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ADVANCED REVIEW

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Effect-based methods in cultured cells—Valuable tools for detection of chemical hazards in drinking water

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Abstract

Chemical contamination of drinking water is of great concern for public health. Chemical analyses are used for monitoring of selected chemicals, however, no information on unknown chemicals or potential toxicity of the mixture of chemicals in a water sample is obtained. Effect-based methods in cells are new high throughput tools, to evaluate the hazard of the whole mixture of chemicals present in drinking water. These methods can be used together with chemical analysis for assessment of the chemical safety of drinking water. This article will review the principle of effect-based methods in cells and how they compare to traditionally used chemical analysis and effect-based methods in whole organisms. Further, this article will give examples from the literature, highlighting how cellular effect-based methods can be used in different practical applications to improve drinking water safety; for example, for monitoring of drinking water quality and evaluation of treatment efficiency in drinking water processing. Finally, this article will review the current regulatory and water sector acceptance of these methods and discuss future expectations on the role of effect-based methods for improved drinking water safety.

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KEYWORDS

chemical hazards, drinking water safety, effect-based methods

1 | INTRODUCTION

Tens of thousands of chemical substances are present in the aquatic environment, both naturally occurring compounds and anthropogenic pollutants. Some of these compounds are hazardous and can pose a risk to human health if they remain in the water after drinking water treatment. Further, hazardous disinfection by-products (DBPs) can be formed during drinking water production, following treatment processes such as ozonation or chlorination (Srivastav et al., 2020) and there is also a risk of contamination from the materials that drinking water come into contact with. Humans consume approximately 2–2.5 L of drinking water per day (EFSA Panel on Dietetic Products, Nutrition, and

Allergies (NDA), 2010). The large consumption, the life-long exposure, and the fact that we often consume drinking water from the same water source for extended periods of our lives (years to decades) indicate that even low levels of chemical contaminants can pose a threat to human health and highlight the importance of drinking water as a potential exposure source for hazardous chemicals. It is also worth mentioning that especially vulnerable parts of the population (i.e., infants and children) are in the risk zone of higher relative exposure to hazardous contaminants in drinking water, as their drinking water intake per body weight is higher as compared to adults (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010).

Drinking water is produced from different water sources, including surface water, groundwater, and artificially recharged groundwater. For example, in Sweden, approximately 50% of the drinking water is produced from surface water while 100% of the drinking water in Denmark is produced from groundwater. Climate change, with extended periods of droughts and more frequent extreme weather events, is expected to put additional pressure on surface water sources and increase the risks of chemical contamination. In most countries, the current drinking water regulation and monitoring efforts are based on parametric values for a very limited number of organic chemicals. The selection of these few chemicals seems to be rather arbitrary and represents examples of carcinogenic substances and/or reflects more so which compounds have been the focus of the debate. In the European Union, the quality of water intended for human consumption is also stating that the drinking water should be "(...) free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, in certain cases, constitute a potential danger to human health (...)" (European Union, 2020). Drinking water utilities regulators struggle with the interpretation of the statement that the drinking water should be free from any compounds that can constitute a potential danger to human health—how should this be monitored?

For the vast majority of all the organic micropollutants that can contaminate our drinking waters, there are no parametric values and there is also very limited knowledge on their occurrence in drinking water and their potential toxicity. Using effect-based testing, where unwanted biological effects of all chemicals in a drinking water sample is measured in biological systems, it has been shown that the well-known and often analyzed chemicals only explain a small fraction of the observed biological effects (Escher et al., 2013). The vast majority, in some cases up to 99%, of the adverse biological effects observed are instead caused by unknown chemicals and/or cocktail effects (Escher, Stapleton, & Schymanski, 2020). Focusing only on target analysis of known compounds can be regarded as looking only at the tip of an iceberg, while overlooking the potentially large risk from unknown chemicals and cocktail effects that are lurking under the surface. In the last decade, a new approach to monitor and detect hazardous chemicals in water has emerged as a result of scientific research, namely effect-based methods (Brack et al., 2019; Dingemans et al., 2019; Escher et al., 2021; Escher, Stapleton, & Schymanski, 2020; Neale et al., 2022). Effect-based methods for water quality monitoring measure the response in whole organisms (in vivo), such as fish, invertebrates, and algae, or in cultured cells (in vitro) targeting specific modes of action, such as endocrine disruption and genotoxicity. The great strength of these methods is that they integrate the effects of all hazardous chemicals in a sample, both known and unknown chemicals as well as potential mixture effects that can arise following simultaneous exposure to multiple compounds. In vitro, methods can detect mixture effects that can arise when multiple compounds are acting via the same molecular mechanism or at least on molecular targets present in the same cell type. For mixture effects involving different cell types or that require more complex biological signaling, in vivo methods would be needed to detect the effects.

The in vitro effect-based methods are based on cultured mammalian cells, yeast cells, or bacteria, that in many cases have been genetically modified to respond to the presence of hazardous chemicals, which have a common mechanism of action, in the water sample to be analyzed. These high-throughput in vitro effect-based methods have been suggested to have great potential to be implemented in the monitoring of chemical hazards in drinking water and thereby contribute to protecting the population from exposure to hazardous chemicals (Dingemans et al., 2019; Ferraro & Prasse, 2021). The aim of this article is to review the effect-based methods most commonly used in drinking water studies, to showcase how these can be used to improve drinking water safety, and to discuss the future role of effect-based methods in drinking water quality control.

2 | CELLULAR EFFECT-BASED METHODS

The common denominator for effect-based methods is that they measure a biological *effect* of an analyzed sample, instead of analyzing the concentrations of single chemicals. Effect-based methods can be utilized in whole organisms (in vivo) or in single cells (in vitro). This review is focusing on the use of effect-based methods in cultured cells.

Effect-based methods can vary from highly engineered assays to study enzyme activities in vitro, single cell organisms, cultured fish, and mammalian cells to more complex multicellular organisms including animals. Effect-based methods based on assays carried out in cultured mammalian cells have attracted much attention in relation to drinking water safety (Neale et al., 2022, 2023). Cells can be used either in an unmodified (naïve) form or after genetic modifications. The genetically modified cells applied in effect-based monitoring are using a technology named reporter gene assay, where the expression of an easily detected reporter protein is under the regulation of a specific biological process that is to be analyzed. For example, in a reporter gene assay for estrogen receptor (ER) activity, a DNA fragment that is sensitive to ligand-activated estrogen receptors is fused with the gene for an easily detected signaling protein. This DNA fragment is introduced into the cultured cells. The cells are then exposed to a water sample, and if the sample is containing any compounds that can activate the estrogen receptor, a complex of compound and receptor will bind to the sensitive DNA fragment and induce the production of the signaling protein. The amount of the signaling protein can then be measured (e.g., via fluorescence or luminescence) and is proportional to the total ER activity from the whole mixture of compounds present in the analyzed sample. The reporter gene assay technology is summarized in Figure 1a.

In some applications, nonmodified (naïve) cultured mammalian cells can be used. The biological effects are then measured as biochemical or morphological changes in or on the cells, instead of via the amount of signaling protein. Cytotoxicity is commonly measured in cells to detect disruption of basal cellular mechanisms and cell integrity, such as disturbed mitochondrial function or physical damage to cell structures, which can lead to cell death (Escher, Henneberger, et al., 2020; Judson et al., 2016). Another example is the first stage of genotoxicity testing, where it is recommended to use a combination of a bacterial test, the Ames test, for detection of gene mutations, and an in vitro micronucleus test, for detection of chromosomal aberrations (EFSA Scientific Committee, 2011; Kirkland et al., 2011). Ames test has been used to evaluate the presence of mutagens in drinking water sources (Guan et al., 2017). Chromosomal aberrations in the form of micronuclei formation, after the exposure to a water sample can be counted

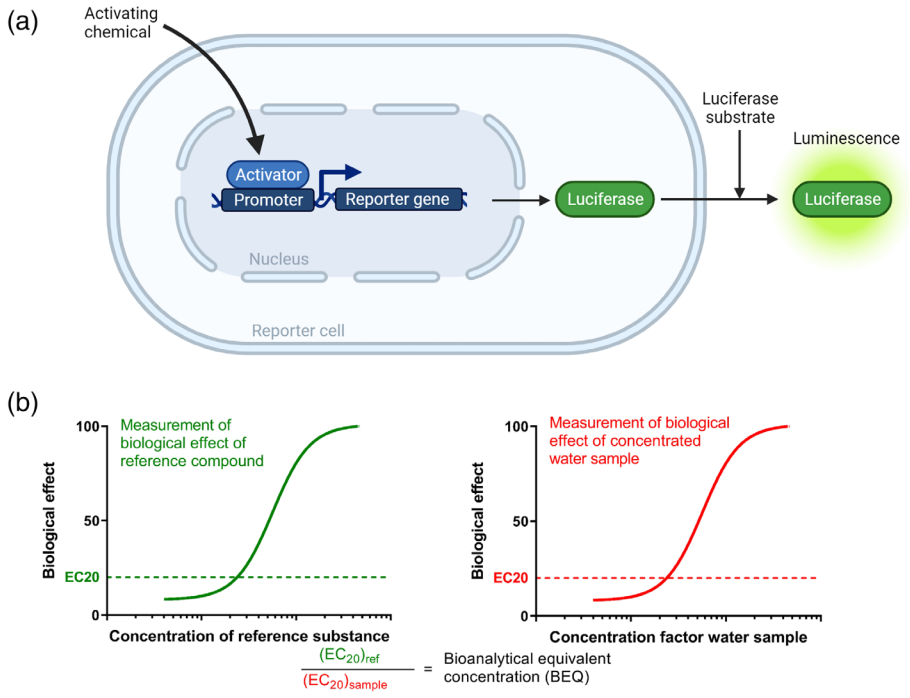

FIGURE 1 (a) Principle of the reporter gene assay technology. A reporter gene (in this case the gene encoding the enzyme luciferase) is under the transcriptional control of one or more regulatory promoter elements. Upon activation by an activating chemical, a receptor or transcription factor (referred to as "activator" in this figure) can bind to these regulatory elements and induce the expression of the reporter gene which will result in an increase of the reporter protein (luciferase in this case). The level of the reporter protein will thereby be proportional to the concentration of activating chemicals in the sample. The reporter protein level can then be measured with different detection systems (in this case by measurement of the enzyme activity of luciferase). (b) Dilution series of water samples and reference compound can be used to calculate effect concentrations (ECs) for both the sample and the reference compound. These EC values can then be used to express the activity in the water sample as bioanalytical equivalent concentrations (BEQs) in the unit of the reference compound. Figure created with BioRender.com.

microscopically or analyzed using flow cytometry (OECD, 2016). This micronucleus test can be performed both with cells cultured in the laboratory (i.e., in vitro) and with cells from an experimental animal that has been exposed to the sample being analyzed (i.e., in vivo) or in cells from humans (Fenech et al., 2020). These, and other assays for genotoxicity testing of drinking water, have been reviewed previously by Ceretti et al. (2016).

The biological mechanisms or targets that are selected for reporter gene assay development for drinking water safety applications are early molecular events (such as ligand-binding to a receptor or activation of a transcription factor) in toxicity pathways that have the potential to lead to adverse effects on the entire organism. The fact that these early molecular events are triggered in cultured cells by a certain chemical or mixture of chemicals does not necessarily mean that the same exposure would lead all the way to an adverse effect in the complex biological system that an entire animal or human constitutes. Kinetics, as it occurs in the entire organism, is not considered, that is, absorption, distribution, metabolism, and excretion of chemicals, or defense or repair mechanisms that would counteract the signals in this toxicity pathway before an adverse effect has appeared. Despite this, the effect-based methods, carried out in cultured cells, are ideal screening tools for the prediction of hazardous effects with a great potential for high-throughput applications which would allow rapid analysis of a large number of samples.

Ideally, the findings from effect-based methods can be quantified and expressed as bioanalytical equivalent concentrations (BEQs; Zhou et al., 2021). To calculate the BEQ for a water sample, the sample is analyzed in parallel with a dilution series of a compound that is a known potent inducer of the biological effect in question. Any effect observed in the water sample can then be quantified as a bioanalytical equivalent concentration of the known potent inducer, that is, the biological effect detected in the water sample is equal to the biological effect caused by a specific concentration of the known potent inducer. Calculation of BEQ values facilitates comparisons between samples and between studies. The principle for calculating BEQ values is presented in Figure 1b.

3 | COMMONLY USED CELL-BASED BIOASSAYS

Effect-based methods carried out in cultured cells are often referred to as in vitro bioassays. The most commonly applied bioassays for effect-based monitoring of chemical hazards in drinking water are related to endocrine disruptive effects (e.g., effects on the estrogen and androgen sex hormone systems, the thyroid, glucocorticoid, and progesterone receptors), oxidative stress, effects on the aryl hydrocarbon receptor (AhR) and the pregnane X receptor and genotoxicity (Neale et al., 2022).

The general workflow for analyzing water samples with effect-based methods starts with sample collection, sample preparation, and sample extraction (e.g., by solid phase extraction, SPE) in a manner that is common with the workflow used for many methods for chemical analysis. The cells are then exposed to the extracted water sample and the presence of hazardous chemicals is then measured via different detection methods (e.g., luminescence, fluorescence, flow cytometry, etc.), depending on the assay in question.

In a key study for the introduction of effect-based studies in water monitoring, Escher et al. (2014) tested 103 different in vitro bioassays, carried out in 20 laboratories, to evaluate 10 water samples, including wastewater treatment plant effluent, recycled water, stormwater, surface water, and drinking water. More than 60% of the bioassays were responsive to at least one of the tested water samples, while only 5% of the bioassays showed responses in the negative control (ultrapure water). The authors concluded that assays related to xenobiotic metabolism, hormone-mediated modes of action, genotoxic effects, and oxidative stress response were among the most responsive health-relevant endpoints. Below, we will review some of the most commonly applied endpoints in effect-based monitoring.

3.1 | Activation of AhR

The AhR has received particular attention because it is activated by many toxic substances, especially by 2,3,7,8-tetrachlorodibenzodioxin (TCDD), whereby metabolizing enzymes are induced (including cytochrome P450 enzymes). However, the AhR has many different physiological functions including in the development of various organ systems and in the regulation of immunity and inflammatory reactions (Bock, 2019). The AhR is activated by a large number of chemicals, such as halogenated organic environmental pollutants, polycyclic aromatic hydrocarbons (PAHs), certain pesticides and pharmaceuticals, and naturally occurring substances such as indoles, stilbenes and

metabolites of tryptophan (Bock, 2019). There are multiple assays available to assess AhR activity in vitro, including mammalian cells stably transfected with a reporter gene under the regulation of a regulatory element that is responsive to ligand-activated AhR (i.e., the cells will produce an easily measurable signal protein upon exposure to compounds that activate AhR) and the EROD assay, where the activity of the AhR induced expression of the cytochrome P450 enzyme CYP1A1 in cells is evaluated with a colorimetric assay (Escher et al., 2021). The principle of reporter gene assay is presented in Figure 1.

3.2 | Alterations of the ER and the androgen receptor activities

Various chemicals can activate or inhibit the estrogen and androgen receptors (AR). Estrogens and androgens have many important physiological functions not only for reproduction but also for the cardiovascular, immune, muscular, and nervous systems (Adeel et al., 2017; Davey & Grossmann, 2016). Examples of chemical contaminants in water that affect sex hormone receptors are natural sex hormones, contraceptives, pharmaceuticals used to treat breast and prostate cancer, as well as isoflavones (so-called phytoestrogens), and certain chemicals used in plastic products (Thacharodi et al., 2023). There are multiple assays available to assess effects on these sex hormone receptors, including mammalian cells stably transfected with a reporter gene under the regulation of a regulatory element that is responsive to ligand-activated ER or AR, respectively (i.e. the cells will produce an easily measurable signal protein upon exposure to compounds that activate the receptor in question). A predecessor to the highly sensitive mammalian cell models mentioned above is the YES/YAS assays, where a similar reporter gene strategy is used, but in cultured yeast cells (Robitaille et al., 2022). The sensitivity of the yeast assays is lower than the mammalian cell models and based on this, and the higher biological relevance of mammalian cells for drinking water testing, the use of YES/YAS assays is no longer recommended (Robitaille et al., 2022). Also, whole-organism tests, such as zebrafish embryonic assays, can be used to monitor estrogenic effects in water samples (Brion et al., 2019).

3.3 | Oxidative stress response

Many environmental pollutants, for example, pesticides, metals, pharmaceuticals, disinfection by-products, and natural substances, for example, curcumin and genistein, can cause oxidative stress. Oxidative stress occurs from excess reactive oxygen radicals and an imbalance in the antioxidant defense system. It is a common mechanism behind various types of adverse effects, for example, inflammatory effects, developmental toxicity, and cancer (Zheng et al., 2020). An important factor that regulates the cells' defense system during oxidative stress is Nrf2 (nuclear transcription factor erythroid 2-related factor 2). Upon induction of oxidative stress, Nrf2 is upregulated, which can be used as a biomarker for chemicals that cause oxidative stress in water samples (Escher et al., 2013). There are different effect-based methods available to measure oxidative stress in water samples. The most commonly used one is a stably transfected mammalian cell line where the expression of a signal protein is regulated by the level of Nrf2 (i.e., the cells will produce the signal protein upon exposure to compounds that cause oxidative stress in the cells; Escher et al., 2021). Alternatively, downstream effects of oxidative stress can be assayed in the form of the expression or activity of protective enzymes that the cells express in the response to oxidative stress, or by direct measurement of reactive oxygen species (ROS).

3.4 | Genotoxicity

Genotoxicity, different types of damage to our DNA, has potentially very serious endpoints of toxicity, including cancer development and fertility impairment (EFSA Scientific Committee, 2011). Different assays are available for different classes of genotoxicity. The micronucleus test and the Comet assay are used to detect structural damages to the DNA, such as chromosomal damage (micronucleus assay) and DNA strand breaks (Comet assay; Ceretti et al., 2016; Kirkland et al., 2011). Both these tests can be carried out both in mammalian cells cultured in the laboratory (in vitro) and in samples from exposed animals (in vivo; Fenech et al., 2020). Ames test, carried out in bacterial cells, is used to detect different types of mutations in the DNA (Kirkland et al., 2011).

4 | ICEBERG MODELING

It has repeatedly been shown that well-known and often analyzed environmental pollutants only explain a small fraction of the biological effects caused by complex mixtures in drinking water, observed with effect-based methods. This has been demonstrated by comparing the observed biological effect (the bioequivalent concentration from the bioassay, BEQ_{bio}) with the sum of effects expected from the concentrations of single chemicals detected with chemical analysis (sum of bioequivalent concentration of single chemicals, BEQ_{chem}). This expected toxicity from the mixture of detected chemicals can be obtained either by calculation from bioactivities of the individual chemicals detected in the mixture with the same assays as used in the bioassays of the complex mixture, or by using information from bioactivity databases such as the ToxCast database. The actual toxicity can be experimentally determined in a mixture composed of the detected chemicals at the detected concentrations. This comparison of observed bioactivity in the whole mixture including unknown chemicals versus expected bioactivity from detected/known chemicals is referred to as iceberg modeling and is reviewed in detail in the book Bioanalytical Tools in Water Quality Assessment (Escher et al., 2021).

The fraction of the biological effects observed in a water sample that can be explained by well-known and often analyzed environmental pollutants vary drastically between water types and the biological endpoint in question. In some cases, a relatively large portion of estrogenic activity in wastewater-impacted surface water has been explained by compounds that could be analyzed in target chemical analysis (König et al., 2017). In other cases, as little as 0.3% of the observed estrogenic effects in treated wastewater could be explained by target chemical analysis of well-known estrogens (unpublished data). It is well known that environmental levels of the natural estrogenic compound 17β-estradiol (E2) is close to or below the detection limit of chemical analyses, while effect-based methods have much higher sensitivity (Conley et al., 2017). A low explanation factor of estrogenic activity in water samples may be due to low sensitivity of chemical analyses of natural estrogens, such as E2, which in many cases is the major contributor to estrogenic activity in water. For other endpoints, well-known and often analyzed chemicals can only explain a very small fraction of the biological effects observed with effect-based methods. Escher et al. (2013) used effect-based methods to measure the oxidative stress response from nine water samples ranging from treated effluent, recycled water, and stormwater to drinking water. The samples were also chemically analyzed for the concentration of 269 organic micropollutants. The 54 organic micropollutants that were detected in the samples were mixed in the detected concentration ratios and these mixtures were also tested in the effect-based method for oxidative stress, to evaluate how much of the observed oxidative stress in the water samples was caused by the detected micropollutants. Strikingly, the mixture of detected micropollutants could explain less than 0.1% of the observed oxidative stress in the water samples. More than 99.9% of the oxidative stress was caused by unknown chemicals, and would have remained undetected if the evaluation would have relied on chemical analysis alone. Similar findings, where well-known environmental pollutants can explain only a very small fraction of observed biological effects in water samples, have been reported repeatedly, especially for toxicity endpoints that can be triggered by a wide range of chemicals, such as oxidative stress and AhR activity. For example, none of the oxidative stress, genotoxic effects, or anti-androgenicity in water samples could be explained by the regulated contaminants and/or well-known organic micropollutants, which were detected by chemical analysis in the same water samples (Oskarsson et al., 2021; Yu et al., 2021). These two cases will be described more in detail in Section 5.2.

5 | APPLICATIONS OF EFFECT-BASED METHODS FOR MONITORING OF DRINKING WATER SAFETY

An overview of how effect-based methods can be used in different steps of drinking water production is presented in Figure 2

5.1 | Monitoring of drinking water quality and evaluation of treatment efficiency in full-scale plants

Effect-based methods have been used to monitor drinking water quality in multiple studies (Enault et al., 2023). For example, Jones et al. (2020), where the authors conducted an effect-based pilot study on raw and finished water from 10 drinking water utilities in Iowa. Most common effects were activation of the aryl hydrocarbon (AhR) and the androgen receptor (40%–72% of all samples). Medlock Kakaley et al. (2020) studied effects of chemical pollutants along a river

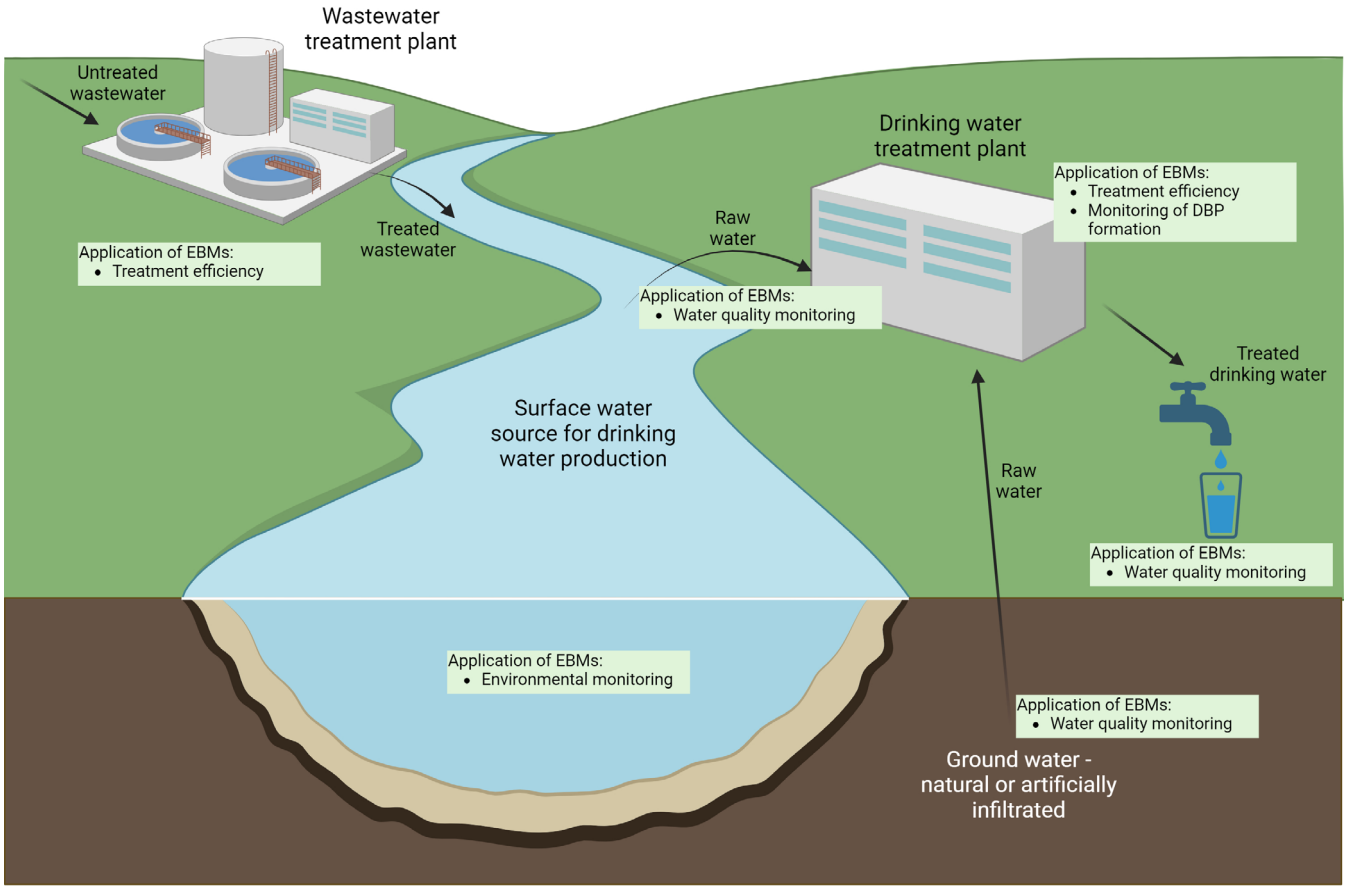

FIGURE 2 An overview of how effect-based methods can be used in different steps of the drinking water production. Treated wastewater can either be directly reused for drinking water production (direct potable reuse) or discharged into a water system that is used as the source for drinking water production (indirect potable reuse). Treatment efficiency evaluations in waste- and/or drinking water treatment plants can be conducted both in the full-scale process or in pilot-scale systems. Figure created with BioRender.com.

in northeastern United States where treated wastewater is discharged into the river, upstream of the intake for drinking water production. The most commonly detected activity was estrogenicity (89% of the samples), while glucocorticoid receptor activity and androgen receptor activity were less common (17% and 11% of the samples, respectively).

By comparing activities from hazardous chemicals in samples after drinking water treatment with those in the corresponding raw water, it is possible to evaluate treatment efficiency in drinking water treatment processes, that is, how efficiently the toxicological activities observed in the raw water are removed with the treatment processes applied. This section will highlight a few examples from the scientific literature, where effect-based methods have been applied to evaluate treatment efficiency in drinking water production.

Neale et al. (2020) evaluated the treatment efficiency in three drinking water treatment facilities in the greater Paris area. The source water for these facilities was collected in three different rivers and the water was treated with different technologies at the facilities, including biological treatment, ozonation, nanofiltration, UV, and granular activated carbon filtration. Estrogenic activities were detected in all raw water samples, in multiple cases above 1 ng E2 equivalents per liter, thus exceeding the guidance value of 1 ng E2/L included in the watch list of substances of concern for water intended for human consumption from the EU Commission (European Commission, 2022a). However, the treatment technologies applied in the facilities could efficiently reduce the estrogenic activities. Oxidative stress was observed in the raw water samples and in some cases, the activity increased after chlorination, which could potentially be explained by the formation of toxic disinfection by-products. Furthermore, the water samples were analyzed for genotoxicity using the Ames test, but no genotoxicity was observed.

Shi et al. (2018) used a combination of chemical analysis and effect-based methods to study the presence of toxic organic pollutants in source and drinking water from Chinese rivers and lakes. The water samples were evaluated using EROD assay, micronucleus assay, Ames test, and an assay for estrogenicity. The untreated water from one of the three

studied water sources was found to be genotoxic in the micronucleus assay, but the genotoxic activity was removed by the water treatment technologies applied, and the finished drinking water did not cause an increase in micronucleus formation. The authors found that all analyzed raw water samples exerted estrogenic effects and that the effects were predominantly caused by known estrogens such as estriol and 17α-ethinylestradiol. The estrogenicity observed in the drinking water samples was considerably lower than in the untreated water, highlighting a high treatment efficiency in the removal of estrogenic effects. However, the drinking water from one of the treatment facilities was still higher (4.2–5.3 ng E2 equivalents per liter) than the proposed trigger values for estrogenic effects in drinking water, which are in the range of 1–3.5 ng E2 equivalents per liter (California State Water Resources Control Board, 2018; European Commission, 2022a; WHO, 2017).

In a study from the United States, Conley et al. (2017) used both chemical analysis and effect-based methods to evaluate the presence of estrogenic compounds in the water from 25 drinking water treatment plants. Estrogenicity was detected in most (17 out of 24) samples of untreated water, while the finished drinking water exerted lower estrogenicity with only 3 out of 24 samples being over the limit of detection, and these three at clearly lower levels than the corresponding untreated waters. This shows a high treatment efficiency for removal of estrogenicity in these drinking water facilities. The study also highlights the high sensitivity of effect-based methods based on mammalian cells to detect estrogenic compounds. The effect-based methods showed the presence of estrogens in almost all raw waters, while the chemical analysis could only detect estrogens above the limit of detection in five of the 24 samples. Relying on only chemical analysis for the assessment of estrogens in water is hence associated with a risk of underestimating the potential risks for human and environmental health.

In another study, Medlock Kakaley et al. (2021) used cell-based bioassays for estrogenic, androgenic, and antiandrogenic effects, as well as for glucocorticoid receptor agonist activity, to evaluate the water quality at three drinking water facilities in Chicago, IL and East Chicago, IN. At two of the sites, water samples were collected from the raw lake water, after drinking water treatment but before distribution and finally after distribution at the consumer tap. At the third site, only raw lake water and water after drinking water treatment but before distribution were analyzed. The authors observed estrogenic activity in the untreated lake water, but not in the distributed water at the consumer tap. Androgenic activity was observed in two of the samples of lake water and in two of the samples collected after drinking water treatment, but only before distribution to consumers. No antiandrogenic or glucocorticoid activities were observed in any of the samples.

Hebert et al. (2018) used an effect-based approach to evaluate the risk of disinfection by-product formation by comparing oxidative stress and p53 activity in drinking water before and after chlorination. Activation of p53, a tumor suppressor gene, is used as an in vitro indicator of genotoxic activity. Oxidative stress was observed in all samples before chlorination and the activity was increased after chlorination, an observation that is attributed to the formation of oxidative stress-inducing disinfection by-products. This study highlights the potential to use effect-based methods to evaluate the treatment efficiency, or risk of by-product formation, over a specific treatment step in a treatment system by comparing samples collected immediately before and after that treatment step.

In a study from our group (Rosenmai et al., 2018), we used a panel of effect-based methods to study the removal efficiency of conventional drinking water treatment technologies in a Swedish drinking water treatment plant. We observed AhR inducing effects, estrogenicity, and oxidative stress (Nrf2 activity) in the untreated water and the conventional treatment technologies applied at this facility had little or no effect on these bioactivities. In another study (Oskarsson et al., 2021), we evaluated the removal efficiency for a panel of effect-based endpoints in seven Swedish drinking water treatment facilities, all using river Göta Älv as their source of water. Most of these facilities showed a high removal efficiency for the bioactivities observed in the untreated water, mainly AhR activity and antiandrogenic effects. AhR activity was observed in all untreated water samples and the activity was removed in most cases by the conventional treatment processes used. Antiandrogenic effects were observed in the untreated water from five of the seven studied facilities. In four of the cases, the antiandrogenic effects were efficiently removed. In the fifth case, the antiandrogenic activity as well as oxidative stress was instead higher after water treatment. This case will be presented more in detail in Section 5.2.

5.2 | Detection of effects from emerging pollutants in drinking water

Effect-based methods are ideal for screening drinking water for unknown emerging pollutants, which will not be revealed by the targeted chemical analyses of regulated or well-known pollutants. Using effect-based methods we have in

two studies detected major toxic effects in water samples, which were acceptable for all the parameters included in the drinking water regulation. In the first example (Oskarsson et al., 2021), we analyzed water samples collected along the Swedish river Göta Älv. This water system is an example of indirect water reuse, where treated wastewater is discharged into the river and raw water for drinking water production is collected, alternately. Water samples were collected at two wastewater treatment plant effluents and at the inlet and outlet of seven drinking water treatment plants collecting their raw water from this river. Surprisingly, at one of the drinking water treatment plants, we observed much higher activities for oxidative stress and antiandrogenic effects in the treated drinking water as compared to the untreated raw water collected from the river, indicating that the water had been contaminated during the treatment process, most likely during the process of artificial infiltration. A parallel study (Tröger et al., 2020) used chemical analysis to investigate the presence of 163 organic micropollutants, of which 27 were detected in the finished drinking water from this plant. We were, however, able to conclude that these compounds were not responsible for the observed oxidative stress and antiandrogenic effects observed with effect-based methods. Interestingly, based on the chemical analyses, Tröger et al concluded that the treatment efficiency in this particular drinking water treatment plant was the highest of the seven plants studied, while our effect-based methods could show that the water in fact was contaminated by compounds with hazardous properties during the treatment process, highlighting the need for effect-based methods in drinking water safety efforts.

In another study (Yu et al., 2021), we observed oxidative stress and genotoxicity, detected by the micronucleus test, in the raw water used for drinking water production at a large Swedish drinking water treatment plant. These effects were observed in the raw water at multiple sampling occasions, and on some occasions also in the treated drinking water being distributed to consumers. The oxidative stress and genotoxicity were observed in the water while all regulated chemical parameters were at acceptable levels, showing that this contamination of the water by compounds with hazardous properties would have remained unknown if only the regulated chemical parameters had been analyzed.

Feretti et al. (2020) studied genotoxicity in raw and finished drinking water in four distribution systems in Sardinia, Italy, using a battery of in vitro tests. They found genotoxicity in raw water, which was not reduced by the pre-oxidant/disinfection treatment. In another system, genotoxicity was introduced by the pre-oxidant/disinfection process from nongenotoxic raw water.

These examples clearly show that the current drinking water regulation for chemical contaminants, focusing on the concentrations of a very limited number of compounds, is insufficient to detect emerging threats to drinking water safety, and that effect-based methods are valuable tools to detect emerging pollutants in raw and drinking water.

5.3 | Evaluation of new treatment technologies in drinking water processing

Effect-based methods can also be used to evaluate new technologies in drinking water treatment, before full-scale investment. This can be done either on a laboratory scale or a pilot scale. Such evaluation of new technologies is important both to study the removal-efficiency of bioactive compounds present in the raw water and to monitor the risk of formation of toxic disinfection by-products and transformation products.

Water shortage and more frequently occurring droughts have increased the interest for direct potable reuse of wastewater, that is, treating wastewater to drinkable standards. Such water treatment typically involves advanced treatment processes and disinfection, which has led to a discussion regarding potential risk of formation of toxic by-products. In a recent study, Lau, Bokenkamp, et al. (2023) used an effect-based approach to evaluate the formation of disinfection by-products in recycled water and compared it to conventional drinking water produced from surface water. The water samples were analyzed for cytotoxic effects, which is a proxy for acute toxicity, assayed by measuring basal cell health parameters. Generally, the cytotoxicity of recycled water was lower than the cytotoxicity of corresponding drinking water conventionally produced from surface water. Furthermore, the authors could evaluate how the cytotoxicity in the recycled water changed over each treatment step in different treatment trains, including reversed osmosis, advanced oxidation, ozonation, biologically active filtration, UV treatment, and granulated active carbon filtration, with generally good removal-efficiency of the cytotoxicity as compared to the incoming water to the process.

In another study, Lau, Feng, et al. (2023) also used cytotoxicity to evaluate two option to reduce formation of toxic DBP in drinking water, namely granular activated carbon treatment (to remove DBP precursors) with postchlorination and chlorination followed by chloramination. They found that the first option always resulted in lower cytotoxicity.

Heringa et al. (2011) used Ames test and Comet assay to evaluate the risk of formation of genotoxic by-products during UV/H_2O_2 treatment followed by GAC filtration, in two pilot-scale systems and one full-scale system. Samples were collected after each treatment step, to allow an evaluation both of the risk of formation of toxic by-products during the UV/H_2O_2 step and the potential removal-efficiency of such compounds over the GAC filtration. No genotoxicity was observed in any of the samples using Comet assay. In one of the bacterial strains used for Ames test, genotoxicity was observed in the water samples after UV/H_2O_2 treatment from all three test sites. However, the subsequent GAC filtration effectively removed the genotoxic activity down to negative control levels (two test sites) or at least to a level lower than before UV/H_2O_2 treatment (one test site). This is a good example of the added value of sampling after each step in a treatment train, as it enables an evaluation of both the risk of formation of toxic by-products during advanced treatment steps, and the potential of subsequent treatment steps to remove such by-products.

In a study from 2019 (Lundqvist et al., 2019), we used effect-based methods to evaluate the potential for the formation of toxic disinfection by-products in raw and drinking water. The study was mainly focused on assays for oxidative stress and genotoxicity, as these are two known pathways for disinfection by-product toxicity. Initially, we evaluated the oxidative stress response in raw water and compared it to finished drinking water from a conventional drinking water treatment facility where the final step of treatment is a low dose of monochloramine. No induced oxidative stress was observed in the finished drinking water as compared to the untreated raw water, indicating that this low dose of monochloramine did not induce the formation of disinfection by-products with a potential to cause oxidative stress. Additionally, we evaluated how a novel drinking water treatment technology, including suspended ion exchange, ozonation, in-line coagulation, ceramic microfiltration, and granular activated carbon altered the disinfection by-product formation potential. Raw water and water samples after each step in the treatment regimen were collected and subjected to a high-dose chlorination, to investigate the formation potential for toxic disinfection by-products. We found that each treatment step clearly decreased the formation potential for oxidative stress-inducing and genotoxic disinfection by-products, most likely by removing the precursors for the by-product formation.

In the study referenced above (Yu et al., 2021), where oxidative stress and genotoxicity were observed in the raw and, at some occasions, also in the finished drinking water, we were also able to use an effect-based approach to evaluate a pilot scale novel treatment technology to remove these unwanted effects from the water. Granular activated carbon filtration either alone or combined with ozonation was found to efficiently remove both the oxidative stress and the genotoxicity observed in samples of raw water and after sand filtration. This example highlights how effect-based methods can be used to guide drinking water treatment plants in the design of new treatment processes to remove unwanted biological effects in the water revealed by effect-based monitoring.

6 | REGULATORY AND WATER SECTOR ACCEPTANCE

Chemical hazards in drinking water, and the need for a more holistic monitoring approach, is gaining attention. For example, in the recently revised European Union Drinking Water Directive (European Union, 2020), a risk-based approach is legislated for the drinking water safety efforts. Dingemans et al. (2019) have highlighted the need for non-targeted methods, such as effect-based bioassays, in this risk-based approach, to ensure that also effects from currently unknown pollutants and cocktail effects can be detected. Recently, the Commission of the European Union published a proposal (European Commission, 2022b) for an update of three directives relating to the protection of ground water and surface water. If accepted, these revised directives would (1) mandate the member states to use effect-based monitoring for estrogenic effects in water bodies, and (2) provide guidance to improve the monitoring of groups/mixtures of pollutants by using effect-based methods. The proposal highlights that the European regulators have embraced the principle of effect-based monitoring to perform a holistic assessment of chemical hazards in the water environment.

While numerous papers (Brack et al., 2019; Dingemans et al., 2019) have pointed to effect-based methods as valuable tools for monitoring of drinking water safety, the use of these methods for routine monitoring and regulation is still in its early implementation phase. Dechesne et al. (2022) recently reported the results from a survey with a global panel of stakeholders from the water sector, addressing the perception and barriers for the implementation of effect-based methods. The majority of the respondents were representing water utilities with regulators being an additional important group of respondents. The survey showed that a majority of the respondents agreed that effect-based methods could improve water quality monitoring and the public confidence in drinking water. Further, the survey concluded that the main barriers for implementation of effect-based methods include the cost of analysis, lack of regulatory acceptance and standardization/guidelines and the challenge of interpretation of results, and lack of trigger values.

California State Water Boards have mandated the use of effect-based methods for two endpoints (estrogenicity and Ah receptor activity) in their Policy for Water Quality Control for Recycled Water (California State Water Resources Control Board, 2018).

7 | CONCLUSION AND FUTURE PERSPECTIVE

To achieve the United Nation's Sustainable Development Goal (SDG) 6, Clean Water and Sanitation, we have to ascertain the chemical safety of drinking water. Climate change and chemical pollutants threaten the public access to clean and safe drinking water on a global scale. Specifically, climate change challenges the access to drinking water due to more frequent droughts and other extreme weather events, which in many regions will severely jeopardize the availability of drinking water. The increasing frequency of droughts will increase water reuse applications, which require more advanced treatment technologies but also better monitoring of chemical hazards in the produced potable water. The application of advanced drinking water treatment technologies comes with an increased risk of formation of unknown and potentially hazardous by-products. Given the vast number of potential chemical contaminants and the increasing pollution pressure on water sources, there is a great need for a new strategy to monitor chemical hazards in drinking water. An extensive body of scientific literature, partly referenced in this review, has shown that the well-known and regulated chemical pollutants only explain a small fraction of the unwanted biological effects in water, including drinking water. The overwhelming majority of unwanted biological effects in water samples is caused by unknown chemicals and/or mixture effects. Hence, there is a strong scientific basis calling for an updated strategy for chemical safety in drinking water and the use of more untargeted analytical methods (e.g., combinations of effect-based methods and nontarget chemical screening) to monitor chemical hazards in the raw water, to monitor the efficiency of the water treatment, to understand treatment processes and the risk of disinfection by-product formation, and to monitor the quality of the finished drinking water.

The effect-based methods are in their nature very different from the analytical–chemical methods currently mainly used to monitor chemical hazards in drinking water, that is, biological assays with living cells versus targeted chemical analysis. Hence, the inclusion of effect-based methods in the drinking water quality control will require the establishment of specialized laboratories for these analyses. The current cost for conducting effect-based monitoring is in the same range as more advanced chemical analyses (e.g., drug residues), but could be expected to decrease over time as the methods are very suitable for high-throughput applications. Further discussions are needed between the scientific community and the regulators, to define the most important toxicity endpoints to include in a standard test panel for drinking water quality control. A *Global Water Research Coalition* report recently proposed a test battery covering estrogenic effects, oxidative stress, AhR activity, and genotoxicity as suitable for different drinking water applications.

Ferraro and Prasse recently suggested that the future monitoring framework for chemical hazards has to be based on a combination of effect-based monitoring and advanced chemical screening (Ferraro & Prasse, 2021). Recently, Neale et al. (2022) argued that effect-based methods can play a pivotal role in water safety planning, by integrating hazards from unknown chemicals and mixture effects. The authors stress that effect-based methods can be valuable tools across most modules in a water safety plan, from system assessment and validation monitoring to operational and verification monitoring, thereby contributing to drinking water safety from source to tap. The recently published proposal from the Commission of the European Union, mandating the use of effect-based methods in the environmental monitoring of hazardous chemicals in groundwater and surface waters highlights that the European regulators are embracing the effect-based approach and we expect that the effect-based methods will soon be included in drinking water regulations across the world.

AUTHOR CONTRIBUTIONS

Johan Lundqvist: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Agneta Oskarsson:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Johan Lundqvist and Agneta Oskarsson are the founders of and stock owners in BioCell Analytica Uppsala AB, a company providing effect-based testing services to the water sector.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Adeel, M., Song, X., Wang, Y., Francis, D., & Yang, Y. (2017). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International*, *99*, 107–119. https://doi.org/10.1016/j.envint.2016.12.010

Bock, K. W. (2019). Aryl hydrocarbon receptor (AHR): From selected human target genes and crosstalk with transcription factors to multiple AHR functions. *Biochemical Pharmacology*, *168*, 65–70. https://doi.org/10.1016/j.bcp.2019.06.015

Brack, W., Aissa, S. A., Backhaus, T., Dulio, V., Escher, B. I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Posthuma, L., Seiler, T.-B., Slobodnik, J., Teodorovic, I., Tindall, A. J., de Aragão Umbuzeiro, G., Zhang, X., & Altenburger, R. (2019). Effect-based methods are key. The European collaborative project solutions recommends integrating effect-based methods for diagnosis and monitoring of water quality. *Environmental Sciences Europe*, *31*(1), 10. https://doi.org/10.1186/s12302-019-0192-2

Brion, F., De Gussem, V., Buchinger, S., Hollert, H., Carere, M., Porcher, J.-M., Piccini, B., Féray, C., Dulio, V., Könemann, S., Simon, E., Werner, I., Kase, R., & Aït-Aïssa, S. (2019). Monitoring estrogenic activities of waste and surface waters using a novel in vivo zebrafish embryonic (EASZY) assay: Comparison with in vitro cell-based assays and determination of effect-based trigger values. *Environment International*, *130*, 104896. https://doi.org/10.1016/j.envint.2019.06.006

California State Water Resources Control Board. (2018). Water quality control policy for recycled water.

Ceretti, E., Moretti, M., Zerbini, I., Villarini, M., Zani, C., Monarca, S., & Feretti, D. (2016). Occurrence and control of Genotoxins in drinking water: A monitoring proposal. *Journal of Public Health Research*, *5*(3), 769. https://doi.org/10.4081/jphr.2016.769

Conley, J. M., Evans, N., Mash, H., Rosenblum, L., Schenck, K., Glassmeyer, S., Furlong, E. T., Kolpin, D. W., & Wilson, V. S. (2017). Comparison of in vitro estrogenic activity and estrogen concentrations in source and treated waters from 25 U.S. drinking water treatment plants. *The Science of the Total Environment*, *579*, 1610–1617. https://doi.org/10.1016/j.scitotenv.2016.02.093

Davey, R. A., & Grossmann, M. (2016). Androgen receptor structure, function and biology: From bench to bedside. *The Clinical Biochemist. Reviews*, *37*(1), 3–15.

Dechesne, M., Dingemans, M., de Baat, M., Canteau, G., Enault, J., Escher, B., Kools, S., Leusch, F., Loret, J., Neale, P., Pronk, G., & Rinck-Pfeiffer, S. (2022). *Effect-based monitoring: Perception & barriers to implementation* (p. 2022). Poster at World Water Congress.

Dingemans, M. M., Baken, K. A., van der Oost, R., Schriks, M., & van Wezel, A. P. (2019). Risk-based approach in the revised European Union drinking water legislation: Opportunities for bioanalytical tools. *Integrated Environmental Assessment and Management*, *15*(1), 126–134. https://doi.org/10.1002/ieam.4096

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). (2010). Scientific opinion on dietary reference values for water. *EFSA Journal*, *8*(3), 1459. https://doi.org/10.2903/j.efsa.2010.1459

EFSA Scientific Committee. (2011). Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal*, *9*(9), 2379. https://doi.org/10.2903/j.efsa.2011.2379

Enault, J., Loret, J.-F., Neale, P. A., de Baat, M. L., Escher, B. I., Belhadj, F., Kools, S. A. E., Pronk, G. J., & Leusch, F. D. L. (2023). How effective are water treatment processes in removing toxic effects of micropollutants? A literature review of effect-based monitoring data. *Journal of Water and Health*, *21*(2), 235–250. https://doi.org/10.2166/wh.2023.235

Escher, B., Neale, P., & Leusch, F. (2021). Bioanalytical tools in water quality. *Assessment*. https://doi.org/10.2166/9781789061987

Escher, B. I., Allinson, M., Altenburger, R., Bain, P. A., Balaguer, P., Busch, W., Crago, J., Denslow, N. D., Dopp, E., Hilscherova, K., Humpage, A. R., Kumar, A., Grimaldi, M., Jayasinghe, B. S., Jarosova, B., Jia, A., Makarov, S., Maruya, K. A., Medvedev, A., … Leusch, F. D. L. (2014). Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environmental Science & Technology*, *48*(3), 1940–1956. https://doi.org/10.1021/es403899t

Escher, B. I., Henneberger, L., König, M., Schlichting, R., & Fischer, F. C. (2020). Cytotoxicity burst? Differentiating specific from nonspecific effects in Tox21 in vitro reporter gene assays. *Environmental Health Perspectives*, *128*(7), 077007. https://doi.org/10.1289/EHP6664

Escher, B. I., Stapleton, H. M., & Schymanski, E. L. (2020). Tracking complex mixtures of chemicals in our changing environment. *Science (New York, N.Y.)*, *367*(6476), 388–392. https://doi.org/10.1126/science.aay6636

Escher, B. I., van Daele, C., Dutt, M., Tang, J. Y. M., & Altenburger, R. (2013). Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. *Environmental Science & Technology*, *47*(13), 7002–7011. https://doi.org/10.1021/es304793h

European Commission. (2022a). Commission Implementing Decision (EU) 2022/679 of 19 January 2022 establishing a watch list of substances and compounds of concern for water intended for human consumption as provided for in Directive (EU) 2020/2184 of the European Parliament and of the Council (notified under document C(2022) 142) (Text with EEA relevance). http://data.europa.eu/eli/dec_impl/2022/679/oj/eng

European Commission. (2022b). Proposal for a Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standard s in the field of water policy.

European Union. (2020). Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. *Official Journal of the European Union*.

Fenech, M., Knasmueller, S., Bolognesi, C., Holland, N., Bonassi, S., & Kirsch-Volders, M. (2020). Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans. *Mutation Research, Reviews in Mutation Research*, *786*, 108342. https://doi.org/10.1016/j.mrrev.2020.108342

Feretti, D., Acito, M., Dettori, M., Ceretti, E., Fatigoni, C., Posadino, S., Zerbini, I., Villarini, M., Moretti, M., Castiglia, P., & Azara, A. (2020). Genotoxicity of source, treated and distributed water from four drinking water treatment plants supplied by surface water in Sardinia, Italy. *Environmental Research*, *185*, 109385. https://doi.org/10.1016/j.envres.2020.109385

Ferraro, P. J., & Prasse, C. (2021). Reimagining safe drinking water on the basis of twenty-first-century science. *Nature Sustainability*, *4*(12), 1032–1037. https://doi.org/10.1038/s41893-021-00760-0

Guan, Y., Wang, X., Wong, M., Sun, G., An, T., Guo, J., & Zhang, G. (2017). Evaluation of genotoxic and mutagenic activity of organic extracts from drinking water sources. *PLoS One*, *12*(1), e0170454. https://doi.org/10.1371/journal.pone.0170454

Hebert, A., Feliers, C., Lecarpentier, C., Neale, P. A., Schlichting, R., Thibert, S., & Escher, B. I. (2018). Bioanalytical assessment of adaptive stress responses in drinking water: A predictive tool to differentiate between micropollutants and disinfection by-products. *Water Research*, *132*, 340–349. https://doi.org/10.1016/j.watres.2017.12.078

Heringa, M. B., Harmsen, D. J. H., Beerendonk, E. F., Reus, A. A., Krul, C. A. M., Metz, D. H., & IJpelaar, G. F. (2011). Formation and removal of genotoxic activity during UV/H_2O_2–GAC treatment of drinking water. *Water Research*, *45*(1), 366–374. https://doi.org/10.1016/j.watres.2010.08.008

Jones, R. R., Stavreva, D. A., Weyer, P. J., Varticovski, L., Inoue-Choi, M., Medgyesi, D. N., Chavis, N., Graubard, B. I., Cain, T., Wichman, M., Beane Freeman, L. E., Hager, G. L., & Ward, M. H. (2020). Pilot study of global endocrine disrupting activity in Iowa public drinking water utilities using cell-based assays. *Science of the Total Environment*, *714*, 136317. https://doi.org/10.1016/j.scitotenv.2019.136317

Judson, R., Houck, K., Martin, M., Richard, A. M., Knudsen, T. B., Shah, I., Little, S., Wambaugh, J., Woodrow Setzer, R., Kothya, P., Phuong, J., Filer, D., Smith, D., Reif, D., Rotroff, D., Kleinstreuer, N., Sipes, N., Xia, M., Huang, R., … Thomas, R. S. (2016). Analysis of the effects of cell stress and cytotoxicity on in vitro assay activity across a diverse chemical and assay space. *Toxicological Sciences*, *152*(2), 323–339. https://doi.org/10.1093/toxsci/kfw092

Kirkland, D., Reeve, L., Gatehouse, D., & Vanparys, P. (2011). A core in vitro genotoxicity battery comprising the Ames test plus the in vitro micronucleus test is sufficient to detect rodent carcinogens and in vivo genotoxins. *Mutation Research*, *721*(1), 27–73. https://doi.org/10.1016/j.mrgentox.2010.12.015

König, M., Escher, B. I., Neale, P. A., Krauss, M., Hilscherová, K., Novák, J., Teodorović, I., Schulze, T., Seidensticker, S., Kamal Hashmi, M. A., Ahlheim, J., & Brack, W. (2017). Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. *Environmental Pollution*, *220*(Pt B), 1220–1230. https://doi.org/10.1016/j.envpol.2016.11.011

Lau, S. S., Bokenkamp, K., Tecza, A., Wagner, E. D., Plewa, M. J., & Mitch, W. A. (2023). Toxicological assessment of potable reuse and conventional drinking waters. *Nature Sustainability*, *6*(1), 39–46. https://doi.org/10.1038/s41893-022-00985-7

Lau, S. S., Feng, Y., Gu, A. Z., Russell, C., Pope, G., & Mitch, W. A. (2023). Cytotoxicity comparison between drinking water treated by chlorination with Postchloramination versus granular activated carbon (GAC) with Postchlorination. *Environmental Science & Technology*, *57*(36), 13699–13709. https://doi.org/10.1021/acs.est.3c03591

Lundqvist, J., Andersson, A., Johannisson, A., Lavonen, E., Mandava, G., Kylin, H., Bastviken, D., & Oskarsson, A. (2019). Innovative drinking water treatment techniques reduce the disinfection-induced oxidative stress and genotoxic activity. *Water Research*, *155*, 182–192. https://doi.org/10.1016/j.watres.2019.02.052

Medlock Kakaley, E., Cardon, M. C., Evans, N., Iwanowicz, L. R., Allen, J. M., Wagner, E., Bokenkamp, K., Richardson, S. D., Plewa, M. J., Bradley, P. M., Romanok, K. M., Kolpin, D. W., Conley, J. M., Gray, L. E., Hartig, P. C., & Wilson, V. S. (2021). In vitro effects-based method and water quality screening model for use in pre- and post-distribution treated waters. *The Science of the Total Environment*, *768*, 144750. https://doi.org/10.1016/j.scitotenv.2020.144750

Medlock Kakaley, E. K., Blackwell, B. R., Cardon, M. C., Conley, J. M., Evans, N., Feifarek, D. J., Furlong, E. T., Glassmeyer, S. T., Gray, L. E., Hartig, P. C., Kolpin, D. W., Mills, M. A., Rosenblum, L., Villeneuve, D. L., & Wilson, V. S. (2020). De facto water reuse: Bioassay suite approach delivers depth and breadth in endocrine active compound detection. *Science of the Total Environment*, *699*, 134297. https://doi.org/10.1016/j.scitotenv.2019.134297

Neale, P. A., Escher, B. I., de Baat, M. L., Dechesne, M., Deere, D. A., Enault, J., Kools, S. A. E., Loret, J.-F., Smeets, P. W. M. H., & Leusch, F. D. L. (2022). Effect-based monitoring to integrate the mixture hazards of chemicals into water safety plans. *Journal of Water and Health*, *20*(12), 1721–1732. https://doi.org/10.2166/wh.2022.165

Neale, P. A., Escher, B. I., de Baat, M. L., Dechesne, M., Dingemans, M. M. L., Enault, J., Pronk, G. J., Smeets, P. W. M. H., & Leusch, F. D. L. (2023). Application of effect-based methods to water quality monitoring: Answering frequently asked questions by water quality managers, regulators, and policy makers. *Environmental Science & Technology*, *57*(15), 6023–6032. https://doi.org/10.1021/acs.est.2c06365

Neale, P. A., Feliers, C., Glauch, L., König, M., Lecarpentier, C., Schlichting, R., Thibert, S., & Escher, B. I. (2020). Application of in vitro bioassays for water quality monitoring in three drinking water treatment plants using different treatment processes including biological treatment, nanofiltration and ozonation coupled with disinfection. *Environmental Science: Water Research & Technology*, *6*(9), 2444–2453. https://doi.org/10.1039/C9EW00987F

OECD. (2016). *Test No. 487: In vitro mammalian cell micronucleus test*. OECD. https://doi.org/10.1787/9789264264861-en

Oskarsson, A., Rosenmai, A. K., Mandava, G., Johannisson, A., Holmes, A., Tröger, R., & Lundqvist, J. (2021). Assessment of source and treated water quality in seven drinking water treatment plants by in vitro bioassays—Oxidative stress and antiandrogenic effects after artificial infiltration. *Science of the Total Environment*, *758*, 144001. https://doi.org/10.1016/j.scitotenv.2020.144001

Robitaille, J., Denslow, N. D., Escher, B. I., Kurita-Oyamada, H. G., Marlatt, V., Martyniuk, C. J., Navarro-Martín, L., Prosser, R., Sanderson, T., Yargeau, V., & Langlois, V. S. (2022). Towards regulation of endocrine disrupting chemicals (EDCs) in water resources using bioassays—A guide to developing a testing strategy. *Environmental Research*, *205*, 112483. https://doi.org/10.1016/j.envres.2021.112483

Rosenmai, A. K., Lundqvist, J., le Godec, T., Ohlsson, Å., Tröger, R., Hellman, B., & Oskarsson, A. (2018). In vitro bioanalysis of drinking water from source to tap. *Water Research*, *139*, 272–280. https://doi.org/10.1016/j.watres.2018.04.009

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. *Environmental Pollution*, *233*, 35–44. https://doi.org/10.1016/j.envpol.2017.10.033

Srivastav, A. L., Patel, N., & Chaudhary, V. K. (2020). Disinfection by-products in drinking water: Occurrence, toxicity and abatement. *Environmental Pollution*, *267*, 115474. https://doi.org/10.1016/j.envpol.2020.115474

Thacharodi, A., Hassan, S., Hegde, T. A., Thacharodi, D. D., Brindhadevi, K., & Pugazhendhi, A. (2023). Water a major source of endocrine-disrupting chemicals: An overview on the occurrence, implications on human health and bioremediation strategies. *Environmental Research*, *231*, 116097. https://doi.org/10.1016/j.envres.2023.116097

Tröger, R., Köhler, S. J., Franke, V., Bergstedt, O., & Wiberg, K. (2020). A case study of organic micropollutants in a major Swedish water source—Removal efficiency in seven drinking water treatment plants and influence of operational age of granulated active carbon filters. *Science of the Total Environment*, *706*, 135680. https://doi.org/10.1016/j.scitotenv.2019.135680

WHO. (2017). Drinking water Parameter Cooperation Project. Support to the revision of Annex I Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption (Drinking Water Directive).

Yu, M., Lavonen, E., Oskarsson, A., & Lundqvist, J. (2021). Removal of oxidative stress and genotoxic activities during drinking water production by ozonation and granular activated carbon filtration. *Environmental Sciences Europe*, *33*(1), 124. https://doi.org/10.1186/s12302-021-00567-y

Zheng, F., Gonçalves, F. M., Abiko, Y., Li, H., Kumagai, Y., & Aschner, M. (2020). Redox toxicology of environmental chemicals causing oxidative stress. *Redox Biology*, *34*, 101475. https://doi.org/10.1016/j.redox.2020.101475

Zhou, S., Peng, S., Brack, W., Doering, J. A., Seiler, T.-B., & Hollert, H. (2021). Bioanalytical equivalents and relative potencies for predicting the biological effects of mixtures. *Science of the Total Environment*, *763*, 143030. https://doi.org/10.1016/j.scitotenv.2020.143030

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