.

Fischer FC, Henneberger L, König M, Bittermann K, Linden L, Goss KU, Escher BI. 2017. Modeling exposure in the Tox21 in vitro bioassays. *Chem Res Toxicol* 30(5):1197–1208.

GBD 2016 DALYs and HALE Collaborators. 2017. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 390(10100):1260–1344.

Hamers T, Legler J, Blaha L, Hylland K, Marigomez I, Schipper CA, Segner H, Vethaak AD, Witters H, et al. 2013. Expert opinion on toxicity profiling—report from a NORMAN expert group meeting. *Integr Environ Assess Manag* 9(2):185–191.

Health Council of the Netherlands. 2014. Risks of prenatal exposure to substances. The Hague (NL): Health Council of the Netherlands. Publication no. 2014/05.

Hollender J, Schymanski EL, Singer HP, Ferguson PL. 2017. Nontarget screening with high resolution mass spectrometry in the environment: Ready to go? *Environ Sci Technol* 51(20):11505–11512.

Houtman C, Kroesbergen J, Behnisch P, Brouwer B, Felzel E. 2017. Bioassays als alternatief voor chemische monitoring van PCB's en PAK's? The Hague (NL): Koninklijk Nederlands Waternetwerk. [cited 2018 August 2]. https://www.h2owaternetwerk.nl/vakartikelen/1461-bioassays-als-alternatief-voor-chemische-monitoring-van-pcb-s-en-pak-s

Jarošová B, Bláha L, Giesy JP, Hilscherová K. 2014. What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? *Environ Int* 64:98–109.

Kienle C, Kase R, Werner I. 2011. Evaluation of bioassays and wastewater quality: In vitro and in vivo bioassays for the performance review in the Project "Strategy MicroPoll". Duebendorf (CH): Swiss Centre for Applied Ecotoxicology, Eawag-EPFL.

Kolkman A, Schriks M, Brand W, Bäuerlein PS, van der Kooi MM, van Doorn RH, Emke E, Reus AA, van der Linden SC, de Voogt P, Heringa MB. 2013. Sample preparation for combined chemical analysis and in vitro bioassay application in water quality assessment. *Environ Toxicol Pharmacol* 36(3):1291–1303.

Kunz PY, Kienle C, Carere M, Homazava N, Kase R. 2015. In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters. *J Pharm Biomed Anal* 106:107–115.

Kunz PY, Simon E, Creusot N, Jayasinghe BS, Kienle C, Maletz S, Schifferli A, Schönlau C, Aït-Aïssa S, Denslow ND, Hollert H, et al. 2017. Effect-based tools for monitoring estrogenic mixtures: Evaluation of five in vitro bioassays. *Water Res* 110:378–388.

Leusch FD, Neale PA, Hebert A, Scheurer M, Schriks MC. 2017. Analysis of the sensitivity of in vitro bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters. *Environ Int* 99:120–130.

Leusch FDL, Snyder SA. 2015. Bioanalytical tools: Half a century of application for potable reuse. *Environ Sci: Water Res Technol* 1:606–621.

Louisse J, Dingemans MML, Baken KA, van Wezel AP, Schriks M. 2018. Exploration of ToxCast/Tox21 bioassays as candidate bioanalytical tools for measuring groups of chemicals in water. *Chemosphere* 209:373–380.

Macova M, Escher BI, Reungoat J, Carswell S, Chue KL, Keller J, Mueller JF. 2010. Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Res* 44(2):477–492.

Macova M, Toze S, Hodgers L, Mueller JF, Bartkow M, Escher BI. 2011. Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. *Water Res* 45:4238–4247.

Maruya KA, Dodder NG, Mehinto AC, Denslow ND, Schlenk D, Snyder SA, Weisberg SB. 2016. A tiered, integrated biological and chemical monitoring framework for contaminants of emerging concern in aquatic ecosystems. *Integr Environ Assess Manag* 12(3):540–547.

Mehinto AC, Jia A, Snyder SA, Jayasinghe BS, Denslow ND, Crago J, Schlenk D, Menzie C, Westerheide SD, Leusch FD, Maruya KA. 2015. Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water Res* 83:303–309.

National Research Council. 2007. Toxicity testing in the 21st century: A vision and a strategy. Washington (DC): National Academies. [cited 2018 August 2]. https://doi.org/10.17226/11970

National Water Quality Management Strategy. 2008. Australian guidelines for water recycling: Augmentation of drinking water supplies. Canberra (AU): Environment Protection and Heritage Council, the National Health and Medical Research Council and the Natural Resource Management Ministerial Council. [cited 2018 August 2]. http://nepc.gov.au/system/files/resources/5fe5174a-bdec-a194-79ad-86586fd19601/files/wq-agwr-gl-adws-corrected-final-200809_1.pdf

Neale PA, Brack W, Aït-Aïssa S, Busch W, Hollender J, Krauss M, Maillot-Maréchal E, Munz NA, Schlichting R, Schulze T, et al. 2018. Solid-phase extraction as sample preparation of water samples for cell-based and other in vitro bioassays. *Environ Sci Process Impacts* 20:493–504.

Osorio V, Schriks M, Vughs D, de Voogt P, Kolkman A. 2018. A novel sample preparation procedure for effect-directed analysis of micro-contaminants of emerging concern in surface waters. *Talanta* 186:527–537.

Pablos MV, Fernández C, del Mar Babín M, María Navas J, Carbonell G, Martini F, García-Hortigüela P, Vicente Tarazona J. 2009. Use of a novel battery of bioassays for the biological characterisation of hazardous wastes. *Ecotoxicol Environ Saf* 72(5):1594–1600.

Power EA, Boumphrey RS. 2004. International trends in bioassay use for effluent management. *Ecotoxicology* 13(5):377–398.

Prasse C, Stalter D, Schulte-Oehlmann U, Oehlmann J, Ternes TA. 2015. Spoilt for choice: A critical review on the chemical and biological assessment of current wastewater treatment technologies. *Water Res* 87:237–270.

Richard AM, Judson RS, Houck KA, Grulke CM, Volarath P, Thillainadarajah I, Yang C, Rathman J, Martin MT, Wambaugh JF, et al. 2016. ToxCast chemical landscape: Paving the road to 21st century toxicology. *Chem Res Toxicol* 29(8):1225–1251.

Schriks M, Baken K, Simon E, Besselink H, van der Linden S, Kienle C, van der Burg B. 2015. Selection criteria to select in vitro bioassays for implementation and use. DEMEAU (FP7) report. Berlin (DE): DEMEAU consortium. [cited 2018 February 5]. http://demeau-fp7.eu/content/d411-selection-criteria-select-vitro-bioassays-implementation-and-use

Schriks M, van Leerdam JA, van der Linden SC, van der Burg B, van Wezel AP, de Voogt P. 2010. High-resolution mass spectrometric identification and quantification of glucocorticoid compounds in various wastewaters in the Netherlands. *Environ Sci Technol* 44(12):4766–4774.

Shi P, Zhou S, Xiao H, Qiu J, Li A, Zhou Q, Pan Y, Hollert H. 2018. Toxicological and chemical insights into representative source and drinking water in eastern China. *Environ Pollut* 233:35–44.

Silva E, Rajapakse N, Kortenkamp A. 2002. Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environ Sci Technol* 36(8):1751–1756.

van der Hoek C, Bannink A, Slootweg T. 2015. An update of the lists with compounds that are relevant for the drinking water production from the river Meuse. Nieuwegein (NL): RIWA. [cited 2018 August 2]. www.riwa-maas.org

Van der Linden S, Heringa M, Man H-Y, Sonneveld E, Puijker LM, Brouwer A, Van der Burg B. 2008. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environ Sci Technol* 42:5814–5820.

Van der Oost R, Sileno G, Janse T, Nguyen MT, Besselink H, Brouwer A. 2017b. SIMONI (Smart Integrated Monitoring) as a novel bioanalytical strategy for water quality assessment: Part II-field feasibility survey. *Environ Toxicol Chem* 36(9):2400–2416.

Van der Oost R, Sileno G, Suárez-Muñoz M, Nguyen MT, Besselink H, Brouwer B. 2017a. SIMONI (smart integrated monitoring) as a novel bioanalytical strategy for water quality assessment: Part I-model design and effect-based trigger values. *Environ Toxicol Chem* 36(9):2385–2399.

Van Zijl MC, Aneck-Hahn NH, Swart P, Hayward S, Genthe B, De Jager C. 2017. Estrogenic activity, chemical levels and health risk assessment of municipal distribution point water from Pretoria and Cape Town, South Africa. *Chemosphere* 186:305–313.

Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, Ottinger MA, et al. 2014. Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142(2):312–320.

Vinken M. 2013. The adverse outcome pathway concept: A pragmatic tool in toxicology. *Toxicology* 312:158–165.

Vughs D, Baken KA, Kolkman A, Martijn AJ, de Voogt P. 2018. Application of effect-directed analysis to identify mutagenic nitrogenous disinfection by-products of advanced oxidation drinking water treatment. *Environ Sci Pollut Res Int* 25(5):3951–3964.

Wernersson A-S, Carere M., Maggi C, Tusil P, Soldan P, James A, Sanchez W, Dulio V, Broeg K, Reifferscheid G, et al. 2015. The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environ Sci Eur* 27:1–11.

Wetmore BA, Wambaugh JF, Allen B, Ferguson SS, Sochaski MA, Setzer RW, Houck KA, Strope CL, Cantwell K, Judson RS, et al. 2015. Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing. *Toxicol Sci* 148(1):121–136.

[WHO] World Health Organization. 2017. Potable reuse: Guidance for producing safe drinking-water. Geneva (CH): World Health Organization. 152 p.

Willemsen A, Vaal MA, De Zwart D. 1995. Microbiotests as tools for environmental monitoring. RIVM report 607042005. Bilthoven (NL): RIVM. [cited 2018 August 2]. https://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/1995/januari/Microbiotests_as_tools_for_environmental_monitoring

Zegura B, Heath E, Cernosa A, Filipic M. 2009. Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere* 75(11):1453–1460.

Zhang G, Truong L, Tanguay RL, Reif DM. 2017. A new statistical approach to characterize chemical-elicited behavioral effects in high-throughput studies using zebrafish. *PLoS One* 12:e0169408.

Zhang Q, Bhattacharya S, Pi J, Clewell RA, Carmichael PL, Andersen ME. 2015. Adaptive posttranslational control in cellular stress response pathways and its relationship to toxicity testing and safety assessment. *Toxicol Sci* 147(2):302–316.

Zwart N, Lamoree MH, Houtman CJ, de Boer J, Kool J, Hamers T. 2018. Development of a luminescent mutagenicity test for high-throughput screening of aquatic samples. *Toxicol In Vitro* 46:350–360.

